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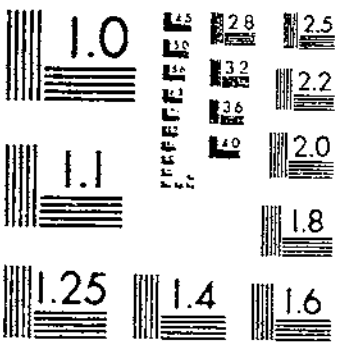
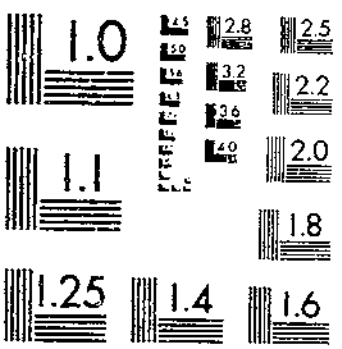


MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A



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PREVENTING
Japanese Beetle Dispersion
by
Farm Products and Nursery Stock

Technical Bulletin No. 1441

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Preventing Japanese Beetle Dispersion by Farm Products and Nursery Stock

By Walter E. Fleming, *collaborator, Entomology Research Division, Agricultural Research Service*

The Japanese beetle (*Popillia japonica* Newman), a native of Japan, was found in a commercial nursery in southern New Jersey in 1916 by inspectors of the New Jersey Department of Agriculture. This infestation probably developed from grubs in the soil about the rhizomes of *Iris kaempferi*, which had been imported by the nursery from Japan prior to 1912. Before its discovery in New Jersey the beetle was known to occur only on the main islands of Japan. (Dickerson and Weiss *1918;* Howard *1918;* Smith and Hadley *1926;* Weiss *1918a, 1918b*)[1]

The beetle has one generation a year. The eggs hatch during the summer. Most of the grubs are full grown in the fall and reach maturity in the spring. Late in the spring the grubs pupate, and finally the adult beetles emerge from the soil. The adult beetle is present during the summer, but one or more of the immature stages are in the soil throughout the year.

In its new environment the beetle found a generally favorable climate, large areas of turf for developing the immature stages, almost 300 plant species to satisfy its voracious appetite, and at that time no important natural enemies. Biological control of the beetle has been discussed by Fleming (*1968*). From this small beginning the beetle multiplied rapidly and spread until by 1970 it had invaded over 100,000 square miles, covering all or parts of 19 States and the District of Columbia in the Eastern United States and part of one Province in Canada. It also became established in several isolated areas beyond this region of natural distribution (Fleming *1970*).

The beetle soon became a threat to American agriculture. The adults seriously damaged orchard crops, small fruits, some garden and field crops, and ornamental trees, shrubs, and vines. The grubs destroyed large areas of turf in lawns, golf courses, parks, and pastures, and they damaged the roots of garden and truck crops and small nursery stock.

[1] The year in italic after the authors' names is the key to the reference in Literature Cited, p. 238.

There was no law prior to 1912 to protect the United States from the entry of foreign plant pests or to control and prevent the distribution of these insects and diseases within the country. Some foreign countries exporting plants found a free market for their products in this country, but for many years they had prohibited the entry of living plants from the United States. (Marlatt *1921*)

The Plant Pest Act of 1912, as amended by Congress, gave broad authority to the Secretary of the U.S. Department of Agriculture to regulate by quarantine the importation and interstate movement of nursery stock, other plants, and plant products carrying foreign plant pests. This law and the Plant Pest Act of 1957 are the basic laws for all plant quarantines in the United States.

Federal quarantines 40 and 48, promulgated by the Secretary in 1920, regulated the interstate movement of all kinds of farm products and plants from the beetle-infested area (U.S. Department of Agriculture *1920a, 1920b*). The infested States imposed quarantines to regulate the intrastate movement of these products and plants.

The general policy in the enforcement of these quarantines was to permit unrestricted movement of all agricultural products within the regulated area and to restrict shipments beyond this area to uninfested products and plants. When the quarantines were promulgated, there was no precedent for treating the many regulated agricultural products. Progress in the development of treatments was slow because plants and plant products were often less tolerant of insecticides than the insect, but eventually many treatments were developed to remove or destroy the insect without seriously injuring the plants and plant products.

Some phases of the investigation were conducted cooperatively by the U.S. Department of Agriculture Japanese Beetle Laboratory, other Federal agencies, and State agricultural experiment stations within the beetle-infested area. The growers and shippers of fruits, vegetables, and nursery and greenhouse plants cooperated by making these products available for experimentation.

Progress reports on the investigation appeared from time to time in Federal and State publications and in various scientific journals. However, much additional information is found in the unpublished progress reports by J. W. Bulger, E. D. Burgess, W. L. Caskey, R. D. Chisholm, L. W. Coles, H. C. Donohoe, W. E. Fleming, C. H. Hadley, H. A. Jaynes, A. C. Johnson, V. A. Johnson, L. Koblitsky, J. W. Lipp, W. W. Maines, A. S.

Mallory, A. C. Mason, M. R. Osburn, L. B. Parker, K. B. Rogers, E. H. Siegler, G. E. Spencer, M. C. Swingle, H. Tashiro, P. A. Vander Meulen, E. R. Van Leeuwen, and C. M. Wible and also in the quarterly and annual unpublished reports of the Japanese Beetle Laboratory by W. E. Fleming and C. H. Hadley on file at the Japanese Beetle Laboratory, Moorestown, N.J. These published and unpublished records have been reviewed here so that information on the development of treatments for agricultural products during 1919–70 [2] might be more available to other entomologists, chemists, and the general public.

QUARANTINE ON FARM PRODUCTS

The quarantine on farm products was in operation from the first emergence of the beetle until none could be found in the area. In southern New Jersey and southeastern Pennsylvania the movement of farm products was usually restricted between mid-June and mid-September. The restrictions were imposed earlier in the more southerly parts and later in the more northerly parts of the region infested by the beetle.

In establishing the limits of a regulated area, the line followed the outer boundaries of political subdivisions, such as townships or counties, where beetles had been found the previous summer by scouting and trapping (Smith *1925a*).

Inspection

The inspection of farm products was not satisfactory because it was difficult to find all the beetles in packages of fruits and vegetables, but for several years it was the only authorized procedure. The inspection, however, did reduce the hazard. In 1925 Smith (*1925a*) reported that in New Jersey 106,832 beetles had been removed from 194,682 packages of corn, 7,223 beetles from 486,880 packages of beans, and 61 beetles from 182,733 packages of miscellaneous products.

Except for the first few years manpower was not available to inspect every package in a shipment, and it was necessary to limit the inspection to those products most likely to carry beetles. The list of products inspected was modified periodically as the beetle invaded new areas and the products shipped out of the

[2] Although the data on which this bulletin is based were collected during 1919–70, the findings are still valid and useful as guidelines for developing additional research to prevent the artificial dispersion of the Japanese beetle and other insects.

regulated area changed. The products inspected prior to 1925 were corn, cabbage, and grapes (Smith and Hadley *1926*); in 1925 the inspected products were corn, beans, peas in the pod, parsley, carrots with tops, onions with tops, lettuce, cabbage, tomatoes, and outdoor-grown flowers (Duryee *1925;* Smith *1925a*); and in 1932 the inspected products were corn, lima beans, string beans, apples, peaches, blackberries, blueberries, huckleberries, and raspberries (Strong *1933*). The shipment of hay, straw, and forage crops from infested farms was prohibited while the beetle was on the wing (Smith *1925a*).

Until the summer of 1923 the products were inspected on the farms. Philadelphia, Pa., was included within the regulated area that year. Since the policy was to permit unrestricted movement of all farm products within the regulated area, thousands of beetles were carried on farm products from New Jersey to the city market. Unrestricted movement of farm products from the city market was permitted within a radius of approximately 25 miles, but shipments from the market beyond that area were prohibited. Farm products moving from the regulated area to uninfested areas were inspected on the farms. (Smith *1925a;* Smith and Hadley *1926*)

In 1924 inspection centers were established at convenient points throughout the regulated area to facilitate the inspection and certification of farm products for shipment to uninfested areas. The number of these centers increased with the expansion of the regulated area; in 1932 there were 67 inspection centers. Many thousands of beetles were removed from farm products at these centers. It is of interest to note that from 1928 to 1933 the inspection centers in Philadelphia were operated between 8 p.m. and 10 a.m., when beetles were not flying, to prevent reinfestation of the inspected products. (Hoyt *1934;* Marlatt *1929;* Smith *1925a;* Smith and Hadley *1926;* Strong *1931, 1933*)

Most of the farm products consigned outside the regulated area were shipped by truck. At the limits of the regulated area all roads were posted with conspicuous signs giving information on the restrictions of the quarantine. Inspectors were placed on the principal roads leaving the area, but inspections might be made at any time or place within or outside the area. Inspectors were stationed at the principal markets outside the regulated area and at the offices of the common carriers in Philadelphia and New York City. The uncertified products were either confiscated by the inspectors or taken back into the regulated area by their owners. There were some violations of the quarantine, but most

were caused by ignorance of the regulations. A few willful violators were arrested, convicted, and fined. In general, the public obeyed the regulations, and the growers and shippers cooperated with the Department in carrying out the regulations. (Duryee *1925;* Hoyt *1934;* Marlatt *1929;* Smith *1925a;* Smith and Hadley *1926;* Strong *1931, 1933;* U.S. Department of Agriculture *1936*)

Nonchemical Treatment of Fruits and Vegetables

Mechanical Removal of Beetles

The commercial grading and packing of apples and peaches eliminated beetles hiding among the fruit. When these operations were supervised by an inspector, the fruit could be certified for shipment outside the regulated area. (Strong *1933*)

The demand for certifying large quantities of string beans from infested fields in southern New Jersey resulted in the development of a mechanical device for separating the beetles from the beans. Several models were constructed and tested by the then Bureau of Plant Quarantine. The model adopted was essentially a sloping cylinder, 7 feet long and 2 feet in diameter, which was constructed of ½-inch mesh hardware cloth and mounted on an iron frame. The beans were poured into a hopper at the higher end of the cylinder and were discharged into the cylinder. As the cylinder revolved the beans tumbled without injury to the lower end of the cylinder and were discharged into a hamper. The beetles were shaken off the beans and fell through the hardware cloth into a container. This device eliminated beetles faster and more efficiently than the best inspectors. It was used extensively for several years throughout the regulated area. (Strong *1933*)

High Frequency Electrostatic Field

Headlee and Burdette (*1929*) and Headlee (*1931*) killed the honey bee (*Apis mellifera* L.), the house fly (*Musca domestica* L.), the imported cabbageworm (*Pieris rapae* (L.)), and the German cockroach (*Blattella germanica* (L.)) by placing them in a high frequency electrostatic field. Swingle (unpublished) exposed Japanese beetles in quart boxes of blackberries and strawberries for 10 minutes in an electrostatic field with a frequency of 12,000 kilocycles per second, a field strength of 3,000 volts, and a flow of 5 amperes between the plates. The treatment had no effect on the beetles.

Ultraviolet Light

Less than 10 percent of the Japanese beetles were killed by an exposure for 60 minutes in quartz test tubes 12 inches away from a quartz mercury vapor arc. The radiation of this lamp extended from 1,850 angstroms in the ultraviolet region to 10,140 angstroms in the infrared region. A fan was directed on the quartz test tubes to remove the heat given off by the lamp. Exposure to the lamp for 60 minutes had no effect on the bean weevil (*Acanthoscelides obtectus* (Say)) or the Colorado potato beetle (*Leptinotarsa decemlineata* (Say)). (Osburn unpublished)

Vacuum and Pressure

Increasing the normal atmospheric pressure from 15 to 150 p.s.i. and retaining that pressure for 10 minutes had no effect on Japanese beetles (Osburn unpublished). Fifty percent of the beetles were killed by an exposure of 160 hours to an atmosphere of 75 p.s.i. and 100 percent by an exposure of 72 hours to 135 p.s.i. (Starkweather and Sullivan *1964*). Reducing the atmospheric pressure from 15 to 5 p.s.i. and retaining that pressure for 17 hours had no effect on the beetles (Mallory unpublished). Reducing the atmospheric pressure from 15 to 5 p.s.i. and then increasing it to 145 p.s.i. or vice versa within 15 minutes killed about 30 percent of the beetles (Mallory unpublished). Reducing the atmospheric pressure from 15 to 2.5 p.s.i. and then increasing it to 200 p.s.i. within 60 minutes killed 42 percent of the beetles (Osburn unpublished).

Centrifugal Force

The beetle was resistant to the stresses imposed by extreme forces of acceleration. Sullivan and McCauley (*1960*) killed 50 percent of the beetles within 10 days by an exposure of 2 minutes to a force of 6,250 g. (times the gravity of the earth), 10 minutes to 5,800 g., and 30 minutes to 4,150 g.

Refrigeration

Many fruits and vegetables were shipped long distances under refrigeration. The usual temperature was about 40° F. Beetles were inactive at that temperature, but an exposure of 96 hours did not kill them. Beetles survived an exposure of 1 hour at 32°, but 42 percent were killed at 23°, 84 percent at 14°, and 100 percent at 5° (Knipling and Sullivan *1957*).

Heat

Although heat cannot be used to treat fresh fruits and vegetables, it could be used to kill beetles hiding in empty trucks, railroad cars, and aircraft and in empty baskets, boxes, and crates. When the relative humidity of the air was between 6 and 21 percent, 54 percent of the beetles were killed by an exposure of 60 minutes at 113° F., and all were killed by an exposure of 30 minutes at 122° or for 15 minutes at 131° (Knipling and Sullivan *1958*). Beetles were also killed by immersion for 40 minutes in water at 110°, for 20 minutes at 112°, for 10 minutes at 114°, and for 1 minute at 122° (Fleming and Baker *1932*).

Fumigation of Fruits and Vegetables

Carbon Disulfide

Carbon disulfide (CS_2) was used for many years to destroy insects infesting grain and other stored products (Chittenden *1913;* Graf *1917;* Hinds *1902, 1917;* Spencer and Strong *1925*). Since then other fumigants or a 1:4 mixture of carbon disulfide and carbon tetrachloride have largely replaced it for the control of stored-products insects (Chisholm *1952;* Cotton *1952*). There was no published record on carbon disulfide being used to fumigate fresh fruits and vegetables.

The commercial grade of carbon disulfide, used as an insecticide, is a volatile, slightly yellowish liquid with a disagreeable fetid odor. It boils at 46.3° C. The specific gravity is 1.263 at 20° and the vapor pressure is 298 mm. at 20°. The vapor is about 2.6 times as heavy as air. It is highly flammable. When mixed with air in the proper proportions, it is explosive. The mixtures may be exploded by a flame or an electric spark. Carbon disulfide ignites spontaneously at 147°. Great care in using this fumigant is essential. (Fleming and Baker *1935*)

Toxicity to Beetles.—At normal atmospheric pressure all beetles were killed by a 2-hour exposure to 2.5 pounds of carbon disulfide in 1,000 cubic feet of air at 95° F. The concentration had to be increased to 7.5 pounds at 90° and to 10 pounds at 80°. The 10-pound treatment killed 96 percent of the beetles at 75°. When the exposure was reduced to 1 hour, all the beetles were not killed by the 10-pound treatment at temperatures below 95°. (Osburn *1930;* Osburn and Lipp *1935*)

The insecticide action was not modified by varying the relative humidity of the air from 1 to 100 percent, but the effectiveness of carbon disulfide was increased by reducing the atmospheric pressure. Only 35 percent of the beetles were killed by a 2-hour exposure at 70° F. to 5 pounds of the chemical in 1,000 cubic feet of air at normal atmospheric pressure of 15 p.s.i. The mortality was increased to 75 percent by reducing the atmospheric pressure to 10 p.s.i. and to 100 percent by reducing it to 5 p.s.i. However, it is more practical to fumigate at normal atmospheric pressure than in a partial vacuum. (Fleming and Baker *1935*)

Fumigation of Fruit.—Beetles were introduced into baskets and crates of blackberries, blueberries, gooseberries, peaches, plums, raspberries, red currants, and strawberries that had been packed for shipment and were exposed for 2 hours at 80° F. to 10 pounds of carbon disulfide in a fumigation chamber of 1,000 cubic feet. All beetles in the baskets and crates and those exposed directly to the vapor were killed by the treatment. The presence of the fruit did not modify the insecticide action. (Osburn *1930;* Osburn and Lipp *1935*)

The treatment did not affect the appearance, flavor, and keeping quality of the fruits with the possible exception of blueberries. After being held for 6 days at 86° F., three varieties of blueberries were slightly less firm than the untreated berries of those varieties. The treatment had no effect on the firmness of the fourth variety. The blackberries were analyzed immediately after removal from the fumigation chamber and again 24 hours later. Traces of carbon disulfide up to 2 p.p.m. were found immediately after fumigation, using the method of Radcliffe (*1909*), but after aeration for 24 hours none of the chemical was detected. (Osburn *1930;* Osburn and Lipp *1935*)

Fumigation of blackberries, blueberries, and raspberries with carbon disulfide at 10 pounds per 1,000 cubic feet and a 2-hour exposure at 80° F. was authorized in 1929 as a basis for certifying the berries for shipment. The growers built fumigation chambers at Hammonton and New Lisbon, N.J. The crates fumigated and certified numbered 9,980 in 1929 (Osburn *1930*), 7,397 in 1930 (Strong *1931*), and 14,966 in 1932 (Strong *1933*). After 1932 when the beetle population declined and fewer plantations were infested, the number of crates fumigated decreased progressively.

Ethylene Oxide

The insecticide properties of ethylene oxide ($(CH_2)_2O$) were discovered by Cotton and Roark (*1928*). Back et al. (*1930*) used ethylene oxide to control insects infesting stored products.

The physical properties of ethylene oxide have been described by Back et al. (*1930*) and Roark and Nelson (*1930*). It is a colorless gas at room temperature. At low temperatures it is a mobile colorless liquid boiling at 10.5° C. at normal pressure. The specific gravity of the liquid is 0.887 at 7°. The vapor is approximately 1.7 times as heavy as air. Concentrations of the vapor up to 3.5 pounds per 1,000 cubic feet of space are nonflammable and nonexplosive. Haenni et al. (*1959*) developed a formulation containing 12 percent of ethylene oxide and 88 percent of dichlorodifluoromethane by weight for use when higher concentrations than 3.5 pounds per 1,000 cubic feet of space were required.

Toxicity to Beetles.—When beetles were exposed directly to the vapor for 1 hour at normal atmospheric pressure, 1 pound of ethylene oxide per 1,000 cubic feet of space killed 54 percent at 75° F. and 93 percent at 80°. With a 2-hour exposure to that dosage, 90 percent were killed at 65°, 96 percent at 70°, and 100 percent at 75°. At 2 pounds per 1,000 cubic feet all beetles were killed within 1 hour at 80° and within 2 hours at 70°. When beetles were in baskets of fresh fruit, it was necessary to use 2 pounds of ethylene oxide per 1,000 cubic feet with a 2-hour exposure at 75° to obtain 100-percent mortality. (Osburn *1931;* Osburn and Lipp *1935*)

Fulton et al. (*1963*) used an ethylene oxide-dichlorodifluoromethane aerosol at 80° F. and killed all beetles exposed for one-half hour to 4 pounds of ethylene oxide per 1,000 cubic feet, for 1 hour to 2 pounds, for 2 hours to 1 pound, and for 4 hours to one-fourth pound. Ethylene oxide was equally as effective against beetles exposed directly to the vapor when it was vaporized or applied as an aerosol. The aerosol was not tested against beetles in boxes and crates of fruit.

Fumigation of Fruit.—Blackberries and raspberries were not injured by exposure for 2 hours at 75° F. to 2 pounds of ethylene oxide per 1,000 cubic feet of space, but the treated blueberries were slightly less firm than the untreated ones. Any ethylene oxide absorbed by the berries had no effect on the odor

or the taste of the berries (Osburn *1931*; Osburn and Lipp *1935*). Fumigation of blackberries, blueberries, and raspberries in this manner was authorized in 1931, but the treatment did not come into general use, probably because the growers were satisfied with the carbon disulfide fumigation.

Conditions at the port of Philadelphia in the early 1930's made it necessary to fumigate railway fruit cars containing green bananas. The authorized ethylene oxide fumigation extensively injured the bananas (Osburn *1931*; Osburn and Lipp *1935*).

Fumigation of Vegetables.—The authorized ethylene oxide fumigation killed all beetles in baskets of corn, lima beans, peppers, and string beans, but it injured lima beans and string beans. Corn and peppers were not injured by the fumigation. (Osburn unpublished) Fumigation of fresh vegetables with ethylene oxide was not authorized.

Hydrocyanic Acid

Hydrocyanic acid (HCN) is a colorless gas at room temperature. It has a strong, characteristic odor like that of bitter almonds, but some people cannot detect it. The boiling point is 26° C., melting point −14°, specific gravity 0.697 at 18°, and vapor pressure 610 mm. at 20°. Its vapors are slightly lighter than air. Hydrocyanic acid is flammable. At concentrations between 5.6 and 40 percent it forms explosive mixtures with air, but in fumigations the concentrations are so low that there is little danger of explosion in the presence of sparks. It is extremely toxic to humans and very dangerous to use. (Chisholm *1952*)

Despite the dangers, hydrocyanic acid is widely used. The liquid may be measured from cylinders with the aid of air pressure. It is generated by adding its sodium or potassium salts to a mixture of sulfuric acid and water. It is released from granular calcium cyanide in the presence of moist air. For special uses it is packaged with an absorbent such as diatomaceous earth, felt, or fiber; the gas is released on distributing these materials in the space to be fumigated. (Chisholm *1952*)

Toxicity to Beetles.—Preliminary experiments by Fleming in 1926 demonstrated that hydrocyanic acid was very toxic to the beetle. Fleming and Burgess (*1943*) studied the dosage-exposure-temperature requirements to kill 100 percent of the beetles exposed directly to the gas. The data are summarized in table 1. With an exposure of 1 hour all the beetles were killed with 2 ounces of hydrocyanic acid in 1,000 cubic feet of air at 95° F., 4 ounces at 65°, 6 ounces at 55°, and 8 ounces at 45°, whereas

TABLE 1.—*Dosage, exposure, and temperature required to kill 100 percent of Japanese beetles exposed directly to hydrocyanic acid (HCN)*

HCN per 1,000 cubic feet (ounces)	Minimum effective exposure	Minimum effective temperature
	Hours	° *F.*
2	1.0	95
	1.5	80
	2.0	75
	3.0	65
4	.5	85
	1.0	65
	1.5	55
	2.0	45
6	.5	70
	1.0	55
	1.5	45
8	1.0	45

with a 2-hour exposure 100-percent mortality was obtained with 2 ounces at 75° and 4 ounces at 45°.

Osburn and Lipp (*1935*) compared the rate of insecticide action of hydrocyanic acid absorbed on granular infusorial earth and on wood paper pulp with that of commercial calcium cyanide (40–50 and 88 percent), using third-instar grubs removed from soil as test insects. The absorbed hydrocyanic acid vaporized rapidly, whereas the gas was released more slowly from calcium cyanide by the action of atmospheric moisture. All the grubs were killed within 30 minutes at 80° F. by 4 ounces of the absorbed hydrocyanic acid in 1,000 cubic feet, but 45 minutes were required for 100-percent mortality with 16 ounces of the calcium cyanide. Two ounces of liquid hydrocyanic acid introduced into a flat pan, from which it vaporized rapidly into 1,000 cubic feet of air, killed all grubs exposed for 90 minutes at 75°.

Fumigation of Bananas.—Large quantities of bananas are brought to Philadelphia for distribution throughout the Eastern States. The green fruit is transferred from the ships to refrigerator cars on the wharves. During 1923–33, migratory flights of beetles occurred during the last part of July and early August across the Delaware River from heavily infested areas in New Jersey into the downtown shopping and marketing districts of Philadelphia. Cars being loaded with bananas when beetles were

abundant on the wharves were not certified for movement outside the regulated area. Attempts to prevent infestation by screening the passage from the ship to the cars were not successful. Problems with labor and railroad schedules made it impractical to transfer the bananas from the ship to the cars at night when the beetles were not flying. (Hoyt *1934*; Smith *1925a. 1925b*) Later the same situation occurred at Wilmington, Del., Baltimore, Md., and New York, N.Y.

Three pounds of calcium cyanide (88 percent) in loaded refrigerator cars of about 2,500-cubic feet capacity for 2 hours at 80° F. killed all the beetles in the cars without injury to the green bananas. One pound of the chemical was dropped into each ice bunker and at the center of the load space. At the completion of the exposure period the cars were iced, the covers of the ice bunkers were fastened in an open position, and these openings were screened to exclude beetles. The cars were aerated during transit. All beetles died within 48 hours after the fumigation period. Experts of the fruit company reported that the fumigation had caused no injury and had not modified the ripening of the fruit. Analyses of the fruit immediately after fumigation by chemists of the fruit company and of the Department, using the method of Viehoever and Johns (*1915*), showed only an occasional trace of cyanide in the skin and none in the pulp of the bananas (Osburn and Lipp *1935*). This fumigation procedure was authorized in 1927 as a basis for certification.

Further research showed that the calcium cyanide fumigation was equally as effective in killing beetles in the cars when the exposure was reduced to 1½ hours and the minimum temperature to 75° F. These changes were made in the authorized treatment.

The calcium cyanide fumigation was very satisfactory with ventilated cargoes where the temperature of the fruit was about the same as that on the wharf, but some injury to the fruit occurred when the cargoes on the ships were refrigerated. The atmospheric moisture condensed on the cold fruit. The injury was eliminated by spreading the calcium cyanide in trays outside the car and then placing these trays on top of the fruit (Osburn and Lipp *1935*).

Large quantities of ventilated and refrigerated bananas were fumigated successfully with calcium cyanide. For example, 122,349 bunches were fumigated during the summer of 1930 (Strong *1931*). The fumigation of bananas on the wharves at Philadelphia was unncessary after 1932 because the dense migratory

flights of beetles from New Jersey had ceased and few beetles were found in the vicinity (Hoyt *1934*), but it soon became necessary to fumigate bananas at Wilmington, Baltimore, and New York.

Osburn and Lipp (*1935*) killed all beetles in loaded cars by introducing 3 ounces of hydrocyanic acid into each ice bunker, 6 ounces per car, when the temperature was not lower than 75° F. and the exposure was for 2 hours. The treatment did not injure the bananas. Hydrocyanic acid was easier to apply than calcium cyanide in that the operator could walk along the tops of the cars and apply the chemical progressively in each bunker, and there was no toxic residue to remove from the cars at the completion of the fumigation period. In 1932 hydrocyanic acid per car cost 37 cents and calcium cyanide $3.45. Fumigation of bananas with hydrocyanic acid, which was authorized in 1932, soon replaced the calcium cyanide treatment. During 1932 hydrocyanic acid was used to fumigate 111 carloads of bananas successfully.

Cars loaded with bananas were fumigated successfully with hydrocyanic acid for several years at Wilmington and at New York, but in 1937 Fleming and Wible (unpublished) found that the treatment was not always effective in killing all the beetles. The temperature of the air in a car could be well above 75° F. during loading, but in about 25 percent of the cars the cold, wet bananas caused the temperature to fall below 75° during the 2-hour exposure. The mortality of the beetles was 100 percent when the minimum temperature was not below 75°, 98 percent at 70°–75°, 91 percent at 60°–70°, and 90 percent below 60°. In addition, the shippers reported that the fumigation had caused some injury to the fruit. The situation had reached an impasse. Neither the dosage nor the exposure could be increased to make the treatment effective at the lower temperatures without increasing the hazard to the bananas. However, by the summer of 1939 the beetle population on the wharves at these ports had reached such a low level that it was possible to discontinue the fumigation of the loaded cars, but as a precaution the empty cars were fumigated in the freight yards before being transferred to the wharves.

Fumigation of empty refrigerator cars, using calcium cyanide or hydrocyanic acid with the same requirements as for loaded cars, was authorized in 1939. Donohoe (*1943a*) demonstrated that the pans in the bunkers into which hydrocyanic acid was poured in the authorized procedure could be eliminated by splashing 4 ounces of the chemical on the end wall of each bunker.

This modification in applying hydrocyanic acid to empty refrigerator cars was authorized in 1943.

Fumigation of Other Fruits and Vegetables.—In a preliminary test Lipp (unpublished) killed all beetles in baskets of apples, cabbage, eggplant, onions, peaches, peppers, and tomatoes by fumigating with 2 ounces of hydrocyanic acid per 1,000 cubic feet for 2 hours at not less than 75° F., but all beetles were not killed in baskets of lima beans and string beans by increasing the dosage to 4 ounces and the exposure to 3 hours.

In more extensive tests Johnson [A. C.], Donohoe, and Bulger (unpublished) found that with a 2-hour exposure the effectiveness of hydrocyanic acid in killing beetles in baskets of fruit and vegetables was modified by the extent to which the chamber was filled and by the penetration of gas into the packed baskets. For example, in fumigating cabbage with 2 ounces, the mortality in bushel baskets was 94 percent in a chamber filled to one-twelfth its capacity, 48 percent to one-third its capacity, and only 31 percent to full capacity. A 3-ounce dosage killed 16 percent of the beetles at the top of bushel baskets of corn, 2 percent of those at the center, and none at the bottom. A 6-ounce dosage killed all beetles in baskets of apples, beets, cabbage, cantaloup, eggplant, and peppers when the chamber was filled to three-fourths or more of its capacity, but not all of them in baskets of carrots, corn, peaches, and string beans. This high dosage did not injure apples, beets, cantaloup, eggplant, peaches, peppers, and string beans, but it definitely injured cabbage, carrots, and corn.

Immediately after fumigation with the 2-ounce dosage, no hydrocyanic acid was found in apples, cabbage, eggplant, onions, peaches, peppers, potatoes, and tomatoes, but 5 p.p.m. was found in spinach, 20 p.p.m. in beets, cucumbers, lima beans, and string beans, 30 p.p.m. in parsley, and 60 p.p.m. in carrots and turnips (Lipp unpublished).

Fumigation of these fruits and vegetables with hydrocyanic acid is not practical and it could be a hazard to human health.

Methyl Bromide

Methyl bromide (CH_3Br) is a colorless gas at room temperature. It is almost odorless. Its boiling point is 4.6° C., specific gravity 1.732 at 0°, and vapor pressure 760 mm. at 4.6°. Its vapors are 3.29 times as heavy as air. It is nonflammable. Its vapor mixed with air cannot be ignited by a flame. It is therefore useful as a fire extinguisher. It is only slightly more toxic to

man than gasoline, chloroform, and carbon tetrachloride with short exposures, but its toxicity increases with prolonged exposure and approaches that of ammonia, carbon monoxide, hydrocyanic acid, and hydrogen sulfide. The lack of a distinctive odor makes it an insidious compound that must be handled carefully. (Chisholm *1952;* Fisk and Shepard *1938;* Mackie *1938*)

While studying the toxicity of fumigants mixed with methyl bromide to overcome their flammability, Le Goupils (*1932*) found that this alkyl halide was more toxic to insects than the compounds with which it was mixed. Vayssiere (*1934*), de Francolini (*1935*), and Lepigre (*1936*) investigated further its insecticide properties. The results obtained by these French investigators stimulated investigations of methyl bromide in this country. Shepard et al. (*1937*) found that the toxicity of methyl bromide to the confused flour beetle (*Tribolium confusum* Jacquelin duVal) was less than that of hydrocyanic acid and between the toxicities of chloropicrin and ethylene oxide, but there was little difference in the toxicities of these fumigants to the granary weevil (*Sitophilus granarius* (L.)). Fisk and Shepard (*1938*) found its insecticide action was modified by temperature and relative humidity. Mackie and Carter (*1937*) and Mackie (*1938*) found methyl bromide was very effective in killing insects infesting grain, dried fruits, and fresh fruits and vegetables without injury to the products.

When methyl bromide was vaporized in a chamber without circulating the air, the heavy vapor was not distributed uniformly throughout the chamber but was more concentrated near the floor. The distribution of 2 pounds of the chemical per 1,000 cubic feet was such that Donohoe et al. (*1940*) killed only 9 percent of the beetles near the ceiling and 100 percent of those near the floor. This situation was overcome by operating a fan or a blower during vaporization of the chemical and for a few minutes thereafter. Fleming et al. (unpublished) found that methyl bromide was distributed uniformly without circulating the air by applying it as an aerosol containing 75 percent of methyl bromide and 25 percent of dichlorodifluoromethane.

The method finally adapted for fumigating refrigerator cars was to discharge the methyl bromide through a disk-type spray nozzle with a $^{1}/_{16}$- to $^{3}/_{32}$-inch orifice into the intake of a blower or into the rear of the blades of a fan mounted in a bunker and blowing through the grill into the load space of the car. When no ice was in a car, the blower was operated during introduction of the fumigants and for an additional 5 minutes.

The fan was operated for an additional 10 minutes after introducing the fumigant. When ice was in the bunkers, the postatomizing period was 10 minutes with the blower and 15 minutes with the fan. The same procedure with modifications was used for fumigating in chambers and in refrigerated and van-type trucks. (Donohoe *1943b;* Donohoe and Gaddis *1942;* Donohoe et al. *1940*)

Fumigation of Fruits and Vegetables.—Donohoe (*1943b,* unpublished; Donohoe et al. *1940*) conducted extensive tests with fruits and vegetables in chambers and in refrigerator cars, using an exposure of 2 hours. The vapor penetrated readily into packages of fruits and vegetables and apparently was not absorbed or adsorbed by any of the products in amounts sufficient to reduce its effectiveness in killing beetles in the packages. There was some absorption of the vapor by the products. In contrast to hydrocyanic acid, methyl bromide was as effective in chambers and refrigerator cars loaded with fruits and vegetables as when they were empty.

Based on the data then available, a dosage of 2 pounds of methyl bromide per 1,000 cubic feet of space was authorized in 1938 for the fumigation of fruits and vegetables in refrigerator cars at 70° F. or above. In 1940 a dosage of 1.6 pounds was authorized for fumigation at 80° or above, and in 1943 dosages ranging from 4 pounds at 40° to 0.8 pound at 95° were authorized. This schedule is given in the following tabulation:

Minimum temperature of load and space (° F.)	*Methyl bromide per 1,000 cubic feet (pounds)*
40	4.0
45	3.6
50	3.2
55	2.8
60	2.4
68	2.0
77	1.6
86	1.2
95	.8

During 1938–42, 9,948 cars of fruits and vegetables were fumigated with methyl bromide.

The dosages of methyl bromide approved for use in refrigerator cars were authorized for the fumigation of produce in chambers, refrigerated trucks, and van-type trucks with tight-fitting doors after Donohoe and Gaddis (*1942*) and Donohoe (*1943b*) had demonstrated their effectiveness under these conditions.

Chisholm and Koblitsky (*1945*) developed a method for rating the tightness of fumigation chambers. An open-arm manometer charged with deodorized kerosene was used to establish the pressure-time relationships. Air was blown into a chamber until a positive pressure slightly more than 50 mm. of kerosene was created in the chamber. The rate of leakage was determined by the time required for the pressure to drop from 50 to 5 mm. A chamber was considered to be sufficiently tight when this change in pressure occurred in not less than 12 seconds.

Usually apples, beets, cabbage, cantaloup, carrots, lima beans, onions, peaches, peppers, potatoes, squash, string beans, sweet corn, sweetpotatoes, turnips, and watermelon were fumigated with methyl bromide without noticeable injury, but cucumbers were injured severely. The effect of the fumigant on these products varied to some extent with the variety, their condition, and the dosage. A product in poor condition was more likely to be injured by the vapor than one in good condition. The higher dosages of the fumigant at temperatures below 70° F. were more likely to injure the products than the lower dosages above this temperature. (Donohoe *1943b;* Donohoe et al. *1940*; Johnson, Donohoe, and Bulger unpublished; Kenworthy *1945*)

Hydrocyanic Acid-Methyl Bromide

Johnson (*1939*) found that a 1:8 mixture of hydrocyanic acid and methyl bromide was much more toxic to the Japanese beetle than anticipated. Fleming and Wible (unpublished) continued the study of mixtures of these fumigants. With an exposure of 30 minutes at 80° F., 50 percent of the beetles were killed with 1 ounce of hydrocyanic acid or 40 ounces of methyl bromide in 1,000 cubic feet of air. The dosages of the mixtures expected to kill 50 percent were calculated from these data and the dosages required were determined experimentally. The results of these tests are summarized in table 2.

All mixtures of hydrocyanic acid and methyl bromide, ranging from 4:1 to 1:66, were more toxic than was anticipated from the toxicity of their components and showed a definite synergistic action. Substituting the 1:66 mixture for methyl bromide reduced the dosage by 52 percent, whereas the 1:21 mixture reduced it by 72 percent, the 1:8 mixture by 85 percent, and the 1:1 mixture by 95 percent. No experiments were conducted to determine how effective mixtures of hydrocyanic acid and methyl bromide were in penetrating into baskets of fruit and vegetables.

TABLE 2.—*Toxicity of mixtures of hydrocyanic acid (HCN) and methyl bromide (CH_3Br) to Japanese beetles exposed for 30 minutes at 80° F.*

Ratio of HCN: CH_3Br in mixture by weight	Amount of mixture per 1,000 cubic feet for 50-percent mortality	
	Expected	Determined
	Ounces	*Ounces*
1:0		1.0
4:1	8.8	.8
3:2	16.6	1.5
1:1	20.5	1.9
2:3	24.4	2.5
1:4	32.2	4.5
1:8	35.6	6.0
1:12	37.0	7.5
1:14	37.6	9.0
1:21	38.2	11.0
1:32	38.8	13.0
1:66	39.4	19.0
0:1		40.0

Ethylene Dibromide

Ethylene dibromide (CH_2BrCH_2Br) is a colorless liquid at room temperature. It has a sharp chloroformlike odor. The boiling point is 131.6° C., melting point 10°, specific gravity 2.1701 at 25°, and vapor weight about 6.5 times that of air. Since ethylene dibromide has neither a flash point nor a fire point, there is no danger of fire or explosion. It is highly toxic to humans. (Chisholm *1952*)

Only exploratory tests were conducted with ethylene dibromide as a fumigant against the beetle. Mason and Chisholm (*1945*, unpublished) heated ethylene dibromide to about 125° F., and as the vapor evolved it was mixed with air and dispersed throughout the chamber by means of a circulating fan. The beetles were killed within 96 hours by exposure for 2 hours to 0.5 pound of ethylene dibromide per 1,000 cubic feet at 50° to 70°. Apples, corn, cucumbers, potatoes, string beans, and tomatoes were not injured by exposure for 6 hours to this dosage at 70°.

When ethylene dibromide was atomized into a chamber at about 70° F., 0.5 pound per 1,000 cubic feet killed the beetles within 77 hours by exposure for 1 hour, within 65 hours for 2 hours, and within 46 hours for 4 or more hours. The 0.25-pound dosage killed the beetles within 125 hours by exposure for 1 hour,

within 71 hours for 2 hours, and within 63 hours for 4 or more hours. Newly dug potatoes were definitely injured by exposure for 4 hours to the 0.25-pound dosage. (Fleming et al. unpublished)

An aerosol containing 25 percent of ethylene dibromide and 75 percent of dichlorodifluoromethane was released in both bunkers of refrigerator cars. With an exposure of 1 hour at 70° to 82° F., 0.5 pound of ethylene dibromide per 1,000 cubic feet killed the beetles throughout the cars within 66 hours, whereas 0.25 pound killed them within 104 hours. The 0.5-pound dosage caused slight injury to newly dug potatoes, but the 0.25-pound dosage caused no injury. (Fleming et al. unpublished)

Ethylene Dibromide Mixed With Diluents

A few exploratory tests were made with ethylene dibromide mixed 1:1 by volume with several diluents and atomized into a chamber at 1 pound of mixture per 1,000 cubic feet at approximately 70° F. One pound of ethylene dibromide was also atomized into the chamber. The exposure of the beetles in the chamber was 1 hour. Ethylene dibromide killed them within 50 hours, whereas 48 hours were required with the ethylene dibromide-ethyl alcohol mixture, 64 hours with the ethylene dibromide-ethylene dichloride mixture, and 65 hours with the ethylene dibromide-carbon tetrachloride mixture. A 2:1:1 mixture of ethylene dibromide, acrylonitrile, and carbon tetrachloride was more toxic, killing the beetles within 34 hours. When 0.5 pound of this mixture was used, less than 50 percent of the beetles died within 120 hours. The mixtures containing acrylonitrile had an objectionable odor. (Fleming et al. unpublished)

Dusts

Preliminary Tests

In preliminary tests with a small duster developed by Chisholm and Fest (*1948*), which used air at a pressure of 50 p.s.i. as the propellent, Fleming et al. (unpublished) dispersed several dusts at 1 ounce per 2,500 cubic feet in chambers at about 70° F. and left the beetles in the chambers for 20 hours. The beetles were killed within 48 hours by the dispersion of 3 grams of chlordane, DDT, or TDE, but cube, pyrethrum, quinhydrone, ryania, and toxaphene were less toxic and slower in killing them. When the dosage was reduced to 0.75 gram, chlordane and toxaphene killed less than 50 percent and TDE 75–90 percent within 120 hours, but DDT killed all of them within 54 hours. DDT was the

only one of these dusts that appeared promising for killing beetles in an enclosed space.

DDT

DDT is 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane.

Toxicity to Beetles.—The residue of a spray containing 1 pound of DDT per 100 gallons of water protected fruit and foliage from attack by beetles throughout the summer. Beetles were killed by coming into contact with the sprayed plants. (Fleming and Chisholm *1944*)

In a preliminary test with a dust Burgess et al. (unpublished) deposited 0.5 to 4.0 μg. of DDT per square centimeter in petri dishes and exposed beetles to the deposits for 4 and 24 hours at 50°, 70 , and 90° F. Except at 50°, where exposure for 4 hours to a 3.5-μg. deposit killed the beetles within 4 days and exposure for 24 hours to a 2.5-μg. deposit killed them within 3 days, prolonging the exposure from 4 to 24 hours did not accelerate the insecticide action. A 2-μg. deposit killed the beetles within 3 days at 70° and 1.5 μg. within 2 days at 90°.

Distribution of DDT in Refrigerator Cars.—Burgess et al. (unpublished) studied the distribution of DDT dust in empty refrigerator cars and in cars loaded with sacks of potatoes. Glass plates were placed at eight to 12 predetermined positions throughout a car before dispersing the dust. The deposits of DDT on these plates were determined by a modification of the method of Umhoefer (*1943*).

In the early tests the dust was applied by an operator with a hand duster walking about the load space of an empty car. Although great care was exercised in distributing the dust, the deposits varied considerably throughout a car and from car to car. Using a hand duster was also laborious and time consuming.

Chisholm et al. (*1947*) developed a duster with carbon dioxide as the propellent. This provided a simple and rapid method for dispersing predetermined dosages of dust at high velocity throughout a car. The duster was placed near the door on the floor or on top of the load with the delivery tubes parallel to the long axis of the car and pointed upward at an angle of 45°. This duster, however, was impractical for rapidly treating large numbers of refrigerator cars. In 1948 Chisholm et al. (*1948*) modified this duster to adapt it better to the conditions in railroad yards.

The distribution of DDT in empty refrigerator cars and in

cars loaded with potatoes in which the dust was dispersed by a hand duster and by the gas-propelled duster is summarized in table 3. The standard deviation in the distribution of 1 ounce of 10-percent DDT dust (3 grams of DDT) in an empty refrigerator car with a capacity of 2,500 cubic feet by the gas-propelled duster was about one-half of that by the hand duster, although about the same average deposit was obtained by both dusters. About the same dispersion of DDT was obtained with 0.5 ounce of dust in cars loaded with potatoes as with 1 ounce of dust in empty cars.

Effectiveness in Refrigerator Cars and Trucks.—Potatoes were passed through a grading machine directly into burlap sacks. This operation eliminated the possibility of beetles being trapped inside the sacks. The cars and trucks became infested by beetles flying into them or by being carried into them clinging to the outside of the sacks. It was not a procedure for killing beetles hiding in baskets or boxes of farm products.

Hadley et al. (unpublished) dispersed 1 ounce of 10-percent DDT dust in 11 empty refrigerator cars and immediately liberated 2,750 beetles in the cars. The following day 2,530 of these beetles were recovered in the cars. Most of them were dead and all except three of those living died within the following 3 days. In addition, the dust was applied to 871 empty cars after cleaning and before loading them. No living beetles were found in 334 of these cars, which were examined 1 or 2 days after dusting. Burgess et al. (unpublished) applied the dust to 16 empty cars. Beetles exposed to the deposit for 4 hours died within

TABLE 3.—*Distribution of 10-percent DDT dust in empty refrigerator cars and in cars loaded with sacks of potatoes*

Applicator	DDT per 2,500 cubic feet	DDT deposit per square centimeter			
		Max.	Min.	Avg.	Standard deviation
	Ounces	*μg.*	*μg.*	*μg.*	*μg.*
	EMPTY CARS				
Hand duster	0.5	3.9	0.6	1.9	0.84
	1.0	5.7	.5	2.4	1.20
Gas-propelled duster	1.0	3.7	1.1	2.3	.69
	LOADED CARS				
Gas-propelled duster	.5	3.1	2.2	2.6	.41
	1.0	5.8	3.5	4.4	.78

7 days, but those introduced into the cars before dusting and left for 18 hours after dusting were all dead 3 days later.

One ounce of 10-percent DDT was dispersed over sacks of potatoes in refrigerator cars. Hadley et al. (unpublished) liberated beetles in four cars before dusting. When these cars reached their destination 7 to 8 days later, the 630 beetles found in the cars were dead. Burgess et al. (unpublished) dusted 39 cars with the bunker hatches open and closed, a factor that did not modify the effectiveness of the treatment. The cars were en route for 3 to 8 days. On arrival at their destination 5,205 dead and 47 living beetles were recovered in the cars. These living beetles died during the following 8 days.

Two ounces of the dust were dispersed in the cars, 1 ounce before loading and another ounce after the sacks of potatoes were in the cars. Hadley et al. (unpublished) dusted 22 cars. When these cars reached their destination 2 to 8 days later, most of the 3,115 beetles recovered were dead. Only one of the few living beetles was not dead 5 days after removal from the cars. Burgess et al. (unpublished) dusted 42 cars, which were en route for 2 to 18 days. At their destination 6,510 dead and 13 living beetles were recovered in the cars. All the living beetles died during the following 7 days.

Although one application of the dust after loading was effective in killing beetles, it was recognized that beetles hiding beneath the floor racks in loaded cars might occasionally escape contact with the deposit of DDT. When the dust was dispersed before and after loading the cars, there was a remote possibility that all beetles in the cars would not come in contact with the insecticide.

Burgess et al. (unpublished) dusted truck loads of sacked potatoes with the 10-percent DDT dust. Most of the trucks reached their destination within 2 days. The beetles in the 11 trucks in which 1 ounce of dust per 2,500 cubic feet was dispersed after loading were not all dead on arrival at their destination, but most of the few alive at that time died during the following 2 or 3 days. The beetles in the 10 trucks dusted before and after loading were all dead within 2 days after the trucks reached their destination.

None of the potatoes were injured by the DDT dust. Analyses of the potatoes in sacks taken from refrigerator cars that had been dusted before and after loading showed that the DDT on the potatoes ranged from less than 0.002 to 0.139 p.p.m., with an average of 0.04 p.p.m. (Burgess et al. unpublished)

Dusting refrigerator cars and trucks with 10-percent DDT dust containing pyrophyllite or nonfibrous talc as the diluent was authorized in 1947 for use at not lower than 50° F. The dust was dispersed by a carbon dioxide-propelled duster. One ounce of dust per 2,500 cubic feet was dispersed in empty cars and trucks. Two ounces, one before and one after loading, were dispersed in cars and trucks loaded with sacks of potatoes. Later the treatment was authorized for sacks of onions. Chisholm et al. (*1948*) reported that over 10,000 refrigerator cars and trucks were dusted during the summer of 1947.

Smoke

Burgess et al. (unpublished) prepared a smoke-producing mixture by blending 30 grams of a 1:1 DDT-pyrophyllite mixture with 30 grams of potassium chlorate, 30 grams of ammonium chloride, and 10 grams of lactose. When 1 ounce of this mixture was ignited, about one-eighth of the original DDT remained in the residue.

In a preliminary test the smoke from 8 ounces of the mixture without DDT had no effect on the beetles. When DDT was in the mixture, exposure for 30 minutes to the smoke from 8 ounces of the mixture per 2,500 cubic feet in a fumigation chamber killed 98 to 100 percent of the beetles within 8 days. The smoke from 10 ounces of the mixture killed all of them in 4 days. In a refrigerator car the smoke from 8 ounces of the mixture killed 97 percent of the beetles within 7 days. Most of the smoke dissipated in about 30 minutes, but a distinct odor remained even after aerating the refrigerator cars for 1½ hours. The use of a DDT smoke was not practical. It was very irritating to the operator, and within the range of dosages tested it could not be depended on to kill all the beetles.

Low-Volume Sprays

Preliminary tests were made with low-volume sprays for killing beetles in chambers and refrigerator cars. One fluid ounce of cyclohexanone, a 1:3 mixture of cyclohexanone and dimethylphthalate, or a 2:1 mixture of ethylene dichloride and carbon tetrachloride, each containing 3 grams of DDT, when atomized into 2,500 cubic feet of space was equally as effective in killing beetles as a dust containing 3 grams of DDT. The addition of chlordane, eugenol, Lethane 384 (2-(2-butoxyethoxy)ethyl thiocyanate), phenol, Thanite (mixture of isobomyl thiocyanoacetate (82 percent) and related compounds), or toxaphene to the DDT

spray did not modify the insecticide action, but the addition of ethylene dibromide retarded it. There seemed to be no advantage in applying DDT as a spray. (Fleming et al. unpublished)

Aerosols

In a preliminary test an aerosol containing 4 percent of DDT, 16 percent of a dispersing agent, and 80 percent of dichlorodifluoromethane was discharged into empty refrigerator cars through the top bunker screens or in the load space. The 5-pound bombs were discharged for 1 and 2 minutes, but the weight of aerosol introduced into the cars was not determined. The discharge of the aerosol for 1 minute killed all beetles in two cars within 3 days, but the mortality in the third car was only 96 percent 7 days later. The discharge for 2 minutes killed all beetles in the cars within 3 days. (Hadley et al. unpublished)

Comparative tests in a chamber showed that 3 grams of DDT per 2,500 cubic feet of space were equally as effective in killing beetles when applied as an aerosol or as a 10-percent dust. The distribution of DDT throughout empty refrigerator cars and cars loaded with potatoes was the same with an aerosol bomb as with a gas-propelled duster. Although an aerosol might be used to advantage in treating refrigerator cars, this method of dispersing DDT was not explored further. (Fleming et al. unpublished)

QUARANTINE ON NURSERY AND GREENHOUSE STOCK

Safeguarding the movement of nursery and greenhouse stock outside the regulated area was the most important phase of the Japanese beetle quarantine because one or more of the immature stages of the beetle are in the soil throughout the year. Nursery and greenhouse stock could be shipped without certification within the regulated area, but it had to be certified at any time of the year for shipment outside this area. (Howard *1918*; Smith and Hadley *1926*)

Since the limits of the regulated area followed the outer boundaries of political subdivisions, such as townships or counties, where beetles had been found the previous summer by scouting and trapping, some uninfested nurseries and greenhouses were included within the area. These establishments were classified in three groups with respect to their infestation:

Group 1.—These nurseries and greenhouses were not infested.

No beetles or grubs were found on or near their premises. The business of these establishments was conducted in the same manner as before they were placed under quarantine. Blanket certification was made for the movement of their products.

Group 2.—One beetle had been found in or near the premises of these establishments, but no grubs were found in the soil. These nurseries and greenhouses could ship plants with soil provided no grubs were found in the nursery blocks in periodic surveys and no grubs were found in the soil about the roots of plants at the time of digging. As an added precaution, the upper 2 or 3 inches of soil about the roots of the dug plants were removed, because during September and October and April and May, the principal shipping periods for evergreens and other field-grown stock, the grubs most likely would be near the surface of the soil.

Group 3.—Beetles and grubs had been found on the premises of these establishments. No shipment of plants with soil was permitted until treatments were developed and authorized to destroy the immature stages in the soil. No movement of nursery and greenhouse stock to establishments in groups 1 and 2 was permitted, except in full compliance with the requirements for shipment outside the regulated area. (Smith *1925a*; Smith and Hadley *1926*)

Preventing Infestation

It was more practical under some conditions to prevent infestation than to destroy the immature stages of the Japanese beetle in the soil.

Screening

One of the first procedures authorized for the production of certified potted plants was to grow them in uninfested soil in greenhouses where all ventilators and doors were screened and in coldframes covered with screening to exclude the adult beetle. Cloth screening was not satisfactory because it was easily torn and difficult to maintain. Copper or galvanized screening was the only type approved. The uninfested soil was obtained outside the regulated area until methods were developed for destroying the immature stages in potting soil. Sanitary procedures were strictly enforced to prevent the introduction of infested soil or plants into the screened greenhouses and cold frames. Many thousands of plants were produced satisfactorily under these conditions. (Smith *1925a, 1925b;* Smith and Hadley *1926*)

Mulch Paper

Several types of mulch paper were spread over the ground and fitted as closely as possible about the base of trees and shrubs in a nursery block to prevent the beetle from laying eggs in the soil. The reduction of the grub population in the fall was related to the extent that the ground was covered by the mulch paper. The normal population in the block was reduced 93 percent under trees with a single trunk where the paper could be fitted closely about the trunk, but it was reduced only 67 percent under shrubs with several shoots. The mulch paper was laborious to apply, difficult to maintain throughout the summer, and did not prevent the soil from becoming infested.

Soil Removal From Plant Roots

It was common practice to ship dormant herbaceous and deciduous plants without soil before nurseries and greenhouses were quarantined. The usual procedure for preparing these plants for shipment did not remove all the soil from the roots. The lumps of soil among the roots were often large enough to harbor grubs. Washing the roots with water was effective in removing the adhering soil, except when the roots were matted or contained cavities packed with soil. The washing procedure was approved in 1923. It was used extensively for many years.

In preparing herbaceous plants for washing, excess soil was removed, the roots were pruned, and large clumps were divided as much as possible without causing injury. The washing was done by shaking the roots in a tub of water until all of the soil was removed. Among the plants treated successfully by washing were species of the following genera: *Allium, Arenaria, Astilbe, Baptisia, Coreopsis, Dahlia, Delphinium, Digitalis, Festuca, Filipendula, Gaillardia, Gypsophila, Hemerocallis, Hibiscus, Hypericum, Iris, Lythrum, Paeonia, Penstemon, Phlox, Polygonum, Rheum, Sedum, Thalictrum,* and *Valeriana* (Fleming and Baker *1930*).

The deciduous shrubs washed successfully by directing a stream of water under slight pressure on the roots included species of *Berberis, Buddleia, Cornus, Deutzia, Euonymus, Forsythia, Hydrangea, Ligustrum, Lonicera, Philadelphus, Spiraea,* and *Weigela* (Fleming and Baker *1930*).

The deciduous trees washed successfully in the same manner as the deciduous shrubs included species of *Acer, Aesculus, Betula, Catalpa, Fagus, Magnolia, Quercus, Salix, Sorbus,* and *Ulmus* (Fleming and Baker *1930*).

The removal of soil by washing the roots seriously retarded or killed the broadleaf evergreens, including species of *Abelia, Buxus, Cotoneaster, Ilex, Kalmia, Pachysandra, and Rhododendron,* and the narrowleaf evergreens, including species of *Chamaecyparis, Juniperus, Picea, Taxus, Thuja,* and *Tsuga,* even when the washed roots were puddled in clay and repacked in uninfested soil (Fleming and Baker *1930;* Jaynes unpublished; Smith *1924*).

Nonchemical Treatments

High Frequency Electrostatic Field

There was a possibility that the immature stages of the beetle in the soil of potted plants could be killed by passing the plants through an electrostatic field. In preliminary tests there was no effect on eggs in moist soil in test tubes when the frequency was less than 2,000 kilocycles per second and the field strength was less than 4,000 volts. With this frequency and field strength the temperature of the soil in the test tubes was raised from 78° to 98° F. in 1 minute, 110° in 2 minutes, 140° in 3 minutes, 145° in 4 minutes, and 172° in 5 minutes. The mortality of the eggs ranged from 12 percent with the shortest exposure to 60 percent with the longest exposure. (Baker unpublished)

Third-instar grubs in test tubes without soil and in test tubes with moist and dry loam, sand, leaf mold, and peat were not affected by exposure for 38 minutes in an electrostatic field with a frequency of 12,000 kilocycles and a field strength of 3,000 volts. Grubs in loose moist soil in small clay pots exposed for 8 minutes in this electrostatic field were all killed by increasing the temperature of the soil from 67° to 133° F. This treatment did not kill the grubs in the pots when the soil was compacted and the temperature increased from 67° to 109°. The heating of soil in the electrostatic field was modified not only by its compactness but by its type, volume, and moisture content. (Swingle unpublished)

Small succulent plants, including species of *Ageratum, Calendula, Dianthus, Helianthus, Lycopersicon, Pelargonium,* and *Salvia,* growing in pots were killed by exposure for less than 1 minute in an electrostatic field with a frequency of 12,000 kilocycles and a field strength of 3,000 volts. Most of the plants wilted after an exposure of a few seconds, but some burst into flames. Inverting a glass beaker over the aerial part of a plant did not protect it from injury, but inverting a potted plant and

immersing the aerial part in water prevented injury even when the exposure was prolonged for 15 minutes. (Swingle unpublished)

The high frequency electrostatic field was of little value for destroying the immature stages in the soil of potted plants because it did not kill all the eggs and grubs in the soil and was very injurious to growing plants.

High Voltage Electrical Treatment

Several devices were proposed in 1932 by electricians for high voltage electrical treatments of potted plants to kill grubs in the soil. An exposure of 5 minutes in 6-inch pots of soil to a two-phase circuit with a potential of 3,800 volts passing through the soil killed no grubs when the soil was dry and only 20 percent of them when it was moist. In view of these results, the effect of the treatment on growing plants in pots was not determined.

Ultraviolet Light

Grubs removed from soil and exposed in quartz test tubes for 60 minutes to the radiation from a quartz mercury vapor arc were not affected by the treatment (Osburn unpublished).

Vacuum and Pressure

Grubs and pupae subjected to an atmospheric pressure of 5 p.s.i. for 17 hours or to 220 p.s.i. for 3 hours and then restored gradually to normal atmospheric pressure were not affected by the change in pressure. When the pressure was reduced to 0.5 pound p.s.i. and then increased to 220 p.s.i. or vice versa, within 15 minutes about 50 percent of the grubs were killed (Mallory unpublished). A change in the atmospheric pressure from 2.5 to 200 p.s.i. in 60 minutes killed less than 10 percent of the grubs (Osburn unpublished).

Potted plants of *Aster* and *Digitalis* species withstood a reduction of the atmospheric pressure to 5 p.s.i. or an increase in the pressure to 220 p.s.i. when the pressure was slowly restored to normal over a period of 20 to 30 minutes, but the plant tissue was ruptured when the pressure was restored to normal within 1 minute (Mallory unpublished).

Refrigeration

Preliminary experiments by Leach (unpublished) during the summer of 1920 showed that low temperatures killed grubs.

Later Fox (*1939*) concluded that 15° F. was the lowest temperature that grubs could withstand under natural conditions. Smith (*1924*) and Smith and Spencer (unpublished) explored the possibility of using refrigeration to kill grubs in the soil of balled and burlapped nursery stock.

Five extensively planted species of evergreens—*Chamaecyparis pisifera, Juniperus virginiana, Picea abies, Pseudotsuga taxifolia,* and *Thuja occidentalis*—were obtained balled and burlapped in the fall and spring from commercial nurseries. The trees were 2 to 6 years old. Third-instar grubs were introduced into the soil balls. The balled trees were placed in refrigerated chambers maintained at predetermined temperatures. The time for the temperature of the soil balls to be reduced from 50° F. to that of the chambers was determined. Usually the trees were left in the chambers for an additional 1½ hours and then transferred to a cool greenhouse for the soil to thaw gradually. The grubs were removed from the thawed soil balls and kept under observation for at least 2 weeks to determine their reaction. The trees were planted in a nursery plot. The reaction of the trees to refrigeration was determined 6 months later. These experiments are summarized in table 4. The species of trees were grouped together in this summary because the number of each species at each temperature was not adequate to establish their individual reactions.

TABLE 4.—*Effect of low temperatures on third-instar Japanese beetle grubs in soil and on balled and burlapped evergreens*

Temperature of chamber (° F.)	Average time to reduce temperature of soil from 50° F.	Average mortality of—	
		Grubs	Evergreens
	Hours	*Percent*	*Percent*
32	3.0	0	0
30	7.0	20	13
28	7.6	35	29
26	8.2	50	40
24	8.8	64	51
22	9.5	74	58
20	10.0	84	63
18	10.5	88	67
16	11.2	95	71
14	11.8	98	75
12	12.4	100	79
10	13.0	100	82

The soil of balled and burlapped nursery stock had to be reduced to 12° F. to kill all the third-instar grubs. This treatment was very detrimental to growing evergreens, killing about four-fifths of the trees. It was obvious that refrigeration could not be used as a certification treatment in commercial nurseries.

In the 1960's there was a problem of how to eliminate all stages of the beetle in samples of soil taken in various parts of the country for determining insecticide residues. The samples could not be certified for shipment to the laboratory until treated to destroy any infestation in the soil. Fleming and Maines (unpublished) found that fumigation with ethylene dibromide left a residue that interfered with bioassays. This residue was not eliminated by aeration. Heating the soil to 130° F. destroyed the immature stages but caused a loss of chlorinated hydrocarbon insecticides in the soil. The eggs, grubs, pupae, and adults in the soil were destroyed without any interference with chemical analyses or bioassays by placing the soil for 24 hours in a quick-freeze locker at between −10° and −20°. The quick-freeze treatment was approved in 1963 for the certification of soil samples taken within the area infested by the Japanese beetle. Later it was authorized for samples of soil taken in areas where the European chafer (*Amphimallon majalis* Razoumowsky), the imported fire ant (*Solenopsis saevissima richteri* Forel), and the white-fringed beetles (*Graphognathus* spp.) were found, except where the burrowing nematode (*Radopholus similis* (Cobb) Thorne), the golden nematode (*Heterodera rostochiensis* Wollenweber), the soybean cyst nematode (*H. glycines* Ichinohoe), and witchweed (*Striga lutea*) occurred.

Hot Water

Leach (*1921*) killed third-instar grubs in 8-inch soil balls by immersing the soil balls in water at 110° F. and holding them in the water for 45 minutes after the temperature of the soil reached that of the water. The treatment seriously retarded the growth of *Rhododendron obtusum* and killed *Chamaecyparis pisifera*. It was considered to be too hazardous to plants. Although the hot-water treatment applied by Leach (*1921*) to kill grubs was very injurious to two species of evergreens, other investigators had found that dormant *Chrysanthemum, Citrus, Gladiolus, Narcissus,* and *Paeonia* species withstood immersion in hot water. By 1926 thousands of herbaceous plants were being washed in commercial nurseries to obtain certification for shipment. Washing the small particles of soil from the roots was slow,

laborious, and costly. Fleming (*1928a, 1931*) and Fleming and Baker (*1928, 1929, 1932*) explored further the use of hot-water immersion to destroy all stages of the beetle in soil among the roots of nursery plants.

Effect on Different Stages of Beetle.—There was a definite relationship between the mortality of the insect, the water temperature, and the immersion period. In a preliminary test with third-instar grubs removed from soil the period of immersion for 100-percent mortality decreased progressively as the temperature of the water was raised from 100° to 122° F., as shown in the following tabulation:

Temperature (° F.)	*Minimum lethal period* (*minutes*)
100	600
104	340
107	170
110	70
112	31
114	16
116	10
118	4
120	3
122	1

The destruction of the grubs below 110° was very slow and often uncertain, but above that temperature the rate of insecticide action became greatly accelerated until at 122° death was almost instantaneous.

Which of these treatments was the least injurious to plants? In a preliminary test dormant roots of *Dahlia* and *Paeonia* species with small particles of soil were subjected to these treatments. The dahlias were killed or seriously retarded by prolonged immersion at 100° and 104° F. but were not affected at 108° and 120°. The peonies were not affected by treatments below 118° but were retarded at 118°. Treatment between 110° and 114° appeared to be the least hazardous to both species of plants.

The reactions of eggs, first–, second–, and third-instar grubs, prepupae, pupae, and adult beetles immersed without soil in water at 110°, 112°, and 114° F. were determined at weekly intervals during their development. The temperature of the water was held within 0.5° of 110°, 112°, and 114° during these treatments. The only seasonal variation in the reactions of the different stages occurred with third-instar grubs, which are in the soil from September until June. The minimum periods of im-

mersion to kill all stages during their development are presented in table 5.

A temperature of 112° F. was about optimum. Immersion for 70 minutes at this temperature was completely effective in killing all stages except eggs. The mortality of the eggs ranged from 96 to 100 percent in the different tests, with an average of 99.7 percent. The few grubs hatching after this treatment were greatly retarded in their development, and most of them were abnormal.

As the temperature of the soil in the field decreased in the fall, third-instar grubs became progressively more resistant to hot water. When removed from soil and immersed in water at 112° F., they were destroyed by immersion for 30 minutes in September and for 40 minutes in October. Although the grubs were quiescent from November through March, the immersion had to be increased to 70 minutes to kill all of them. As they became active in the spring their susceptibility to the treatment increased. Immersion for 50 minutes was effective in April and for 35 minutes in May and June.

Preheating Period.—All stages of the insect in soil were destroyed by immersing the soil in water at 112° F. and continuing the treatment for 70 minutes after the soil masses had been heated throughout to the temperature of the water. The preheating period contributed to the insecticide action so that the more resistant stages were killed many times in less than 70 minutes after the temperature of the soil reached that of the water. The preheating period gave further assurance of the effectiveness of the treatment.

When a mass of soil was immersed in water at 112° F., the hot water flowed quickly into the soil, displacing practically all the

TABLE 5.—*Periods of immersion without soil in hot water for 100-percent mortality of various stages of Japanese beetle*

Stage	Length of immersion for 100-percent mortality at—		
	110° F.	112° F.	114° F.
	Minutes	*Minutes*	*Minutes*
Egg	160	85	50
First-instar grub	60	40	30
Second-instar grub	60	40	30
Third-instar grub	130	70	40
Prepupa	90	45	30
Pupa	130	70	40
Adult	40	20	10

air and raising the soil temperature by 20° to 30°. After this initial increase in temperature, the heat penetrating from the surrounding water gradually raised the soil temperature to 112°. The time required to heat a mass of soil under these conditions depends on many factors, among which the most important are the volume, type, temperature, and absorptive capacity of the soil.

The volume of soil affected the rate of heating more than the other factors. The time required to heat 2 to 1,728 cubic inches (1 cubic foot) of moist clay loam from 70° to 112° F. was as follows:

Soil volume of— (cubic inches)	*Minutes*
2	15
4	18
8	25
16	35
32	45
64	60
128	75
512	90
1,024	140
1,728	200

A preheating period up to 60 minutes was practical to use in commercial nurseries; the treatment could then be completed within 130 minutes. This would limit the volume of a mass of clay loam to not more than 64 cubic inches. Other tests showed that with this preheating period a mass of a sandy loam could have a volume of 92 cubic inches and a mass of peat not more than 40 cubic inches.

The temperature of the soil before immersion also affected the preheating period. Soils with temperatures of 35° to 50° F. required from 10 to 30 minutes' longer treatment depending on the volume than did soils of 65° to 75°.

The extent to which a soil was saturated with water was another factor modifying the preheating period. Soils saturated with water before being placed in hot water heated slower than soils only partially saturated with water. The heat capacity of water is approximately five times that of dry particles of soil (Patten *1909*). Thus such soils as peat, which absorb a high percentage of water, heated slower than did sandy soils with a limited absorptive capacity.

There are many intrinsic factors involved in the penetration

of heat into a mass of soil immersed in hot water. It was impossible to predict accurately the time required to heat the soils about the roots of different nursery plants. It was necessary to determine the preheating period for each batch of plants by inserting thermometers in several plants in such a manner that the mercury bulbs were approximately at the center of each mass of soil and roots.

Reaction of Plants.—Most of the tests to determine the reaction of plants to immersion in water at 112° F. for 70 minutes after the soil mass had reached that temperature were conducted with dormant or semidormant plants in commercial nurseries. The treatment so far as possible was made an extra step in the usual procedure of a nursery in preparing plants for the market. Herbaceous plants were prepared for treatment by removing loose soil, dividing large clumps, and pruning the tops and roots. Deciduous plants were prepared for treatment by removing loose soil and pruning the roots. The soil about the roots of evergreens was reduced as much as possible without damaging the plants. There was no preparation for potted plants. The herbaceous plants were completely immersed in water. Only the roots of the other plants were immersed. Each variety was handled after treatment according to the usual practices of the nursery.

The following species withstood the hot-water treatment successfully:

Ajuga reptans
Allium schoenoprasum
Amsonia tabernaemontana
Aquilegia skinneri
Arenaria montana
Aster subcoeruleus
Astilbe sp.
Baptisia australis
Campsis grandiflora
Dahlia spp.
Dianthus caryophyllus
Dianthus deltoides
Dicentra formosa
Digitalis lanata
Digitalis purpurea
Elymus glaucus
Euonymus fortunei
Euphorbia corollata
Filipendula purpurea
Filipendula ulmaria
Forsythia suspensa
Franklinia alatamaha
Geum chiloense
Gypsophila repens
Helenium hoopesi
Heliopsis helianthoides
Hemerocallis dumortieri
Hemerocallis fulva
Hosta caerulea
Humulus lupulus
Hypericum moserianum
Iberis sempervirens
Iris cristata
Iris kaempferi
Iris pallida
Iris pseudacorus
Iris sibirica
Iris variegata
Kerria japonica
Kniphofia uvaria
Liatris pycnostachya
Limonium latifolium

Lychnis chalcedonica
Lychnis coronaria
Lythrum salicaria
Mentha spicata
Monarda didyma
Nierembergia rivularis
Paeonia lactiflora
Paeonia officinalis
Phlox amoena
Phlox glaberrima
Phlox paniculata
Physostegia virginiana
Polygonum cuspidatum
Potentilla nepalensis
Saponaria ocymoides
Scabiosa japonica
Spiraea bumalda
Spiraea prunifolia
Symphoricarpos orbiculatus
Syringa vulgaris
Thalictrum glaucum
Tradescantia virginiana
Tritonia undulata
Trollius europaeus
Tunica saxifraga
Vaccinium spp.
Valeriana officinalis
Veronica incana
Veronica maritima
Veronica spicata
Veronica spuria
Weigela florida

The following species were retarded or killed by the treatment:

Achillea filipendulina
Achillea ptarmica
Adiantum pedatum
Aquilegia chrysantha
Aquilegia flabellata
Aquilegia vulgaris
Arrhenatherum elatius
Aster novae-angliae
Berberis thunbergii
Calimeris incisa
Callicarpa dichotoma
Canna indica
Centaurea dealbata
Centaurea montana
Centranthus ruber
Chelone glabra
Chelone lyoni
Chrysanthemum coccineum
Chrysanthemum maximum
Cibotium schiedei
Clematis heracleaefolia
Convallaria majalis
Coreopsis lanceolata
Coreopsis rosea
Echinops ritro
Erigeron coulteri
Eryngium maritimum
Eryngium planum
Eupatorium urticaefolium
Festuca ovina
Gaillardia aristata
Hedera helix
Hydrangea arborescens
Hydrangea macrophylla
Iris ochroleuca
Limonium carolinianum
Lonicera japonica
Malva moschata
Penstemon barbatus
Penstemon laevigatus
Phalaris arundinacea
Physalis alkekengi
Picea pungens
Polemonium pulcherrimum
Polypodium vulgare
Rhododendron catawbiense
Rhododendron indicum
Rhododendron obtusum
Rudbeckia laciniata
Rudbeckia maxima
Rudbeckia subtomentosa
Sanguisorba obtusa
Sedum spectabile
Senecio pulcher
Sidalcea candida
Silene schafta
Silphium perfoliatum
Solidago altissima
Solidago shorti
Stachys grandiflora
Stokesia laevis
Thuja occidentalis
Thymus serpyllum
Veronica repens

About one-half of the species treated and handled according to the usual procedures of the nurseries were not injured. However, many of them retarded under commercial conditions had been treated successfully in preliminary tests. The factors causing this discrepancy were not established. The tests did show that many herbaceous and deciduous species would withstand immersion of their roots in hot water.

Immersion in water at 112° F. for 70 minutes after the mass of roots and soil had reached that temperature was authorized in 1927 as a basis for certifying the shipment of dormant herbaceous and deciduous plants with little soil among their roots (Smith *1928*). The treatment soon became generally used by commercial nurseries. During the dormant season of 1927–28 the treatment was used for about 164,000 plants (Fleming and Baker *1929*).

Steam

Heating soil by steam to destroy soil-inhabiting insects and other pests was a common practice at many nurseries and greenhouses. Fleming and Baker (*1932*) considered the thermal death point to be the lowest temperature at which death of the insect resulted after immersion in water for 1 minute. The thermal death point of the beetle during its embryonic development was 128° F. The grub after it emerged from the egg was destroyed at 122°. There was little change in susceptibility to heat during the first and second larval instars. After the second postembryonic molt the thermal limit was raised to 126°. It remained at this point until the third-instar grub approached maturity when it decreased to 122°. After the grub changed into a prepupa, the thermal death point was raised to 126°. During the pupal stadium the thermal limit was 128°, but when the pupa transformed into the adult, the insect was destroyed at 122°.

Fleming and Baker (*1930*) killed all stages of the beetle in friable soil to be used for potting plants by injecting steam at a pressure of 70 p.s.i. to raise the temperature of the soil to 130° F. Steam at lower pressures did not disperse as readily throughout the soil and tended to make it muddy. This treatment with steam was authorized in 1927. To assure adequate heating of

the soil in commercial nurseries, it was required that the temperature be maintained at 130° for 30 minutes.

Vapor Heat

Van Leeuwen (unpublished) removed third-instar grubs from soil and exposed them to air at 112° F. and a relative humidity of 93 percent. The vapor heat was much slower in killing grubs than immersion in hot water, as shown in table 6. Furthermore, 105 minutes were required for vapor heat at 112° to raise the temperature of 64 cubic inches of a moist clay loam from 70° to 112°, whereas this was accomplished in 60 minutes by immersion in water at 112°. These preliminary tests demonstrated that vapor heat was not a satisfactory substitute for immersion in hot water.

TABLE 6.—*Relative effectiveness of vapor heat and water at 112° F. in killing third-instar Japanese beetle grubs removed from soil*

Month	Exposure for 100-percent mortality with—	
	Vapor	Water
	Minutes	*Minutes*
April	100	50
May	60	35

Fumigation in Chamber

Preliminary Tests

Preliminary tests were made by Fleming (*1925a*), Mason (unpublished), and Osburn (unpublished) to determine the effectiveness of various chemicals as fumigants against third-instar grubs, which were removed from soil and exposed directly to the vapors. The results of these tests are summarized in table 7. The minimum effective dosage is shown when all the grubs were killed in replicated tests, and the maximum dosage tested is shown when it did not kill all the grubs.

TABLE 7.—*Preliminary tests with chemicals as fumigants against third-instar Japanese beetle grubs removed from soil*

Chemical	Dosage per 1,000 cubic feet	Length of exposure at 80° F.	Mortality of grubs	Source
	Pounds	*Hours*		
Acetaldehyde diethyl acetal	8.0	2	Incomplete	Osburn unpub.
Acetone	5.6	24	Complete	Fleming *1925a.*
	20.0	2	do	Osburn unpub.
Amylene (2-methyl-2-butene)	8.0	2	Incomplete	Do.
Aniline	1.5	24	Complete	Fleming *1925a.*
	30.0	2	Incomplete	Osburn unpub.
Benzene	7.5	2	Complete	Do.
Benzylamine	15.5	24	do	Fleming *1925a.*
Benzyl chloride (α-chlorotoluene)	.25	24	do	Do.
	7.5	2	do	Osburn unpub.
Bromobenzene	5.6	24	do	Fleming *1925a.*
Bromoform	2.5	2	do	Osburn unpub.
Butyl acetate	2.5	2	do	Do.
Butyl alcohol	30.0	2	Incomplete	Do.
Calcium cyanide	1.0	24	Complete	Fleming *1925a.*
Carbon disulfide	2.75	24	do	Do.
	7.5	2	do	Osburn unpub.
Carbon tetrachloride	10.0	2	do	Do.
Chlorobenzene	4.0	2	do	Do.

Chloroform	10.0	2	..... do	Do.
Chloropicrin	.5	2	..... do	Do.
o-Cresol	.5	2	..... do	Fleming *1925a.*
Cyclohexane	8.0	2	Incomplete	Osburn unpub.
m-Cymene	5.6	24	Complete	Fleming *1925a.*
p-Cymene	3.75	[1]24	Incomplete	Mason unpub.
D-D (dichloropropene-dichloropropane mixture)	2.5	[1]2	Complete	Do.
Dichloroacetone (1,3-dichloro-2-propanone)	.5	2	..... do	Osburn unpub.
o-Dichlorobenzene	30.0	2	..... do	Do
p-Dichlorobenzene	1.5	24	..... do	Fleming *1925a.*
Diethylamine	2.0	2	..... do	Osburn unpub.
p, *α*-Dimethylstyrene	4.5	[1]24	Incomplete	Mason unpub.
Epichlorohydrin (1-chloro-2,3-epoxypropane)	.5	2	Complete	Osburn unpub.
	2.5	[1]24	..... do	Mason unpub.
Ethyl alcohol	30.0	2	Incomplete	Osburn unpub.
Ethyl benzylaniline (*N*-benzyl-*N*-ethylaniline)	30.0	2	..... do	Do.
Ethyl formate	4.0	2	Complete	Do.
Ethyl valerate	8.0	2	..... do	Do.
Ethylene chlorohydrin (2-chloroethanol)	2.5	2	..... do	Do.
Ethylene dichloride	1.5	[2]24	..... do	Mason unpub.
	5.0	2	..... do	Osburn unpub.
Ethylene oxide	1.0	2	..... do	Do.
Formaldehyde	2.75	24	..... do	Fleming *1925a.*
Furfural	2.75	24	..... do	Do.
	5.0	2	..... do	Osburn unpub.
Hexachloro-1,3-butadiene	4.5	[3]24	Incomplete	Mason unpub.
Hexachloroethane	7.75	24	Complete	Fleming *1925a.*
Hexachloropropylene	1.0	[3]24	..... do	Mason unpub.

See footnotes at end of table.

TABLE 7.—*Preliminary tests with chemicals as fumigants against third-instar Japanese beetle grubs removed from soil*—Continued

Chemical	Dosage per 1,000 cubic feet	Length of exposure at 80° F.	Mortality of grubs	Source
	Pounds	*Hours*		
Isobutyl acetate	4.0	2	do	Osburn unpub.
Isopentyl acetate	2.5	2	do	Do.
Isopentyl alcohol	30.0	2	Incomplete	Do.
Isopropyl formate	8.0	2	do	Do.
p-Menthane	3.5	[1] 24	do	Mason unpub.
Mesityl oxide (4-methyl-3-pentene-one)	1.0	2	Complete	Osburn unpub.
p-Methyl acetophenone	4.5	[1] 24	Incomplete	Mason unpub.
Methyl alcohol	10.0	2	Complete	Osburn unpub.
Methyl ethyl ketone (2-butanone)	6.0	2	do	Do.
Methyl formate	4.0	2	do	Do.
Methylene chloride	8.0	2	Incomplete	Do.
Naphthalene	.5	24	Complete	Fleming *1925a.*
Nicotine	1.0	24	do	Do.
Nitrobenzene	1.5	24	do	Do.
o-Nitrotoluene	2.75	24	do	Do.
Paraldehyde (2,4,6-trimethyl-1,3,5-trioxane)	15.5	24	do	Do.
Phenol	.25	24	do	Do.
Propyl acetate	4.0	2	do	Osburn unpub.
Propyl chloride (1-chloropropane)	16.0	[2] 24	do	Mason unpub.
Propylene dichloride	8.0	2	do	Osburn unpub.

Pyridine	7.75	24	do	Fleming *1925a.*
	30.0	2	Incomplete	Osburn unpub.
1,1,2,2-Tetrachloroethane	2.0	2	Complete	Do.
	7.0	[2]24	Incomplete	Mason unpub.
Tetrachloroethylene	7.5	2	Complete	Osburn unpub.
Thymol	2.75	24	do	Fleming *1925a.*
Toluene	2.5	2	do	Osburn unpub.
o-Toluidine	1.0	24	do	Fleming *1925a.*
p-Toluidine	.5	24	do	Do.
1,1,1-Trichloroethane	2.5	2	do	Osburn unpub.
Trichloroethylene	8.0	2	Incomplete	Do.
Xylene	2.5	2	Complete	Do.
Xylidine	30.0	2	Incomplete	Do.

[1] 50° F.
[2] 70° F.

Hydrocyanic Acid

Fleming and Burgess (*1943*) determined the dosage, exposure, and temperature relationship of hydrocyanic acid to the mortality of third-instar Japanese beetle grubs removed from soil and exposed directly to the gas. These tests are summarized in table 8. All grubs were killed at 75° F. by exposures of 3 hours to 2 ounces, 2 hours to 4 ounces, and 1.5 hours to 6 ounces of hydrocyanic acid per 1,000 cubic feet. A 3-hour exposure to 4 ounces killed the grubs at 45°.

Hydrocyanic acid is very soluble in water so that much of the gas is absorbed by the outer layer of soil and the penetration throughout the soil mass is poor. Sasser and Sanford (*1918*) placed 5-inch pots of soil infested with third-instar grubs in a chamber, reduced the atmospheric pressure to 2 p.s.i. in 15 minutes, introduced hydrocyanic acid at 10 ounces per 1,000 cubic feet, re-

TABLE 8.—*Dosage, exposure, and temperature required to kill 100 percent of third-instar Japanese beetle grubs removed from soil and exposed directly to hydrocyanic acid (HCN) in chamber*

HCN per 1,000 cubic feet (ounces)	Minimum effective exposure	Minimum effective temperature
	Hours	*° F.*
2	2	80
	3	75
4	1	90
	1.5	85
	2	75
	3	45
6	1	80
	1.5	75
	2	60
	3	45
8	1	60
	2	55
	3	45
10	1	55
	2	50
	3	45

stored the atmospheric pressure to normal, and exposed the infested soil to the gas for 1½ hours at 80° F. All the grubs were killed in dry and moist soil, but none of them in wet soil. Increasing the exposure to 3 hours did not kill any grubs in wet soil.

Lipp (unpublished) exposed soil balls 8 inches in diameter to 8 ounces of hydrocyanic acid per 1,000 cubic feet for 2 hours at room temperature and normal atmospheric pressure. The treatment killed 88 percent of the third-instar grubs in dry loam but only 22 percent of them in moist loam.

Although the gas is highly toxic to the grubs, its poor penetration into masses of soil made it of little value in destroying the grubs in the soil.

Ethylene Oxide

Osburn (*1931*) killed all grubs in 6-inch pots of moist soil by an exposure for 2 hours to 7.5 pounds of ethylene oxide per 1,000 cubic feet at 80° F. or for 3 hours to 2 pounds at that temperature. A 3-hour exposure to 10 pounds was required to kill all the grubs in 14-inch pots of soil. *Hydrangea macrophylla* and *Rhododendron indicum* were severely injured by exposure to the 2 pounds for 2 hours. The foliage turned black, curled, and fell within a few days.

Mason (unpublished) did not kill all the grubs buried in 1 inch of moist soil by a 6-hour exposure to 2 pounds at 50° F.

Naphthalene

Naphthalene ($C_{10}H_8$) is a white crystalline compound occurring in coal tar. It is obtained by crystallization from the fraction boiling between 180° and 300° C. The crystals are shining plates with a characteristic odor and a bitter aromatic taste. Naphthalene is readily soluble in alcohol, benzene, and other organic solvents, but it is insoluble in cold water and very slightly soluble in hot water. It melts at 80.1° and boils at 217.9°. The vapor pressure is low, ranging from 0.02 mm. at 0° to 0.08 mm. at 20°, 0.11 mm. at 30°, and 0.32 mm. at 40°. The vapor is about 4.4 times as heavy as air. It was calculated that 1,000 cubic feet of air became saturated with 0.01 pound of naphthalene at 0°, 0.035 pound at 20°, 0.06 pound at 30°, and 0.13 pound at 40°. The specific gravity of air saturated with naphthalene at 25° and normal pressure as compared with air is 1.0004. The vapor burns with a luminous but smoky flame. (Chisholm *1952*; Fleming and Baker *1934*; Roark and Nelson *1929, 1930*)

Toxicity to Immature Stages.—Fleming and Baker (*1934*) studied the toxicity of naphthalene vapor to eggs, grubs, and pupae. When the creamy-white eggs and grubs were exposed to naphthalene vapor, they became reddish, ranging in intensity from light pink to mahogany brown depending on the period of exposure. Possibly the change in color is due to the reaction of the vapor with the fat bodies since naphthalene is a fat solvent. After the grubs were exposed to the vapor for several hours, they were unable to walk, but there was a convulsive movement of the head and body. As the exposure was prolonged the grubs gradually became completely paralyzed and finally died.

None of the eggs hatched after exposure in a saturated atmosphere for 72 hours at 70° F. An exposure of 48 hours killed all third-instar grubs, but 120 hours killed only 54 percent of the pupae.

The temperature modified the rate of insecticide action. The exposure to naphthalene vapor needed to kill all third-instar grubs was 120 hours at 50° F., 72 hours at 60°, 48 hours at 70°, and only 12 hours at 80°.

The rate of insecticide action was also modified by the relative humidity. The mortality of third-instar grubs exposed to the vapor for 18 hours at 70° F. was 24 percent with 26-percent relative humidity, 40 percent with 56-percent relative humidity, and 86 percent with 100-percent relative humidity. However, an exposure of 48 hours was required to kill all the grubs when the relative humidity was from 0 to 100 percent.

When third-instar grubs in masses of moist sandy loam, approximately 3 inches in diameter, were placed in a chamber where the relative humidity was 90 to 95 percent and the air was saturated with naphthalene vapor, all the grubs were killed by an exposure of 96 hours at 75° F., 72 hours at 80°, and 48 hours at 90°. When the grubs in 3-, 4-, and 6-inch clay pots of soil were placed in the chamber at 80°, an exposure of 144 hours killed 99 percent in the 3- and 4-inch pots but only 81 percent in the 6-inch pots. With its low vapor pressure the penetration of naphthalene vapor throughout the soil was slow and uncertain.

Hartzell (*1929*) tested the tolerance of 150 species and varieties of plants and found most of them were not injured by naphthalene vapor. By carefully controlling the concentration of the vapor Hartzell and Wilcoxon (*1930*) fumigated without injury several species of plants that previously had not tolerated the treatment.

In view of the tolerance of many species of plants to naphthalene vapor in greenhouses, Fleming and Baker (*1934*) did not expect that *Berberis thunbergii*, *Hydrangea macrophylla*, and *Rhododendron obtusum* would be seriously injured by exposure to a saturated atmosphere in a chamber where the relative humidity was 90 to 95 percent and the temperature 80° F. The leaves of these plants showed some injury by the vapor after a 24-hour exposure, and at the end of 48 hours the plants were damaged so seriously that they were of no commercial value. They were less tolerant of the vapor than the grubs in the soil. This discrepancy in the reaction of plants in greenhouses and in the chamber may be attributed to the fumigation period in greenhouses being only 6 hours and possibly to the greenhouses being less gastight than the chamber.

Carbon Disulfide

Toxicity to Immature Stages.—Fleming and Baker (*1935*) studied the toxicity of carbon disulfide vapor to the immature stages of the beetle. When they were removed from soil and exposed to the vapor for 2 hours at 80° F., 38 pounds of carbon disulfide per 1,000 cubic feet were required to prevent hatching of eggs, 24 pounds to kill pupae, and 15 pounds to kill third-instar grubs. These dosages are excessive because of the short exposure.

The dosage required was modified by the temperature and the period of exposure to the vapor. The dosages to kill third-instar grubs at 40° to 100° F. and exposures from 2 to 72 hours are given in table 9. Not more than 10 pounds of carbon disulfide per 1,000 cubic feet were required to kill the grubs in 2 hours at not less than 90°. That dosage was effective in 6 hours at 70°, in 12 hours at 60°, in 18 hours at 50°, and in 24 hours at 40°. Prolonging the exposure at each temperature decreased progressively the dosage required.

The air in the chambers at 80° F. was conditioned to predetermined relative humidities by circulating the air through saturated aqueous salt solutions, as recommended by Headlee (*1921*), for 24 hours before introducing third-instar grubs and carbon disulfide at 3 pounds per 1,000 cubic feet. The relative humidity, ranging from 1 to 100 percent, did not modify significantly the velocity of insecticide action.

The purity of the carbon disulfide was not a factor in the insecticide action against third-instar grubs. Analyses of the chemically pure and the commercial grades, according to the

TABLE 9.—*Exposure, minimum dosage per 1,000 cubic feet, and temperature required to kill 100 percent of third-instar Japanese beetle grubs removed from soil and exposed to carbon disulfide vapor*

Exposure (hours)	Dosage at indicated temperatures (° F.)						
	40	50	60	70	80	90	100
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
2				20	15	10	4
4				15	12	8	..
6			22	10	9	5	3
8			13		8	4	..
12		14	9	6	6	3	2
18	22	8	6	5	3	2	..
24	9	6	4	3	3	2	1
48	4				2		..
72	3	3	2	2	1		..

procedure of Collins et al. (*1927*), showed that the C.P. grade had a boiling range of 46°–47° C. and contained 0.001 percent of nonvolatile matter and no foreign sulfides, sulfates, sulfites, or water, whereas the commercial grade had a boiling range of 45°–48° and contained 0.016 percent of nonvolatile matter and traces of foreign sulfides, sulfates, sulfites, and water. When the grubs were exposed to the vapor for 24 hours at 70° F., the LD_{50}[3] of the C.P. grade was 1.51 pounds per 1,000 cubic feet and that of the commercial grade was 1.57 pounds, a nonsignificant difference.

Effectiveness in Soil.—Nurserymen considered a fumigation of more than 2 hours to be impractical because it would seriously disrupt the routine of preparing plants for shipment. That limitation made it necessary to use higher dosages of carbon disulfide and to vaporize the chemical rapidly by means of a device similar to the "gasifier" described by Weigel et al. (*1927*). With this device all the carbon disulfide was vaporized in a chamber within a few minutes.

Fleming and Baker (*1935*) killed third-instar grubs in 3-inch clay pots of moist, friable soil by exposing the pots for 2 hours at 90° F. in a chamber containing 8.5 pounds of vaporized carbon disulfide per 1,000 cubic feet. Ten pounds of the chemical at 85°, 15 pounds at 80°, and 20 pounds at 70° were required to kill the grubs. As 80° was the maximum temperature usually

[3] Lethal dose that kills 50 percent of grubs.

recommended for fumigating living plants, further tests were conducted at this temperature. The dosage necessary to destroy the grubs increased progressively with the increment in the mass of soil. Eighteen pounds were needed to be effective against the grubs in 4-inch pots. The dosage had to be increased to 20 pounds for 5-inch pots, 23 pounds for 6-inch pots, and 25 pounds for 8- and 12-inch pots. The vapor penetrated more readily through masses of soil wrapped with burlap than through soil in clay pots, but the dosage was modified by the volume of the soil in about the same manner as with the clay pots.

The moisture content of the soil was the most important factor limiting the effectiveness of the fumigation. The fumigation was successful when the soil was friable and partially saturated with water, but only a few grubs were killed when the pore spaces of the soil were filled with water.

Tolerance of Plants.—Carbon disulfide vapor in insecticide concentration was very injurious to nursery and greenhouse plants. The adverse reaction was the same with a 2-hour exposure at 80° F. to 15 or more pounds of vaporized carbon disulfide per 1,000 cubic feet as with a 24-hour exposure to 3 pounds of the chemical. In preliminary experiments Leach and Fleming (*1925*) found that *Calendula officinalis* and *Salvia splendens*, two species killed by exposure to the vapor in a fumigation chamber, were not injured when the aerial part of the plants was immersed in water during fumigation.

Fleming and Baker (*1935*) explored further this method of fumigating plants with carbon disulfide, using a tank 10 feet square and 9 feet deep in which water was maintained to a depth of 6 feet. The tank was gastight and was equipped with a vaporizer and a fan for circulating the air. The surface layer of water could be removed constantly, aerated, and returned to the tank. The pots and soil balls of the inverted plants were supported by a rack just above the surface of the water.

Tests were made to determine the method of operating this tank whereby the vapor would be uniformly distributed in the air and the smallest quantity of the chemical absorbed by the water. Carbon disulfide was vaporized in the tank at 25 pounds per 1,000 cubic feet of air; the temperature of the air was 90° F. and the water 60°. Two hours later samples of air and water were taken and analyzed according to the procedure used by Delachanal and Mermet (*1877*), Gastine et al. (*1884*), and Radcliffe (*1909*). The distribution of carbon disulfide throughout the tank when the air and water were quiet and when one or both were circulated is summarized in table 10.

TABLE 10.—*Distribution of carbon disulfide 2 hours after vaporizing in covered tank filled two-thirds with water*

Layers above and below surface of water (inches)	Carbon disulfide recovered when—			
	Air quiet		Air circulated	
	Water quiet	Water circulated	Water quiet	Water circulated
	Percent	*Percent*	*Percent*	*Percent*
AIR ABOVE WATER				
20–30	26.2	22.4	20.0	20.2
10–20	29.0	25.0	20.0	20.1
0–10	32.7	27.8	20.0	20.1
BENEATH SURFACE OF WATER				
0–10	5.5	2.9	10.2	6.1
10–20	1.3	2.6	8.3	5.6
20–30	.8	3.0	8.4	6.0
30–40	.8	2.9	5.0	5.0
40–50	.7	2.8	3.0	4.5
50–60	.6	2.6	2.8	4.5
LOST FROM SYSTEM				
	2.4	8.0	2.3	7.9

The maximum concentration of carbon disulfide in the air and the minimum in the water occurred when both the air and water were quiet, although there was stratification of the chemical in the air and in the water. Circulating the air made the distribution in the air uniform, but it reduced the concentration in the air and increased the absorption by the water. Circulating the water tended to make the distribution uniform in the water. The aeration system was inadequate to remove the chemical from the water. The best method of fumigating plants was with the air and the water quiet in the tank.

The soil of *Hydrangea macrophylla*, variety Mme. Chautard, in 6-inch pots and *Rhododendron indicum*, varieties Mme. Petrick and Mme. Vandercruyssen, in 5-inch pots was fumigated for 2 hours with the aerial parts of the plants immersed in water. The air in the tank was 80° F. and the water was 60°. The dosage for the 6-inch pots was 25 pounds per 1,000 cubic feet of air and that for the 5-inch pots 20 pounds, amounts sufficient to destroy the grubs in the soil. The plants were forced in the

greenhouse according to the usual commercial practice. The treatment had no effect on the *Hydrangea*. It did not affect the quality of the blooms on the *Rhododendron*, but the number of blooms on the variety Mme. Petrick was reduced by 10 percent and on the variety Mme. Vandercruyssen by about one-third.

The reactions of other species of plants to fumigation with carbon disulfide in this manner were not determined because the nurserymen considered the procedure too complicated for general use in nurseries.

Ethylene Dichloride

Ethylene dichloride, or 1,2-dichloroethane (CH_2ClCH_2Cl), is a colorless liquid at room temperature. It has an odor like that of chloroform. The boiling point is 83.7° C., the melting point −35.3°, the specific gravity 1.257 at 20°, and the vapor pressure 62.9 mm. at 20°. The vapor is about 3.5 times as heavy as air. Ethylene dichloride supports combustion with difficulty and burns with a smoky flame. Mixtures of 6 to 16 percent with air are flammable. It is not dangerously explosive. When used as a fumigant ethylene dichloride is usually mixed with carbon tetrachloride. (Chisholm *1952*)

In preliminary tests with a 3:1 mixture of ethylene dichloride and carbon tetrachloride by volume, Mason et al. (*1943*, unpublished) killed third-instar grubs removed from soil and exposed for 2 hours to 1 pound of the mixture per 1,000 cubic feet at 80° F. One and one-half pounds were required at 70°, 3 pounds at 60°, and 6 pounds at 40° to kill the grubs. Grubs in dry and moist soil in 4- and 5-inch pots of azaleas and wrapped soil balls were killed by 1½ pounds at 80°, 2 pounds at 70°, and 3 pounds at 60°. The grubs in the soil were removed for observation 24 hours after the 2-hour fumigation. The insecticide action was variable when the soil was wet. Many of the grubs appeared to be normal at completion of the fumigation. Most of them died during the following 2 or 3 weeks, but an occasional grub lingered for 5 weeks.

Plants, including several varieties of *Cissus* sp., *Dracaena* sp., *Pandanus* sp., and *Rhododendron* spp., were not injured by exposure for 2 hours to 2¼ pounds of the mixture per 1,000 cubic feet at 60° and 70° F., but they were injured in varying degrees when the dosage or the exposure was increased. Although the preliminary results with the ethylene dichloride-carbon tetrachloride mixture were promising, the possibilities of this fumigant were not explored further.

Methyl Bromide

Toxicity to Immature Stages.—Donohoe and Johnson (unpublished) killed all stages of the beetle among the roots of bare-root deciduous stock, in pots not larger than 8 inches in diameter, and in soil balls not larger than 8 inches in diameter by exposing the infested soil in a fumigation chamber for 2.5 hours to 2.5 pounds of methyl bromide per 1,000 cubic feet at 63° F. The treatment was effective in all types of friable soil but was not always completely effective when the soil was saturated with water. Eggs, grubs, and pupae removed from soil after fumigation did not seem to be affected by the vapor, but the eggs did not hatch and the grubs and pupae died during the following 3 weeks. The velocity of the insecticide action was enhanced by leaving the insects in the fumigated soil.

Later Donohoe and Johnson (unpublished) found that this fumigation was effective in pots and soil balls up to 14 inches in diameter and in open packages of plants with the minimum dimension not more than 14 inches.

The dosage and the duration of fumigation were modified according to the temperature to avoid subjecting plants to excessive treatment. Donohoe and Johnson (unpublished) determined the dosages needed to kill the insect at temperatures from 40° to 75° F. with an exposure of 2.5 hours. Fleming and Maines (unpublished) increased the exposure at temperatures below 70° and decreased Donohoe and Johnson's dosages to kill the insect at these temperatures. These schedules of fumigation are given in table 11.

Tolerance of Plants.—Donohoe and Johnson (*1939*) deter-

TABLE 11.—*Temperature, dosage, and exposure required to kill immature stages of Japanese beetle in masses of soil up to 14 inches in diameter by methyl bromide fumigation*

Minimum temperature (° F.)	Schedule 1		Schedule 2	
	Methyl bromide per 1,000 cubic feet	Exposure	Methyl bromide per 1,000 cubic feet	Exposure
	Pounds	*Hours*	*Pounds*	*Hours*
40	5.0	2.5	3.5	4.5
50	4.0	2.5	3.0	4.0
60	3.0	2.5	2.5	3.0
70	2.0	2.5	2.0	2.5
75	1.5	2.5	1.5	2.5

mined the tolerance of about 500 species and horticultural varieties of plants to exposure for 2.5 hours to 2.5 pounds of methyl bromide per 1,000 cubic feet at temperatures above 63° F. The fumigation did not injure 92 percent of the plants; 6.6 percent were injured sufficiently to make them unsalable, and 1.4 percent of them were severely injured or killed. The species marked with an asterisk (*) in the following tabulation were injured or killed.

*Abelia grandiflora
Acer palmatum
Adiantum cuneatum
Adiantum tenerum
*Aglaonema simplex
Ajuga reptans
Aloe humilis
Alyssum sp.
Anemone japonica
Aquilegia sp.
Arabis alpina
Araucaria excelsa
Arctostaphylos uva-ursi
Arenaria montana
Armeria maritima
Aronia arbutifolia
Artemisia dracunculus
Asparagus plumosus
*Aspidistra elatior
Asplenium nidus
Aster novibelgii
Astilbe rosea
Aucuba japonica
Begonia rex-cultorum
Berberis gagnepaini
Berberis julianae
Berberis thunbergi
Berberis triacanthophora
Berberis verruculosa
*Billbergia nutans
Bouvardia humboldti
Brunnera macrophylla
Buddleia sp.
Buxus microphylla
Buxus sempervirens
Caladium bicolor
*Calathea vandenheckei
Calceolaria crenatiflora
Calluna vulgaris
Calycanthus floridus
Campanula carpatica
Campanula garganica
Campanula isophylla
Campanula medium
Campsis grandiflora
*Capsicum frutescens
Caryopteris incana
Cedrus atlantica
Cerastium biebersteini
Cereus peruvianus
Chaenomeles lagenaria
Chamaecyparis obtusa
*Chamaecyparis pisifera
Chrysalidocarpus lutescens
Chrysanthemum arcticum
Chrysanthemum coccineum
Chrysanthemum frutescens
Chrysanthemum morifolium
*Cibotium schiedei
Cissus rhombifolia
Cissus striata
Citrus sinensis
Citrus taitensis
Clematis jackmani
Clivia miniata
Codiaeum variegatum
Convallaria majalis
Cordyline indivisa
*Cordyline terminalis
Cornus alba
Cornus florida
Cornus stolonifera
Cotoneaster congesta
Cotoneaster dielsiana
Cotoneaster divaricata
Cotoneaster francheti
Cotoneaster horizontalis
Cotoneaster lucida
Cotoneaster microphylla
Cotoneaster pannosa
Cotoneaster racemiflora
Cotoneaster rotundifolia
Cotoneaster salicifolia
Cotoneaster simonsi

**Crassula arborescens*
Cryptanthus zonatus
Cyclamen persicum
Cyperus alternifolius
Cyrtomium falcatum
Cytisus canariensis
Cytisus scoparius
Daphne sp.
Davallia fijiensis
Delphinium sp.
Dennstaedtia adiantoides
Deutzia gracilis
Deutzia lemoinei
Deutzia scabra
Dianthus barbatus
Dicentra spectabilis
Dieffenbachia picta
Dracaena fragrans
Dracaena godseffiana
Elaeagnus pungens
Enkianthus campanulatus
Epimedium musschianum
Erica melanthera
Erodium sp.
Euonymus alatus
Euonymus fortunei
Eupatorium coelestinum
Euphorbia fulgens
Euphorbia pulcherrima
Fagus sylvatica
Fatsia japonica
Ferocactus sp.
Ficus elastica
**Ficus pandurata*
Ficus pumila
Fittonia verschaffelti
Forsythia intermedia
Forsythia suspensa
Gaillardia aristata
Gardenia jasminoides
Genista pilosa
Ginkgo biloba
Hedera helix
Helenium tenuifolium
Helianthemum sp.
Hemerocallis thunbergi
Heuchera sanguinea
Hibiscus syriacus
Hosta caerulea
Howea belmoreana
**Howea forsteriana*
Hydrangea arborescens
Hydrangea macrophylla
Hydrangea paniculata
Hydrangea xanthoneura
Hypericum moserianum
Ilex aquifolium
Ilex crenata
Ilex glabra
Ilex opaca
Iris kaempferi
Jasminum nudiflorum
**Juniperus chinensis*
Juniperus communis
Juniperus excelsa
Juniperus horizontalis
Juniperus sabina
Juniperus virginiana
Kalanchoe blossfeldiana
Kalmia latifolia
Kerria japonica
Kniphofia uvaria
Kolkwitzia amabilis
Liatris pycnostachya
Ligustrum amurense
Ligustrum lucidum
Ligustrum ovalifolium
Lithospermum sp.
Lobelia cardinalis
Lonicera fragrantissima
Lonicera japonica
Lonicera nitida
Lonicera periclymenum
Lonicera pileata
Lonicera tatarica
Magnolia kobus
Magnolia soulangeana
Magnolia stellata
**Maranta leuconeura*
Miscanthus sinensis
Monarda didyma
Mondo jaburan
**Monstera deliciosa*
Myosotis scorpioides
Nandina domestica
Nepeta cataria
Nephrolepis exaltata
**Nephthytis afzelii*
Opuntia sp.
Oxydendrum arboreum
Pachysandra terminalis
Paeonia officinalis

**Pandanus veitchii*
Papaver orientale
Parthenocissus quinquefolia
Parthenocissus tricuspidata
Pelargonium hortorum
Peperomia argyroneura
Peperomia maculosa
Peperomia obtusifolia
Philadelphus grandiflorus
Philadelphus laxus
Philadelphus nivalis
**Philodendron cordatum*
Phlox subulata
Phoenix humilis
Physocarpus opulifolius
Physostegia virginiana
Pieris floribunda
Pieris japonica
**Pinus bungeana*
Platanus orientalis
Polygonum auberti
Polypodium aureum
Primula malacoides
Primula veris
Pteris cretica
Pteris ensiformis
Phyracantha angustifolia
Pyracantha coccinea
Quercus alba
Rhododendron calendulaceum
Rhododendron carolinianum
Rhododendron catawbiense
Rhododendron indicum
Rhododendron molle
Rhododendron mucronulatum
Rhododendron obtusum
Rhodotypos scandens
Rhus aromatica
Rosa spp.
Rudbeckia laciniata
Saintpaulia kewensis
Salix discolor
Schlumbergera truncata
Scindapsus aureus
**Sedum adolphi*
Sedum sieboldi
Sedum spectabile
Sedum stoloniferum
Selaginella braunii
Selaginella emmeliana
Sempervivum grandiflorum
Sempervivum tectorum
Senecio cruentus
Spiraea bumalda
Spiraea cantoniensis
Spiraea thunbergii
Spiraea vanhouttei
Stephanandra incisa
Stokesia laevis
Stranvaesia davidiana
**Strelitzia reginae*
Symphoricarpos albus
Symphoricarpos chenaulti
Symphoricarpos orbiculatus
Syringa vulgaris
Tamarix africana
Taxus baccata
Taxus cuspidata
Teucrium chamaedrys
Thuja occidentalis
Thuja orientalis
**Thuja plicata*
Thymus serpyllum
Trollius europaeus
**Tsuga canadensis*
Tunica saxifraga
Veronica spicata
Viburnum bitchiuense
Viburnum dentatum
Viburnum lantana
Viburnum molle
Viburnum opulus
Viburnum rhytidophyllum
Viburnum trilobum
Vinca minor
Viola cornuta
Viola odorata
Vitex agnus-castus
Weigela florida
Wistaria floribunda
Wistaria sinensis

Livingstone and Swank (*1941*), using 0.5 to 1 pound of methyl bromide per 1,000 cubic feet for 3 to 5 hours at 84° to 91° F., fumigated the following species of greenhouse plants for the

control of scale and other insects. The plants marked with an asterisk (*) were injured by the treatment.

Aspidistra elatior
Camellia japonica
Camellia sasanqua
Chrysalidocarpus lutescens
**Chrysanthemum* sp.
Codiaeum variegatum
**Cordyline terminalis*
Dracaena fragrans
Ficus elastica
Ficus retusa
Gardenia sp.
**Hibiscus* sp.
Ligustrum sp.
Livistona chinensis
Nephrolepis exaltata
Pandanus veitchii
Phoenix humilis
Pittosporum sp.
Rhododendron sp.
Schlumbergera truncata
**Scindapsus aureus*

English (*1943*), using 3 pounds of methyl bromide per 1,000 cubic feet for 3 hours at 60° F., 2 pounds for 3.5 hours at 70°, 2 pounds for 2.5 hours at 80°, and 2 pounds for 1.5 hours at 90°, fumigated many varieties of azaleas and camellias. Only two varieties of azaleas were injured by the treatment.

Latta and Johnson (*1944*) determined the tolerance of coniferous evergreens—*Chamaecyparis pisifera, Juniperus communis, Picea pungens, Taxus cuspidata, Thuja occidentalis,* and *T. orientalis*—to fumigation with methyl bromide, using an exposure of 2.5 hours and adjusting the dosage according to the temperature as given in table 11. The plants were not injured while dormant during the winter, but severe injury occurred in the spring and the fall, the normal shipping periods in the nurseries.

Use in Fumigation Chamber.—Fumigation with 2.5 pounds of methyl bromide per 1,000 cubic feet and an exposure of 2.5 hours at temperatures not lower than 63° F. was authorized in 1939 for the treatment of bare-root nursery stock and plants in pots and with soil balls not larger than 8 inches in diameter. In 1941 the dosage was modified according to the temperature, and the maximum mass of soil that could be fumigated was increased to 12 inches in diameter. Later adjustments were made in both the dosage and the exposure at temperatures below 70°, and the maximum for the mass of soil was increased to 14 inches. Provision was made for fumigating unsealed packages not more than 14 inches in the minimum dimension. The plants could be certified after removal from the fumigation chamber.

Fumigation with methyl bromide was used extensively by nurseries and greenhouses. Middleton and Cronin (*1952*) reported that during 1939–50 a total of 15,192,561 plants had been fumigated and certified for shipment.

Insecticide Dips

General Considerations

Immersion of the soil of balled and potted plants in an insecticide dip might appear to be an easy, dependable, and economical method for destroying the immature stages of the Japanese beetle among the roots of plants. It is not difficult to kill the immature stages by removing them from soil and immersing them in dilute solutions or emulsions of many chemicals. This entails only the adjustment of the dosage, temperature, and exposure. It is more difficult to destroy the insect embedded in soil among the roots without damage to the plants because of the porosity of a soil, its absorptive and adsorptive capacity, and the susceptibility of plants to the chemicals.

Leach and Thomson (*1921*), Fleming (*1926a*). Fleming and Baker (*1935*), and others have observed that when a mass of soil is immersed in a solution or a dilute emulsion the liquid flows rapidly into the soil, displacing the air there. The quantity of liquid penetrating a soil is dependent on the volume of the interstitial spaces within the soil and on the extent to which these spaces are already filled by water and roots. The liquid taken up may be 35 percent of the weight of a sandy soil, 65 percent of the weight of a clay soil, and equivalent to the weight of a peat. The addition of organic matter and soil conditioners to a mineral soil modifies its normal capacity for taking in liquids. Under optimum conditions for growing plants about one-half the pore space in a soil is filled with water. The development of roots in the interstitial spaces modifies further the penetration of a liquid. The soil of a potbound plant may take in very little of the surrounding liquid.

Fleming and Baker (*1935*) immersed tarred brass cylinders of a sandy loam and a peat in an emulsion containing 630 mg. of carbon disulfide per liter and determined the quantity of the chemical in different parts of the soil and peat at intervals up to 25 hours after immersion. The average quantity of chemical found at different depths is summarized in table 12.

Most of the carbon disulfide was carried into the loam and peat as the emulsion flowed into the interstices to replace air. Additional carbon disulfide diffused into the masses during the next 5 hours. The concentration of the chemical in the interstices declined progressively during the following 20 hours because of absorption and adsorption by the particles of loam and peat.

Five hours after immersion the concentration of carbon disulfide in the interstices of the upper inch of moist loam was 59

TABLE 12.—*Penetration of emulsified carbon disulfide into interstitial spaces of sandy loam and peat immersed in water containing 630 mg. of chemical per liter*

Immersion (hours)	Carbon disulfide per liter in—			
	Sandy loam		Peat	
	Moist	Wet	Moist	Wet
	Mg.	*Mg.*	*Mg.*	*Mg.*
	0–1 INCH BELOW SURFACE			
1	810	250	1,190	400
5	1,000	700	1,325	500
10	900	410	1,275	450
15	800	325	1,230	400
20	700	300	1,150	375
25	650	300	1,150	375
	1–3 INCHES BELOW SURFACE			
1	560	100	400	100
5	900	400	575	100
10	775	225	425	100
15	625	110	340	100
20	590	100	280	100
25	590	100	240	100
	3–5 INCHES BELOW SURFACE			
1	410	100	80	80
5	760	200	80	80
10	600	150	80	80
15	480	100	80	80
20	440	100	80	80
25	440	100	80	80

percent higher than in the surrounding emulsion. It was 43 percent higher at a depth of 1 to 3 inches and 21 percent higher at a depth of 3 to 5 inches. Twenty hours later the concentration in the upper inch was about the same as in the emulsion, but it was 6 percent less at the 1- to 3-inch depth and 30 percent less at the 3- to 5-inch.

Smaller quantities of carbon disulfide penetrated into wet loam. Five hours after immersion the concentration was 11 percent higher in the interstices of the upper inch than in the emulsion,

36 percent less at a depth of 1 to 3 inches, and 68 percent less at a depth of 3 to 5 inches. Twenty-five hours after immersion there was 52 percent less carbon disulfide in the upper inch and 85 percent less in the 1- to 3- and 3- to 5-inch layers than in the emulsion.

Most of the carbon disulfide did not penetrate more than 3 inches into moist peat. Five hours after immersion there was 110 percent more of the chemical in the interstices of the upper inch than in the surrounding emulsion, but 9 percent less in the 1- to 3-inch layer and 88 percent less in the 3- to 5-inch layer. Twenty hours later the interstices in the upper inch contained 79 percent more of the chemical and the 1- to 3-inch layer 62 percent less than in the emulsion.

Little carbon disulfide penetrated into wet peat. Five hours after immersion the concentration in the interstices of the upper inch of peat was 21 percent less than in the surrounding emulsion and in the 1- to 3-inch layer 84 percent less.

These tests demonstrated that the friableness of soil and peat at the time of immersion in the emulsion was an important factor in the penetration of carbon disulfide.

Preliminary Tests

Beginning in 1920 and continuing for several years, tests were made to find solutions and emulsions of chemicals that would kill the immature stages of the beetle in the soil of potted plants and balled nursery stock without seriously injuring the plants. In general, these tests were to determine (1) the quantity of a chemical in solution or emulsion needed to kill third-instar grubs removed from soil and immersed at between 60° and 80° F., (2) the effectiveness of the dip in killing grubs embedded in soil, and (3) the reaction of various plants to the immersion of their roots in the dip. Many of these tests were not completed because of the death of F. E. Baker in 1937.

After the grubs without soil had been immersed in a dip for a predetermined period, they were placed on moist untreated soil containing germinating seed and their reaction was observed periodically during the following 2 or 3 weeks. Grubs embedded in soil were usually transferred to untreated soil about 1 week after immersion. Only a few tests were made with plants. The results of these preliminary tests with solutions and emulsions as dips to kill grubs are summarized in table 13.

TABLE 13.—*Preliminary tests with solutions and emulsions of chemicals as dips to kill Japanese beetle grubs*

Chemical	Amount per liter of water	Immersion period	Mortality of grubs when—		Effect on plants	Source
			Removed from soil	In soil		
	Mg.		*Percent*	*Percent*		
Acetaldehyde (diethyl acetal)	250	24 hours	23			Baker unpub.
Acetone	250	do	18			Do.
Acetonitrile	250	do	13			Do.
Acetophenone	250	do	19			Do.
Aldrin	24	Soil saturated		100	None	Mason unpub.
Allyl bromide	250	24 hours	100			Baker unpub.
Allyl isothiocyanate	Saturated	do	100	67		Leach and Thomson *1921*.
Do	do	30 minutes	100			Do.
Do	3,000	10 seconds		100		Mason unpub.
Do	1,500	do		88		Do.
Do	250	24 hours	100			Baker unpub.
2-Amino-1,3-dimethylamine benzene	250	do	29			Do.
Aniline	1,590	do	98			Do.
Do	1,000	do	65			Fleming *1925a*.
p-Anisaldehyde	250	do	78			Baker unpub.
Ascaridole (1,4-epidioxy-*p*-menth-2-ene)	165	6 hours	100			Leach and Johnson *1925*.
Benzaldehyde	Saturated	1 hour	66	0	Killed	Do.
Do	300	24 hours	100			Baker unpub.
Benzene	650	do	100			Do.
Benzene + *p*-dichlorobenzene 7:5	12,000	5 seconds		100	None	Burgess et al. unpub.
Do	6,000	do		60	do	Do.
Benzonitrile	250	24 hours	22			Baker unpub.
Benzyl acetate	250	do	100			Do.
Benzyl alcohol	2,500	do	86			Do.
Benzylamine	600	do	100			Do.
Benzyl benzoate + ethylene dichloride 1:1	3,000	5 seconds		96		Mason et al. unpub.
Benzyl bromide (α-bromotoluene)	40	24 hours	100			Baker unpub.

Benzyl chloride (*α*-chlorotoluene)	750	10 seconds		100		Mason et al. unpub.
Do	370	do		92		Do.
Do	40	24 hours	100			Baker unpub.
N-Benzyl-*N*-ethylaniline	250	do	97			Do.
N-Benzyl-*N*-ethyl-*o*-toluidine	250	do	86			Do.
Benzylidene chloride (*α,α*-dichlorotoluene)	300	do	100			Do
N-Benzylidenemethylamine	250	do	99			Do.
Bromobenzene	300	do	100			Do.
Bromotrichloromethane	190	Soil saturated		70		Fleming and Maines unpub.
Butyl alcohol	250	24 hours	20			Baker unpub.
Butylamine	250	Soil saturated	23			Do.
Butyl bromide (1-bromobutane)	250	do	59			Do.
Butyl chloride (1-chlorobutane)	250	do	18			Do.
Butyl chlorocarbonate (butyl chloroformate)	250	do	81			Do.
Butyl iodide (1-iodobutane)	250	do	80			Do.
Butyl nitrite	250	do	100			Do.
Butyl thiocyanate	250	do	100			Do.
Camphor	Saturated	2 hours	0			Leach and Thomson *1921*.
Carbon disulfide	do	24 hours	100	0		Do.
Do	do	30 minutes	100			Do.
Do	1,000	18 hours		100	Killed	Fleming *1926a*.
Do	625	15 hours	100	100	Retarded	Leach and Johnson *1925*.
Do	250	24 hours	100			Baker unpub.
Carbon tetrachloride	250	do	71			Do.
Chlordane	1,200	10 seconds	67			Fleming and Maines unpub.
Do	48	Soil saturated		100	None	Mason unpub.
Do	24	do		60		Fleming and Maines unpub.
Chlorobenzene	6,000	10 seconds		92		Mason unpub.
Do	400	24 hours	100			Fleming *1925a*.
1,1,1-Chloroethane	250	do	47			Baker unpub.
1,1,2,2-Chloroethane	250	do	23			Do.
bis-(2-chloroethyl) carbonate	250	do	100			Do.
bis-(2-chloroethyl) ether	3,000	5 seconds		92		Mason et al. unpub.
Do	1,250	Soil saturated		100		Mason unpub.
2-Chloroethyl *p*-toluene sulfonate	250	24 hours	100			Baker unpub.
2-Chlorofluorene	2,500	10 seconds		70		Mason unpub.

TABLE 13.—*Preliminary tests with solutions and emulsions of chemicals as dips to kill Japanese beetle grubs* —Continued

Chemical	Amount per liter of water	Immersion period	Mortality of grubs when—		Effect on plants	Source
			Removed from soil	In soil		
	Mg.		*Percent*	*Percent*		
Chloroform	Saturated	15 minutes	100			Leach and Thomson *1921.*
Do	250	24 hours	15			Baker unpub.
1-Chloronaphthalene	250	do	94			Do.
2-Chloropropane	250	do	98			Do.
Copper sulfate	50,000	do	0			Leach and Thomson *1921.*
o-Cresol	5,000	Soil saturated		97		Mason unpub.
Do	2,000	24 hours		Incomplete		Fleming *1926a.*
Do	600	do	100			Fleming *1925a.*
Crotonaldehyde	250	do	100			Baker unpub.
Cyclohexane	250	do	98			Do.
m-Cymene	1,000	do	90			Fleming *1925a.*
p-Cymene	650	do	99			Baker unpub.
DDT	8,000	20 minutes	100	91		Fleming et al. unpub.
Do	4,000	do	100	87		Do.
Do	2,000	do	97	76		Do.
Do	1,000	do	78	59		Do.
Dibenzylamine	250	24 hours	100			Baker unpub.
Dibutyl oxalate	250	do	52			Do.
m-Dichlorobenzene	3,000	5 seconds		86		Mason et al. unpub.
Do	300	24 hours	100			Baker unpub.
o-Dichlorobenzene + ethylene dichloride 1:1	3,000	5 seconds		82		Mason et al. unpub.
1:2	3,000	do		81		Do.
1:5	3,000	do		70		Do.
p-Dichlorobenzene	400	24 hours	100			Baker unpub.
Do	330	do	100			Fleming *1925a.*
Dichloroethyl formal (bis-(2-chloroethoxy)-methane)	1,250	Soil saturated		100		Mason unpub.

2,2-Dichloropropane	250	24 hours	100			Baker unpub.
Dieldrin	24	Soil saturated		100	None	Mason unpub.
Diethylamine	250	24 hours	27			Baker unpub.
N,N-Diethyl aniline	250	do	98			Do.
Diisopentyl phthalate	250	do	31			Do.
Dimethyl aniline	250	do	19			Do.
Epichlorohydrine	250	do	99			Do.
Do	120	10 seconds		78		Mason unpub.
Ethyl alcohol	250	24 hours	24			Baker unpub.
N-Ethylaniline	250	do	37			Do.
Ethylbenzene	400	do	100			Do.
Ethyl benzoate	350	do	100			Do.
Ethyl bromide	250	do	33			Do.
Ethyl isothiocyanate	250	do	100			Do.
N-Ethyl-l-naphthylamine	250	do	100			Do.
Ethyl sulfide	250	do	100			Do.
Ethyl thiocyanate	250	do	100			Do.
Ethyl valerate	250	do	93			Do.
Ethylene dibromide	250	do	99			Do.
Do	48	Soil saturated		100		Mason unpub.
Ethylene dibromide + chlordane 2:1	48	do		100		Fleming and Maines unpub.
Ethylene dibromide + heptachlor 2:1	48	do		100		Do.
Ethylene dichloride	370	10 seconds		100		Mason unpub.
Do	250	24 hours	19			Baker unpub.
Ethylidene chloride	250	do	21			Do.
Ethylidene chlorohydrin	250	do	15			Do.
Formaldehyde	50,000	1 hour	100	0	Killed	Leach and Johnson *1925*.
Furfural	30,000	do	0	0	do	Do.
Do	250	24 hours	34			Baker unpub.
Geraniol	250	do	98			Do.
Heptachlor	24	Soil saturated		88	None	Fleming and Maines unpub.
Hexachloroethane	160	24 hours	100			Fleming *1925a*.
Iodobenzene	400	do	100			Baker unpub.
Isobutylacetate	250	do	32			Do.
Isobutyl butyrate	250	do	90			Do.
Isopentyl acetate	250	do	34			Do.
Isopentyl alcohol	250	do	19			Do.

TABLE 13.—*Preliminary tests with solutions and emulsions of chemicals as dips to kill Japanese beetle grubs* —Continued

Chemical	Amount per liter of water	Immersion period	Mortality of grubs when		Effect on plants	Source
			Removed from soil	In soil		
	Mg.		*Percent*	*Percent*		
Isopentylamine	250	do	99	---	----	Do.
N-Isopentylaniline	250	do	96		------	Do.
Isopentyl butyrate	250	do	74		----	Do.
Isopentyl chlorocarbonate (isopentyl chloroformate)	250	do	80		...	Do.
Isopentyl nitrate	250	do	29		...	Do.
Isopentyl nitrite	250	do	90		...	Do.
Isopentyl propionate	250	do	98		...	Do.
Kerosene	250	do	100		...	Do.
Mercuric chloride	1,000	1 hour	0	0	Injured	Leach and Johnson *1925.*
Mesitylene	650	24 hours	100		...	Baker unpub.
Mesityl oxide (4-methyl-3-pentene-2-one)	250	do	31		...	Do.
Methyl alcohol	250	do	21		...	Do.
N-Methylaniline	250	do	18		...	Do.
o-Methylcarbonate	250	do	22		...	Do.
Methylcyclohexane	250	do	98		...	Do.
N-Methyldiphenylamine	250	do	95		------	Do.
Methyl ethyl ketone	250	do	17		----------	Do.
Methyl formate	250	do	34		...	Do.
N-Methyl-1-naphthylamine	250	do	100		------	Do.
Methyl salicylate	250	do	56		------	Do.
Methyl sulfide	250	do	40		------	Do.
Methyl thiocyanate	250	do	100		------	Do.
Methylene chlorobromide (bromochloromethane)	48	Soil saturated	...	53	------	Mason unpub.
Naphthalene	150	24 hours	100		----------	Fleming *1925a.*
1-Naphthol	Saturated	1 hour	100	75	Killed	Leach and Johnson *1925.*
2-Naphthol benzoate	do	do	0	0	None	Do.

Nicotine	1,600	3 hours	0			Leach and Thomson *1921.*
Do	250	24 hours	99			Baker unpub.
Nitrobenzene	1,200	do	100			Do.
Do	800	do	100			Fleming *1925a.*
Nitromethane	250	do	12			Baker unpub.
m-Nitrotoluene	600	do	100			Do.
o-Nitrotoluene	600	do	100			Baker unpub.; Fleming *1925a.*
Paraldehyde (2,4,6-trimethyl-1,3,5-trioxane)	80,000	1 hour	0	0	Killed	Leach and Johnson *1925.*
1-Pentene	250	24 hours	83			Baker unpub.
Pentylamine	250	do	21			Do.
Petroleum ether	Saturated	1 hour	0	0	None	Leach and Johnson *1925.*
Phenol	2,000	24 hours		Incomplete		Fleming *1926a.*
Do	600	do	100			Fleming *1925a.*
Phenylacetonitrile	800	do	100			Baker unpub.
2-Picoline	250	do	21			Do.
Piperidine	250	do	22			Do.
Potassium fluoride	100,000	2 hours	0			Leach and Thomson *1921.*
Propionitrile	250	24 hours	28			Baker unpub.
Propyl acetate	250	do	19			Do.
Propyl alcohol	250	do	18			Do.
Propyl bromide (1-bromopropane)	250	do	53			Do.
Propyl *p*-toluenesulfonate	250	do	90			Do.
Propylene dichloride	3,000	5 seconds		25		Mason unpub.
Pyrethrin + piperonyl butoxide 4:40	4,400	Soil saturated		97		Fleming and Maines unpub.
Pyridine	30,000	1 hour	0	0		Leach and Johnson *1925.*
Do	250	24 hours	33			Baker unpub.
Quinaldine	250	do	43			Do.
Sodium chloride	50,000	15 hours	0			Leach and Thomson *1921.*
Sodium cyanide	2,000	24 hours		100	Killed	Fleming *1926a.*
Do	1,000	2 hours	0			Leach and Thomson *1921.*
Do	150	24 hours	100			Fleming *1925a.*
Sodium ethylxanthate	15,000	1 hour	0	78	Killed	Leach and Thomson *1921.*
Sodium ethylxanthate + 36-percent acetic acid 1:1	20,000	do	100	100	do	Do.
Sodium sulfocarbonate	3,950	do	33	100	do	Do.

TABLE 13.—*Preliminary tests with solutions and emulsions of chemicals as dips to kill Japanese beetle grubs* —Continued

Chemical	Amount per liter of water	Immersion period	Mortality of grubs when		Effect on plants	Source
			Removed from soil	In soil		
	Mg.		*Percent*	*Percent*		
Sodium sulfocarbonate + 36-percent acetic acid 1:1	7,900	do	100	56	do	Do.
Tetrachloroethylene	250	24 hours	77			Baker unpub.
Tetraethylammonium hydroxide	250	do	26			Do.
Tetrahydronaphthalene	500	do	100			Do.
Tetramethylammonium hydroxide	250	do	39			Do.
Thymol	Saturated	do	100	11		Leach and Johnson *1925*.
Toluene	do	1 hour	100	33	Retarded	Do.
Do	450	24 hours	100			Baker unpub.
Toluene trichloride (α,α,α-trichlorotoluene)	350	do	100			Do.
o-Toluidine	1,000	do	100			Fleming *1925a*.
Do	250	do	28			Baker unpub.
o-Tolyl benzoate	250	do	75			Do.
1,2,4-Trichlorobenzene	400	do	100			Do.
Trichloroethylene	250	do	27			Do.
Valeronitrile	250	do	28			Do.
Wormseed oil	Saturated	6 hours	100	100	Retarded	Leach and Johnson *1925*.
o-Xylene	200	24 hours	100			Baker unpub.
Zinc chloride	50,000	2 hours	0	0	Killed	Leach and Johnson *1925*.

Wormseed Oil

Wormseed oil is a volatile oil distilled from the entire plant of *Chenopodium ambrosioides anthelminticum*. It is a colorless or pale yellow liquid with a specific gravity varying from 0.955 to 0.980 at 25° C. The oil contains at least 60 percent of ascaridole and 30 to 40 percent of a mixture of cymene, terpenes, lower fatty acids, and methyl salicylate. Ascaridole ($C_{10}H_{16}O_2$), considered to be the active constituent of the oil, has a specific gravity of 1.0024 at 25°, a disagreeable benumbing odor, and a disagreeable taste. The oil was used as an anthelmintic; the dosage is based on the ascaridole content (Henry and Paget *1921*). Nelson (*1920*) developed an approximate method for assaying the ascaridole content of the oil. This was essentially a determination of part of the oil that dissolved in 60-percent acetic acid.

Leach and Johnson (*1925*) emulsified the oil and based the dosage on the ascaridole content. All third-instar grubs removed from soil and immersed in water containing 1 ml. of ascaridole in 6 liters were killed by immersion for 18 hours at 50° F., 12 hours at 60°, and 6 hours at 65° and 70°. It was the practice in commercial nurseries to dig iris, phlox, peonies, and sedum in the fall, divide the clumps, and remove most of the soil from the roots by thorough shaking. When this operation was completed, up to 10 cc. of soil per plant remained among the roots of iris, phlox, and sedum and up to 16.4 cc. (1 cubic inch) of soil per plant in the cavities of the peony roots. Immersion for 12 hours at 70° in the 1-ml. dip killed the grubs in masses of soil up to 10 cc. in volume, but the dosage had to be increased to 2 ml. to kill them in a cubic inch of soil. The 2-ml. dosage was not effective in larger masses of soil.

Dormant roots of iris, phlox, and sedum were not injured by immersion for 15 hours at 70° F. in a dip containing 1 ml. of ascaridole in 6 liters of water. Dormant roots of peonies were not injured when the concentration of ascaridole in the dip was doubled.

Immersion for 15 hours at 70° F. in 6 liters of water containing 1 ml. of ascaridole was authorized in 1922 for the treatment of divided clumps of dormant iris, phlox, sedum, and other plants with similar root systems to kill grubs in small masses of soil among their roots. After removal from the dip the plants were held at 70° for 48 hours before being certified for shipment. During 1922 and 1923 the treatment was used for about 75,000

plants of this type in the commercial nurseries. (Leach et al. *1924;* Leach and Johnson *1925;* Smith *1925c*)

Carbon Disulfide

Formulations.—Carbon disulfide is only slightly soluble in water. An aqueous solution was prepared by agitating a small quantity of the chemical with a large volume of water. Solutions of carbon disulfide were used by Leach (*1918*) in experiments to control the woolly apple aphid (*Eriosoma lanigerum* (Hausmann)) on the roots in orchards and by Leach and Thomson (*1921*) in preliminary experiments with Japanese beetle grubs. The solutions were not satisfactory because the quantity of carbon disulfide that disssolves in water is appreciably affected by the temperature. At normal atmospheric pressure 1 liter of water can dissolve the following quantities of carbon disulfide (Seidell *1919*):

° C.	*Grams*
0	2.04
10	1.94
20	1.79
30	1.55
40	1.11

The quantity of carbon disulfide in a dip can be controlled more accurately by emulsifying the chemical and adding the required quantity of the emulsion to water. Fleming and Baker (*1935*) gave the composition of many of the stock formulations used in experimentation. Only the formulations approved for use in a commercial treatment are given here.

A creamlike stock emulsion was prepared by agitating carbon disulfide with a soap solution until the chemical emulsified. The formulas of the best stock emulsions of this type are as follows:

	Parts by volume
Formula 1 (Leach and Johnson *1925*):	
Carbon disulfide	5
Rosin-fish oil soap solution	2
Dissolve 125 grams of "Old Style" rosin-fish oil soap in 875 ml. of water by heating.	
Formula 2 (Leach *1925*; Leach and Johnson *1923*; Leach and Lipp *1927a*):	
Carbon disulfide	5
Rosin-fish oil soap solution	2
Dissolve 1 volume of cold-water soluble rosin-fish oil soap in 3 volumes of water.	

Formula 3 (Leach and Fleming *1927*; Leach and Lipp *1927a*; Lipp *1927*):

Carbon disulfide	7
Soap solution	3

To 135 ml. of warm 7-percent sodium hydroxide solution add 50 grams of powdered rosin gradually. Add 450 ml. of hot water and agitate the mixture until the rosin has dissolved. Add 50 ml. of oleic acid and continue the agitation until the mixture is homogeneous.

The emulsifying power of the rosin-fish oil soaps in formulas 1 and 2 was uncertain owing to the varied composition of different batches of the same soap. It was necessary, therefore, to determine the emulsifying power of each batch of commercial soap and to modify the formulas accordingly before satisfactory stock emulsions could be prepared. Furthermore, the stock emulsions prepared with these soaps were stable for only a few days; emulsified carbon disulfide tended to settle to the bottom, leaving a layer of soap solution at the top. It was necessary to agitate these emulsions thoroughly before use in order to assure homogeneity. On long standing or on exposure to a temperature below 40° F., the films of soap around the globules of carbon disulfide tended to rupture, causing some of the globules to coalesce and the emulsion to separate into three distinct layers—soap solution, emulsified carbon disulfide, and unemulsified carbon disulfide. When the emulsion separated in this manner, it could not be restored to homogeneity by agitation.

The uncertainty with the commercial soaps was overcome by using a sodium oleate-rosin soap as the emulsifier (formula 3). The ingredients of this soap were readily available and there was no variation in emulsifying power. The emulsion prepared with this soap was stable for several months provided it was not exposed to low temperatures. It was very viscous and difficult to measure small quantities accurately by volume. This disadvantage was largely overcome by mixing an equal volume of water with the stock emulsion before measuring. Fleming and Baker (*1935*) found that this diluted stock emulsion stratified readily on standing undisturbed. Agitation restored homogeneity but caused foaming and thus made the meniscus difficult to read in a glass cylinder and caused some loss of carbon disulfide.

More satisfactory stock formulations were prepared by replacing the water with an organic solvent that was miscible with carbon disulfide. Ethyl alcohol was the most satisfactory solvent for the soaps. Sodium oleate dissolved in alcohol produced a flocculant precipitate when added to carbon disulfide, but alco-

holic solutions containing not more than 60-percent potassium oleate formed clear translucent solutions with carbon disulfide. It was necessary to have more oleic acid than needed to react with the potassium hydroxide to avoid the presence of free alkali and the conversion of some of the carbon disulfide to less toxic *o*-ethyl dithiocarbonate. Sufficient potassium oleate was needed to disperse the carbon disulfide in water and sufficient alcohol to prevent the precipitation of the potassium oleate in the carbon disulfide.

Two formulations containing alcoholic potassium oleate remained stable for at least 6 months under normal conditions. The formulas of these solutions are as follows:

	Parts by volume
Formula 4 (Fleming *1926b*):	
Carbon disulfide	7
Alcoholic potassium oleate	3
Dissolve 15 grams of potassium hydroxide in 214 ml. of ethyl alcohol and add 86 ml. of oleic acid.	
Formula 5 (Fleming *1925b*, *1926b*):	
Carbon disulfide	7
Alcoholic potassium oleate plus cottonseed oil	3
Dissolve 13.5 grams of potassium hydroxide in 193 ml. of ethyl alcohol and add 77 ml. of oleic acid and then 30 ml. of cottonseed oil.	

After standing undisturbed for several months some of the emulsifier in formula 4 tended to crystallize and an odor of potassium *o*-ethyl dithiocarbonate developed. The formula was modified as in formula 5, but these modifications did not overcome these objectionable features. Considerable care had to be exercised in diluting these mixtures with water in order to produce a satisfactory dilute emulsion. When poured into water, only a part of the carbon disulfide dispersed in the water and the remainder collected on the bottom of the container. When an equal volume of water was added to the mixtures and agitated, the emulsions produced did disperse readily upon pouring a large volume of water.

Mixtures of carbon disulfide with alcoholic solutions of partially saponified vegetable oils were similar in appearance to the mixture of carbon disulfide and an alcoholic solution of potassium oleate, but they dispersed more readily in water. The mixtures containing cottonseed oil, coconut oil, palm oil, peanut oil, and corn oil were unsatisfactory because of the appearance of a sediment and the formation of potassium *o*-dithiocarbonate

on standing for several weeks. No sediment or potassium *o*-dithiocarbonate developed in the mixture containing blown castor oil. The formula for the mixture of carbon disulfide and alcoholic blown castor oil soap used in nurseries is as follows:

	Parts by volume
Formula 6 (Fleming and Baker *1935*; Fleming and Wagner *1928*):	
Carbon disulfide	35
Alcoholic blown castor oil soap	65

Dissolve 37 grams of potassium hydroxide in a mixture of 86 grams of ethyl alcohol and 45 grams of water and add to 832 grams of blown castor oil in a closed kettle or in a three-necked flask equipped with a reflux condenser to prevent loss of alcohol. Gradually increase the temperature to 200° F. (93.3° C.) and hold at this temperature until the alkali has reacted with the oil, agitating the mixture during the procedure. In small batches, 2 hours at 200° F. were sufficient. Saponification is completed when a mixture of the soap and carbon disulfide poured into water turns milky white and gradually diffuses throughout the water.

The carbon disulfide-castor oil soap formulation was mixed in the proportion 35:65 by volume because the 70-percent stock mixtures approved previously were diluted with an equal volume of water before measuring volumetrically. It is a mobile, translucent liquid that does not form a heavy foam when shaken. It can be poured easily and measured accurately in small quantities. It mixes readily with water in all proportions, forming a milky emulsion.

Toxicity to Immature Stages.—The emulsifiers given in this bulletin did not modify significantly the toxicity of emulsified carbon disulfide. When third-instar grubs were removed from soil and immersed for 24 hours, the median lethal concentration of carbon disulfide emulsified by sodium oleate rosin soap (formula 3) was 0.13 ml. per liter. It was 0.14 ml. with alcoholic blown castor oil soap (formula 6) as the emulsifier and 0.17 ml. with alcoholic potassium oleate (formula 4) as the emulsifier. (Fleming and Baker *1935*)

The grubs were more susceptible than the other immature stages to immersion in emulsified carbon disulfide. When removed from soil and immersed for 24 hours at 70° F., 1 ml. of emulsified carbon disulfide per liter of water was required to prevent hatching of eggs, 0.5 ml. to kill pupae, and 0.3 ml. to kill third-instar grubs (Fleming and Baker *1935*). It seemed advisable to limit

the use of a carbon disulfide dip to the periods of the year when only grubs were present to avoid increasing the potential hazard of the treatment to plants.

The mortality of the grubs was modified by the dosage, temperature, and period of immersion. In a preliminary test Leach and Johnson (*1925*) killed third-instar grubs removed from soil by immersing them in water containing 0.5 ml. of emulsified carbon disulfide per liter for 4 hours at 70° F. and for 12 hours at 50°. The results at intervening temperatures were erratic. Fleming and Baker (*1935*) investigated the dosage-temperature-immersion relationship more extensively. When the temperature was 70°, the grubs were killed by the following immersions and dosages:

Immersion (hours)	*Carbon disulfide per liter (ml.)*
24	0.3
10	.4
5	.5
4	.6
3.5	.8
3	1
2	1.2
1	2

When immersion was for 24 hours, the grubs were killed by the following temperatures and dosages:

° F.	*Carbon disulfide per liter (ml.)*
90	0.08
80	.16
70	.3
60	.4
50	.5
45	.6
40	.8

The optimum temperature for killing grubs was about 70°.

Effectiveness Against Grubs in Soil.—Leach and Johnson (*1925*) killed third-instar grubs embedded in soil in the cavities of peony roots by immersing the roots for 15 hours at 70° F. in water containing 0.5 ml. of emulsified carbon disulfide per liter. The mass of soil in these cavities was not more than 1 cubic inch. Fleming and Baker (*1935*) found that this dosage was effective in killing grubs in the soil of potted plants when the volume of soil was not more than 14 cubic inches and the immersion was for 24 hours. A dosage of 0.6 ml. was effective in soil balls up to 6 inches in diameter, but it was only partially

effective in larger masses of soil. The grubs were killed in larger masses of soil by increasing the dosage, but preliminary tests showed that dosages above 0.6 ml. were often very injurious to plants. This reaction of the plants limited the application of the treatment to masses of soil not more than 6 inches in diameter.

Effect on Soil Micro-Organisms.—Fleming (*1929*) studied the effect of saturating soil with water and with dilute carbon disulfide emulsion on the number of bacteria and fungi and on the accumulation of ammonia and nitrates in the soil.

Saturating the soil with water had no effect on the number of bacteria. The number of fungi was reduced while the soil was waterlogged, but soon thereafter the population reached its initial density. The production of ammonia ceased while the soil was saturated with water, but it became normal as the soil was restored to optimum moisture. The quantity of nitrates in the soil did not change.

When dilute carbon disulfide was used in place of water, the number of bacteria and fungi decreased markedly, followed by an increase in these micro-organisms. Seventeen days after treatment there were 10 times as many bacteria as initially in the soil. The fungi increased more slowly and did not reach their initial density until 59 days after the application of the emulsion. The production of ammonia ceased while the soil was saturated and then increased so rapidly that 30 days after treatment there was 10 times as much ammonia as initially in the soil. The dilute emulsion did not affect the accumulation of nitrates in the soil. The carbon disulfide had no permanent detrimental effect on the bacteria and fungi in the soil.

Reaction of Plants.—In cooperative studies with several commercial nurseries Fleming and Baker (*1935*) determined the reaction of dormant and semidormant ornamental plants to the immersion of their roots for 24 hours at 70° F. in water containing 0.6 ml. of emulsified carbon disulfide per liter. After treatment the plants were handled according to the usual nursery practice.

The ornamental grasses—*Arrhenatherum elatius, Arundo donax, Cortaderia selloana, Elymus glaucus, Miscanthus sinensis, Pennisetum alopecuroides,* and *Phalaris arundinacea*—were injured only superficially by the immersion.

The roots of garden rhubarb *Rheum rhaponticum* were not injured.

The herbaceous perennials—*Convallaria majalis, Dahlia* spp., *Hemerocallis dumortieri, H. fulva, Iris cristata, I. germanica, I. kaempferi, I. ochroleuca, I. pseudacorus, I. sibirica, Paeonia*

albiflora, P. officinalis, Phlox amoena, P. paniculata, and *Sedum spectabile*—were not seriously retarded in their subsequent growth, although sometimes the bud scales and the sprouts were blackened by the treatment.

The deciduous shrubs were not injured by immersing their roots in the dilute emulsion. The shrubs treated included *Berberis thunbergii, Buddleia davidi, Callicarpa dichotoma, Calluna vulgaris, Clethra alnifolia, Cornus florida, Crataegus oxycantha, Deutzia gracilis, Euonymus alatus, Forsythia suspensa, Hibiscus syriacus, Hydrangea arborescens, Ligustrum ovalifolium, Lonicera involucrata, Philadelphus coronarius, Rhus typhina, Rosa* spp., *Spiraea* spp., *Symphoricarpos orbiculatus, Syringa vulgaris, Viburnum* spp., and *Weigela florida.*

The deciduous trees tolerated the treatment, including *Acer platanoides, Aesculus hippocastanum, Betula pendula, Fagus sylvatica, Fraxinus ornus, Magnolia virginiana, Morus alba, Quercus falcata, Salix babylonica, Sorbus aucuparia, Tilia europaea,* and *Ulmus americana.*

The broadleaf evergreens were seriously injured or killed by immersing their roots in the dilute emulsion, including *Buxus sempervirens, Ilex crenata, Kalmia latifolia, Pachysandra terminalis, Pyracantha coccinea, Rhododendron catawbiense, R. indicum,* and *R. japonicum.*

The narrowleaf evergreens did not tolerate the treatment, including *Abies concolor, Chamaecyparis obtusa, C. pisifera, Juniperus chinensis, J. communis, J. horizontalis, J. sabina, J. virginiana, Picea abies, P. glauca, P. pungens, P. rubens, Pinus mugo, P. nigra, P. sylvestris, Taxus baccata, T. canadensis, T. cuspidata, Thuja occidentalis, T. orientalis,* and *Tsuga canadensis.*

Use in Nurseries.—Immersion of the roots of dormant *Paeonia* spp. for 15 hours at 70° F. in water containing 0.5 ml. of emulsified carbon disulfide per liter was authorized in 1922 to kill grubs in the small quantities of soil in the cavities of the roots. The roots could be certified for shipment 48 hours after removal from the dip (Leach and Johnson *1925;* Smith *1925c*). In 1929 immersion of the roots of dormant and semidormant herbaceous plants and deciduous trees and shrubs for 24 hours at 70° in water containing 0.6 ml. of emulsified carbon disulfide per liter was authorized to destroy grubs in masses of soil up to 6 inches in diameter. The treatment was used extensively in the commercial nurseries, but the number of plants treated is not available.

Ethylene Dichloride

Formulation.—Mason et al. (*1943*, unpublished) prepared several clear emulsifiable mixtures of ethylene dichloride and fatty acid soaps. The best of these mixtures had the following composition:

	Parts by weight
Ethylene dichloride	60
Alcoholic potassium oleate soap	40

Dissolve 2.5 pounds of potassium hydroxide in a mixture of 14 pounds of ethyl alcohol and 6 pounds of water, add 17.5 pounds of oleic acid, and stir intermittently for about 10 minutes.

Each 100 ml. of this formulation contained 60 grams of ethylene dichloride. The miscible mixture remained homogeneous during storage at room temperature, but it separated into two layers at temperatures below 40° F. When stratification occurred, homogeneity was restored by warming to room temperature and stirring. To dilute this mixture, small quantities of water were added successively with stirring until a creamlike emulsion was formed. Then the remainder of the water was added. It was not difficult to dilute this formulation, except when only hard water was available. The hard water converted some of the potassium oleate to an insoluble soap.

Chisholm et al. (*1944*) overcame this objectionable feature by replacing the soap with a surface active agent, Tween 20, a commercial polyoxyalkylene derivative of sorbitan monolaurate. The modified formulation was prepared by dissolving 2.5 pounds of Tween 20 in 97.5 pounds of ethylene dichloride. This product was a clear liquid that withstood low temperatures and diluted uniformly with hard water. In diluting this emulsifiable mixture, one volume of the mixture was shaken with an equal volume of water for about 1 minute to form an emulsion. This emulsion dispersed readily in water.

A dilution of 1:100 by volume of the 60-percent formulation with water contained 6 grams of ethylene dichloride per liter, whereas a dilution of 1:200 of the 97.5-percent formulation contained 6.1 grams per liter. Six grams of ethylene dichloride will dissolve completely in a liter of water at room temperature. The dilution prepared with the 60-percent formulation was opaque because of the presence of potassium oleate, but that prepared with the 97.5-percent formulation was substantially clear. Mason

(unpublished) found that water dilutions of these formulations were equally toxic to grubs.

Effectiveness Against Grubs in Soil.—Mason et al. (unpublished) immersed azalea plant balls containing third-instar grubs in water containing from 1.5 to 9 grams of ethylene dichloride per liter for 10 seconds. A week later the grubs were removed from the treated soil and placed in untreated soil at 80° F. for observation. When they were removed from treated soil, the mortality was 6 percent with 1.5 grams of ethylene dichloride per liter, 14 percent with 3 grams, 31 percent with 6 grams, and 75 percent with 9 grams. All grubs subjected to the 9 grams were dead within 1 week. The 6 grams killed all of them in 2 weeks. At that time the mortality was 99 percent with the 3-gram and 69 percent with the 1.5-gram treatment.

The 6-gram dosage was not completely effective against pupae and eggs. Pupae in the early stages of development were killed as readily as the grubs; up to 10 percent of the pupae approaching the adult stage were not affected by the treatment. About two-thirds of the eggs in the early stages of development hatched, but most of the nearly mature eggs were killed by the treatment. It was necessary therefore to restrict the application of the 6-gram dosage to those periods of the year when only grubs were in the soil. (Mason et al. *1948*)

Immersion of the plant balls for 5 seconds in the 6-gram dip was less consistent but almost as effective in killing third-instar grubs as immersion for 10 seconds. The mortality of the grubs after immersion for 5 seconds was 29 percent when removed from soil, 88 percent 1 week later, 96 percent 2 weeks later, and 98 percent 3 weeks later, whereas the mortality following immersion for 10 seconds was 44, 95, 99, and 100 percent, respectively. (Mason et al. *143*, unpublished)

The temperature and the holding conditions modified the insecticide action. Infested azalea plant balls were immersed for 10 seconds in a dip containing 6 grams of ethylene dichloride per liter at between 40° and 80° F. and held at these temperatures for 5 days before removing the grubs for observation. When the treated plant balls were placed on a bench or on the floor so that they were separated from each other to permit circulation of air around each plant ball, the mortality 3 weeks later ranged from 92 to 98 percent. There was no relationship between the temperature and the mortality; the loss of the chemical by aeration apparently was the dominant factor. When the plant balls were placed in a compact mass, 100-percent mortality was obtained in 3 weeks at between 40° and 50°, in 2 weeks between 50°

and 70°, and in 1 week between 70° and 80°. Placing the plant balls in a compact mass after treatment reduced the loss of ethylene dichloride from the soil. (Mason et al. *1943*, unpublished)

The period the grubs were left in the plant balls after immersion modified the rate of insecticide action. When the grubs were removed from the plant balls 1 day after immersion, 25 days elapsed before all were dead, but when removed 2 to 14 days after immersion, all were dead within 12 to 15 days after immersion. Holding the plant balls in a compact mass for 2 days after immersion was sufficient to reach the maximum rate of insecticide action. (Mason et al. unpublished)

The treatment was not completely effective when the plant balls were saturated with water at the time of immersion in the dip. The mortality of the grubs in the wet plant balls was 93 percent 3 weeks after they were removed from the plant balls for observation. When the plant balls were only partially saturated with water, the treatment killed all of them within 2 weeks. (Mason et al. unpublished)

The type of soil was not a limiting factor in the effectiveness of the ethylene dichloride dip. Grubs in muck and a heavy loamy soil were all dead within 1 week after being removed from the treated soils for observation. From 2 to 3 weeks elapsed before those removed from treated sandy loam and peat were dead. The insecticide was probably retained longer in the heavy soils than in the light soils. (Mason et al. unpublished)

The size of the plant balls—up to 16 inches in diameter and up to 14 inches in depth—did not limit the effectiveness of the dip. Grubs in 2- to 6-inch plant balls, in pots, or wrapped in burlap or other suitable material were dead 3 weeks after immersion in the dip; those in 6- to 10-inch plant balls died within 2 weeks after treatment, and those in 16-inch plant balls died within 9 days after immersion. The dip was not completely effective in killing grubs in larger masses of soil, although the mortality approached 100 percent. (Mason unpublished; Mason and Coles unpublished; Mason et al. unpublished)

Reaction of Plants.—Through the cooperation of commercial greenhouses and nurseries, Mason et al. (*1943*, unpublished) immersed the soil balls of many varieties of greenhouse and nursery plants for 10 seconds in water containing 6 grams of ethylene dichloride per liter at from 40° to 75° F. After treatment the plants were handled according to the normal procedures. Observations over several months showed that, with the exception of *Genista* sp., injury to the plants by this treatment was negligible.

The following greenhouse plants were treated successfully:

Adiantum sp.
Aglaonema simplex
Araucaria araucana
Asplenium nidus
Aucuba chinensis
Aucuba japonica
Begonia gracilis
Begonia rex-cultorum
Beloperone sp.
Bouvardia sp.
Caladium bicolor
Calceolaria crenatiflora
Cibotium schiedei
Cissus rhombifolia
Citrus reticulata
Clivia miniata
Cotoneaster sp.
Crassula sp.
Croton spp.
Cyclamen persicum
Dieffenbachia picta
Dracaena deremensis
Dracaena fragrans
Erythea sp.
Euonymus japonicus
Euphorbia pulcherrima
Ficus pandurata
Fuchsia sp.
Gardenia jasminoides
Geranium sp.
Hedera helix
Heliotropium arborescens
Howea sp.
Hydrangea macrophylla
Lantana camara
Monstera deliciosa
Nephrolepis exaltata
Onoclea sensibilis
Opuntia microdasys
Osmanthus sp.
Pandanus veitchi
Peperomia sp.
Philodendron cordatum
Phoenix sp.
Piper ornatum
Polygonum cuspidatum
Pothos loureiri
Primula sp.
Saintpaulia sp.
Sansevieria sp.
Senecio cruentus
Spathyphyllum sp.

The following perennials tolerated immersion of their roots in the dip:

Abelia zanderi
Achillea tomentosa
Ajuga genevensis
Althaea rosea
Alyssum saxatile
Arabis alpina
Armeria maritima
Astilbe rosea
Brunnera macrophylla
Campanula medium
Centaurea montana
Convallaria majalis
Cotoneaster horizontalis
Daphne genkwa
Delphinium hybridum
Dianthus barbatus
Dianthus caryophyllus
Dicentra formosa
Digitalis purpurea
Doronicum plantagineum
Geum chiloense
Gypsophila paniculata
Helleborus niger
Heuchera sanguinea
Hosta plantaginea
Iberis sempervirens
Lavandula officinalis
Lupinus polyphyllus
Mondo jaburan
Myosotis scorpioides
Pachysandra terminalis
Primula veris
Rosmarinus officinalis
Salvia officinalis
Santolina chamaecyparissus
Thymus britannicus
Trollius sp.
Vinca minor
Viola cornuta

The following shrubs, trees, and vines were treated successfully:

Berberis mentorensis
Berberis thunbergii
Buxus rempervirens
Camellia japonica
Cephalotaxus drupacea
Chamaecyparis obtusa
Chamaecyparis pisifera
Clematis jackmani
Clematis lawsoniana
Clematis paniculata
Elaeagnus pungens
Euonymus japonicus
Ilex cornuta
Ilex crenata
Juniperus chinensis
Juniperus communis
Laurus nobilis
Ligustrum japonicum
Ligustrum lucidum
Lonicera henryi
Magnolia grandiflora
Nandina domestica
Picea abies
Pinus nigra
Prunus caroliniana
Pyracantha sp.
Rhododendron indicum
Rhododendron obtusum
Rhododendron sanderi
Rosa spp.
Syringa persica
Syringa vulgaris
Thuja occidentalis
Tsuga canadensis

Use in Nurseries.—Authorization was given in 1942 for the immersion of soil in unglazed clay pots and wrapped soil balls up to 10 inches in diameter for 10 seconds in water containing 6 grams of ethylene dichloride per liter at between 40° and 75° F. Later soil masses up to 16 inches in diameter and up to 14 inches in depth could be treated. The treatment could be used in the fall, winter, and spring when only grubs were in the soil. The plants could be certified for shipment after holding them in a compact mass for 24 hours. Middleton and Cronin (*1952*) reported that during 1942–50 over 6,826,000 plants in commercial nurseries and greenhouses had been treated by this procedure and certified for shipment.

Ethylene Dibromide

Formulations.—Since ethylene dibromide is a solid at temperatures below 10° C. (50° F.), Chisholm et al. (*1946a*), Chisholm and Mason (*1948a*), and Mason and Chisholm (*1945*, unpublished) mixed it with solvents having lower melting points and added a surface-active agent to disperse the mixtures in water. The composition of the three best formulations is as follows:

	Percent by weight
Formula 1:	
Ethylene dibromide	16.0
Ethylene dichloride	81.5
Tween 20 (a polyoxyalkylene derivative of sorbitan monolaurate)	2.5

	Percent by weight
Formula 2:	
Dowfume W–85 (83 percent ethylene dibromide)	19.3
Ethylene dichloride	78.0
Tween 20	2.7
Formula 3:	
Dowfume W–85	24.1
Isopropyl alcohol (99 percent)	55.9
Tween 20	20.0

The ingredients of these formulations were mixed together to form clear emulsifiable liquids. Each formulation contained 20 grams of ethylene dibromide per 100 ml. One volume of a formulation was shaken with two volumes of water to form a milky emulsion, and then additional water was added with stirring to obtain the concentration desired. Since 4.3 grams of ethylene dibromide dissolve in 1 liter of water at 30° C. (86° F.), substantially clear solutions were obtained when the quantity of the dibromide in the final dilution did not exceed this amount.

In 1956 circumstances involving patents and other restrictions made it necessary to replace ethylene dibromide with Dowfume W–85, a commercial preparation containing 83 percent of ethylene dibromide and 17 percent of a light petroleum fraction. The modified formulation (formula 2) was adjusted so that it contained the same weight of ethylene dibromide per 100 ml. as the original formulation. The substitution of Dowfume W–85 did not affect the toxicity to grubs.

Although ethylene dichloride was the dominant insecticide in formulations 1 and 2, it was much less toxic to grubs than ethylene dibromide, and because of the small quantities of the formulations used, it had a minor role in killing grubs. As noted previously, a dosage of 6 grams of ethylene dichloride per liter of water was needed to kill grubs in soil. When applied with ethylene dibromide, there was usually less than 1 gram of ethylene dichloride per liter, a dosage that had little effect on the grubs. Furthermore, the substitution of isopropyl alcohol, a compound of very low toxicity, for the ethylene dichloride did not modify the toxicity of the formulation. Therefore in all these formulations ethylene dibromide was considered to be the active insecticide ingredient.

Toxicity to Grubs.—When third-instar grubs were removed from soil and immersed for 10 seconds, 1.5 grams of ethylene dibromide per liter were required to kill them within 3 weeks after immersion (Mason and Chisholm *1945*). However, grubs

out of soil are not found even among the roots of bare-root nursery stock.

The largest mass of soil usually found among the roots of bare-root nursery stock is about a cubic inch. Third-instar grubs in 1-inch cubes of moist, friable sandy loam, clay, and peat were killed by immersion for 10 seconds in water containing 0.25 gram of ethylene dibromide per liter at 40° F., 0.13 gram at 50°, and 0.06 gram at 70°. When these small masses of soil were saturated with water at the time of immersion, the dosage had to be increased to 0.5 gram per liter to kill the grubs at 40° and above. In these tests the grubs were removed from the cubes of soil 1 to 5 days after immersion to observe their reaction. Many of them at that time appeared to be normal, but with these treatments all were dead within 3 weeks. The rate of insecticide action was accelerated by leaving the grubs in the treated soil for more than 1 day. To assure their destruction among the roots of bare-root nursery stock, the dosage was increased to 0.6 gram of ethylene dibromide per liter for use at 40° to 70°. (Mason and Chisholm *1945;* Mason et al. unpublished)

Third-instar grubs in 4-inch azalea plant balls were killed by immersing the plant balls for 10 seconds in water containing 0.10 gram of ethylene dibromide per liter at 40° F. and in water containing 0.05 gram per liter at 50°. The 0.10 gram was effective in killing grubs in 10- and 16-inch plant balls when the plant balls were immersed until saturated, as indicated by no air bubbling from the soil, but 0.25 gram was not completely effective in larger masses of soil. All grubs were dead within 3 weeks after removal from the treated soil, but the mortality was accelerated by leaving the grubs in the treated soil for more than 2 days, indicating some residual action of ethylene dibromide in the soil. To demonstrate the residual action, grubs were introduced into plant balls 1 to 3 weeks after immersion in the 0.10-gram dip. All grubs introduced into the plant balls 1 week after immersion were killed; the mortality was 95 percent with grubs introduced after 2 weeks and 92 percent with those introduced after 3 weeks. (Mason unpublished; Mason and Chisholm unpublished; Mason et al. unpublished)

Toxicity to Pupae.—Pupae removed from soil and immersed for 10 seconds in water containing 0.5 gram of ethylene dibromide per liter continued to develop normally and emerged as adult beetles. Plant balls containing pupae were dipped for 10 seconds and then held for 30 days before examining to determine the effect on the pupae. Thirteen percent of them completed their development after immersion in the 0.13-gram dosage,

1 percent after immersion in the 0.25-gram dosage, and none after immersion in the 0.5-gram dosage. When the plant balls were immersed until saturated, all pupae were killed by the 0.10-gram dosage. (Mason and Chisholm unpublished; Mason et al. unpublished)

Toxicity to Eggs.—Eggs removed from soil and immersed for 24 hours in water containing 0.15 gram of ethylene dibromide per liter did not hatch, but when the dosage was reduced to 0.075 gram per liter, 16 percent of the eggs hatched. Eighty to 91 percent of the eggs hatched after soaking in water for 24 hours. (Mason and Chisholm unpublished)

The mortality of eggs in plant balls immersed for 10 seconds in water containing 0.25 gram of ethylene dibromide per liter was 74 percent when the eggs were removed from the soil 2 days after immersion and 100 percent when the eggs were left in the treated soil for 8 days. When plant balls were immersed until saturated in the 0.10-gram dosage and the eggs were removed 2 days later for observation, 62 percent of the eggs in a loamy soil and 99 percent of them in a peat were killed. No eggs hatched when left in the loamy soil and peat for 8 days. (Mason and Chisholm unpublished; Mason et al. unpublished)

Reaction of Bare-Root Plants.—Dormant bare-root deciduous shrubs were removed from a heeling-in bed in April and their roots were immersed for 10 seconds in water containing 2.8 and 5.6 grams of ethylene dibromide per liter. These dosages greatly exceeded those needed to kill grubs immersed without soil and in 1-inch cubes of soil. The dipped plants were held for 24 hours under canvas covers in the packing shed and then planted in the field. All the shrubs grew normally. (Mason et al. unpublished)

The species of plants treated successfully were as follows:

Berberis thunbergii	*Prunus amygdalus*
Calycanthus floridus	*Ribes triste*
Celastrus scandens	*Spiraea bumalda*
Cercis canadensis	*Spiraea thunbergii*
Deutzia scabra	*Symphoricarpos albus*
Forsythia suspensa	*Syringa vulgaris*
Hydrangea arborescens	*Vitis* sp.
Hydrangea paniculata	*Weigela florida*

The roots of 12 varieties of roses, not identified in the report, were immersed for 10 seconds in water containing 0.5 gram of ethylene dibromide per liter. After holding in storage for about a month, the roses were potted and placed in a greenhouse. Three months later 10 of the varieties were growing normally, but two of them were retarded. (Mason et al. unpublished)

Herbaceous perennial plants with roots practically free of soil were immersed for 10 seconds in water containing 0.5 gram of ethylene dibromide per liter. After holding in storage for 24 hours in the packing shed, half of the plants were packed and sent to the laboratory and the other half were potted at the nursery. The plants sent to the laboratory were potted soon after arrival. The treatment did not affect the growth of *Aster novae-angliae, Chrysanthemum maximum, Lavandula officinalis,* and some varieties of *Papaver orientale,* but one variety of *P. orientale* and of *Viola* sp. were retarded. (Mason et al. unpublished)

Several nurseries dipped bare-root trees, shrubs, and perennial plants in the 0.5-gram dosage. Information is not available on the varieties treated, but no injury to the plants was reported. (Mason et al. unpublished)

Reaction of Balled and Potted Plants.—Mason et al. (unpublished) in a preliminary test immersed the soil balls of several potted plants, with the pots removed, for 10 seconds in water containing 0.18, 0.35, and 0.7 grams of ethylene dibromide per liter. *Geranium* sp. tolerated 0.18 gram but was injured by 0.35 gram. *Rhododendron obtusum* was not injured by 0.35 gram, but 0.7 gram caused some defoliation. *Asparagus plumosus, Hedera helix, Nephrolepis exaltata,* and *Pandanus australis* were not affected by 0.7 gram.

Many varieties of azaleas in commercial nurseries were not injured by immersing the plant balls for 10 seconds or until saturated in water containing 0.25 gram of ethylene dibromide per liter. One nursery immersed the plant balls of 141 varieties of greenhouse plants and reported that those subjected to the 0.13-gram dosage grew normally, whereas many of the varieties were retarded or killed by 0.25 and 0.5 grams. Other nurseries reported no injury to greenhouse plants by 0.13 gram. Information is not available now to identify these varieties. (Mason et al. unpublished)

The evergreens—*Abies concolor, Juniperus communis, Picea abies, Pinus mugo, Pseudotsuga taxifolia, Taxus baccata,* and *Thuja occidentalis*—were injured or killed by immersing their plant balls in water containing 0.5 gram of ethylene dibromide per liter. The reaction of the plants to lower dosages was not determined. Probably dormant evergreens would not be seriously injured by the 0.10-gram dosage. (Mason et al. unpublished)

Use in Nurseries.—The immersion of the roots of bare-root nursery stock, with only small quantities of soil adhering to the roots, for 10 seconds in water containing 0.6 gram of ethylene

dibromide per liter at between 50° and 75° F. was authorized in 1945 as a basis of certification. The treatment was restricted to the period of the year when only grubs were in the soil. Later when the bare-root stock was immersed until the soil was saturated, it was possible to reduce the minimum temperature to 40° and to remove the seasonal limitation. The treated stock could be certified for shipment 24 hours after immersion.

The treatment of balled nursery stock with soil balls not more than 16 inches in diameter by immersing the soil in water containing 0.1 gram of ethylene dibromide per liter was authorized in 1947 for use at between 40° and 75° F. Initially the treatment was restricted to the period of the year when only grubs were in the soil, but later the seasonal restriction was removed. The treated stock could be certified for shipment 24 hours after immersion.

During 1945–50, 3,522,333 plants in the commercial nurseries and greenhouses were certified for shipment after their roots had been immersed in the dilute aqueous solutions of ethylene dibromide (Middleton and Cronin *1952*).

Ethylene Dibromide-Chlordane

Formulations.—The formulation used in all but the preliminary tests had the following composition:

	Percent by weight
Ethylene dibromide	13.0
Chlordane (technical)	6.5
"Cellosolve" (ethylene glycol monoethyl ether)	6.5
Tween 20 (a polyoxyalkylene derivative of sorbitan monolaureate)	6.5
Isopropyl alcohol (99 percent)	67.5

The ingredients were mixed in the order given to form a clear, dark mixture. Each 100 ml. of the formulation contained 12 grams of ethylene dibromide and 6 grams of chlordane. The formulation was diluted by pouring it into water. In most of the dilutions used, the ethylene dibromide was in solution and the chlordane was dispersed as an emulsion of very small particles. (Chisholm and Koblitsky *1951;* Chisholm and Mason *1948b;* Mason and Chisholm *1949*)

As with the ethylene dibromide formulations, it was necessary in 1956 to substitute Dowfume W–85 for ethylene dibromide in this formulation. Chisholm (unpublished) increased the quantities of ethylene dibromide and chlordane in the new formulation so as to have the same weight per volume of ethylene

dibromide in the formulation as in the ethylene dibromide formulations. The composition of the revised formulation was as follows:

	Percent by weight
Dowfume W–85	24.1
Chlordane	10.0
Cellosolve	10.0
Triton X–100 (octylphenoxypoly (ethyleneoxy) ethanol)	20.0
Isopropyl alcohol (99 percent)	35.9

Each 100 ml. of the formulation contained 20 grams of ethylene dibromide and 10 grams of chlordane.

Toxicity to Grubs.—When third-instar grubs in 1-inch cubes of friable soil were immersed for 10 seconds in water containing 0.6 gram of ethylene dibromide and 0.3 gram of chlordane per liter and they were removed from the cubes 2 days later for observation, all were dead within 7 days after immersion. One-half of this dosage killed all the grubs within 14 days, but one-fourth of the dosage was not completely effective. The ethylene dibromide-chlordane mixture was more toxic and killed grubs faster than did ethylene dibromide alone. (Fleming et al. unpublished)

Third-instar grubs in plant balls up to 10 inches in diameter were immersed until the soil was saturated in water containing 0.1 gram of ethylene dibromide and 0.05 gram of chlordane per liter. The treatment was used at 40° to 70° F. The grubs were left in the treated soil balls from 1 to 14 days. All were dead within 7 days after removal from the soil balls. (Mason unpublished; Mason and Chisholm *1949*)

The effectiveness of the ethylene dibromide-chlordane treatment persisted in the plant balls for several weeks. Third-instar grubs inserted into the plant balls at intervals up to 21 days after immersion were killed during a 14-day period in the treated soil. First-instar grubs died within 5 days after they were placed in soil from plant balls that had been immersed as long as 4 weeks previously. (Mason and Chisholm *1949*, unpublished)

Toxicity to Pupae.—Pupae in plant balls were immersed until the soil was saturated in water containing 0.1 gram of ethylene dibromide and 0.05 gram of chlordane per liter. The plant balls after treatment were placed in cages. A few beetles emerged from the plant balls during the 2 days after treatment, but they were abnormal and died within 48 hours after their emergence. No living pupae or adults were found in the plant balls 15 days after immersion. (Mason and Chisholm *1949*, unpublished)

Toxicity to Eggs.—Eggs in plant balls were subjected to the same treatment as the grubs and pupae. Less than half of the eggs hatched when they were left in the treated soil for 1 day. An occasional egg survived for 7 days in the treated soil, but no living eggs or grubs were found in the treated plant balls left undisturbed for 3 weeks. (Mason and Chisholm *1949*)

Reaction of Plants.—In cooperative studies with several nurseries and greenhouses, many varieties of plants were subjected to the ethylene dibromide-chlordane treatment. The bare-root stock was treated by immersing its roots until the small lumps of soil adhering to the roots were saturated by water containing 0.6 gram of ethylene dibromide and 0.3 gram of chlordane per liter. The potted and balled plants were treated by immersing the soil in water containing 0.1 gram of ethylene dibromide and 0.05 gram of chlordane per liter. No injury to the plants was reported. The treatment was used successfully on the following species of plants:

Abelia grandiflora
Allium schoenoprasum
Artemisia pontica
Begonia semperflorens
Berberis thunbergii
Buddleia asiatica
Callicarpa dichotoma
Carya cordiformis
Chamaecyparis pisifera
Cyclamen sp.
Cymbidium sp.
Cytisus praecox
Delphinium sp.
Deutzia gracilis
Erica darleyensis
Euonymus fortunei
Euphorbia pulcherrima
Hedera helix
Hemerocallis middendorffi
Hydrangea macrophylla
Ilex opaca
Juniperus chinensis
Juniperus horizontalis
Juniperus sabina
Kerria japonica
Lavandula officinalis
Ligustrum ovalifolium
Mentha arvensis
Mentha citrata
Mentha pulegium
Mentha spicata
Monarda didyma
Pelargonium graveolens
Pelargonium limoneum
Pelargonium ordoratissimum
Pelargonium quercifolium
Pelargonium tomentosum
Philodendron cordatum
Phlox sp.
Picea glauca
Pyracantha coccinea
Rhododendron dauricum
Rhododendron gandavense
Rhododendron obtusum
Rhododendron ponticum
Rhododendron sanderi
Rosa spp.
Rosmarinus officinalis
Saintpaulia ionantha
Salvia officinalis
Santolina chamaecyparissus
Stachys lanatana
Stephanotis floribunda
Taxus cuspidata
Teucrium chamaedrys
Thuja occidentalis
Thymus vulgaris
Viburnum burkwoodi
Viburnum trilobum
Weigela wagnerii

After treatment the plants were held at the nurseries under the same conditions as untreated plants until their reaction could be determined. The final observation of some species was made within 2 months after immersion. The reaction of other species was determined at the end of the growing season.

Use in Nurseries.—The immersion of bare-root nursery stock in water containing 0.6 gram of ethylene dibromide and 0.3 gram of chlordane per liter and immersion of balled and potted plants with soil masses not more than 16 inches in diameter or more than 14 inches in depth in water containing 0.1 gram of ethylene dibromide and 0.05 gram of chlordane per liter until the soil was saturated were authorized as a basis for certification in 1948. The treatment could be used at 40° to 75° F. There was no seasonal limitation on its use. The plants could be certified for shipment 24 hours after immersion of their roots in the dip.

Although chlordane, a residual insecticide, was introduced into the soil, nothing was known about its distribution and longevity in the different masses of soil under various conditions. The ethylene dibromide-chlordane mixture was considered to be only a substitute for other soil fumigants.

During 1948–50, 731,214 plants were certified for shipment after their roots had been immersed in the ethylene dibromide-chlordane dips (Middleton and Cronin *1952*).

Aldrin

Aldrin contains not less than 95 percent of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene. It is a white solid with a melting point of 104°–104.5° C. It is practically odorless at room temperature, but it has a pinelike odor when warm. It is soluble in the common organic solvents but practically insoluble in water. (Bowen and Hall *1952*)

Formulation.—Chisholm (unpublished) prepared an emulsifiable mixture of aldrin of the following composition:

	Percent by weight
Technical aldrin	3.6
Triton X–100	14.0
Isopropyl alcohol (99 percent)	82.4

It was a clear liquid that emulsified readily upon being poured into water. Each 100 ml. of the formulation contained 3 grams of aldrin. Some sediment developed after the formulation had been on the laboratory shelf for 17 months. Fleming et al. (unpublished) found that the formation of this sediment did not affect the emulsification or the toxicity of the formulation.

Toxicity to Grubs.—When the soil of potted plants was immersed until saturated at 60° F., 0.15 gram of aldrin per liter of water killed all grubs within 2 weeks. Dosages of 0.038 and 0.019 grams killed them within 3 weeks. The 0.038-gram dosage was effective in killing third-instar grubs in plant balls up to 16 inches in diameter and in pots up to 14 inches deep, but 0.019 gram of aldrin was not always effective in these larger masses of soil. (Fleming et al. unpublished)

The average deposit of aldrin in the soil after immersion in the 0.038-gram dosage was 0.2 gram per cubic foot. This was equivalent to approximately 5 pounds of aldrin mixed with the upper 3 inches of an acre of soil. In the field treatments (p. 94), 3 pounds of aldrin per acre killed newly hatched grubs after the chemical had weathered for 4 years. It would be expected that the residue from the dip treatment would be effective in killing newly hatched grubs for that period of time. (Fleming et al. unpublished)

Reaction of Plants.—In preliminary tests *Pelargonium* sp. was not affected by immersing the soil of the potted plants until saturated in water containing 0.15 gram of aldrin per liter, the highest dosage used. The 0.038-gram dosage did not injure *Hedera helix, Hydrangea arborescens, H. paniculata, Nephrolepis exaltata, Rhododendron indicum,* or *R. obtusum.* Other plants were treated successfully with 0.038 gram of aldrin in commercial nurseries and greenhouses, but information on the varieties tested is not now available. (Fleming et al. unpublished)

Use in Nurseries.—Water containing 0.038 gram of emulsified aldrin per liter was authorized in 1959 for the treatment of balled and potted plants. Plant balls not more than 16 inches in diameter or more than 14 inches in depth were immersed in the dip until saturated and then held in a compact mass for 2 weeks at not less than 60° F. before being certified for shipment. The treatment could be used at any time of the year. The treated plants in pots could be in a certified status for 4 years.

Heptachlor

Heptachlor contains about 72 percent of 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene and about 28 percent of related compounds. It is soluble in the common organic solvents but practically insoluble in water.

Formulation.—Chisholm (unpublished) prepared an emulsifiable mixture of heptachlor of the following composition:

	Percent by weight
Technical heptachlor	3.5
Triton X-100	14.0
Isopropyl alcohol (99 percent)	82.5

It was a clear liquid that emulsified readily upon being poured into water. Each 100 ml. of formulation contained 3 grams of heptachlor.

Toxicity to Grubs.—Third-instar grubs in the soil of pottted plants were killed within 3 weeks by immersing the soil until saturated in water containing 0.075 gram of heptachlor per liter at 60° F. Dosages of 0.038 and 0.019 grams killed them within 4 weeks. The 0.038-gram dosage was effective in killing the grubs in plant balls up to 16 inches in diameter and in pots up to 14 inches in depth. The 0.019 gram of heptachlor was not always effective in these larger masses of soil. (Fleming et al. unpublished)

The average deposit of heptachlor in the soil after immersion in the 0.038-gram dosage was 0.2 gram per cubic foot. This was equivalent to approximately 5 pounds of heptachlor mixed with the upper 3 inches of an acre of soil. In the field treatments (p. 108) 3 pounds of heptachlor per acre were effective in killing newly hatched grubs after the chemical had weathered for 4 years. It would be expected that the residue from the dip treatment would be effective in killing newly hatched grubs for that period of time. (Fleming et al. unpublished)

Reaction of Plants.—In preliminary tests *Pelargonium* sp. was not injured by immersing the soil of the potted plants until saturated in water containing 0.15 gram of heptachlor per liter, the highest dosage used. The 0.038-gram dosage did not affect *Hedera helix, Hydrangea arborescens, H. paniculata, Nephrolepis exaltata, Rhododendron indicum,* or *R. obtusum.* Other plants were treated successfully with the 0.038-gram dosage in commercial nurseries and greenhouses, but information on the varieties used is not now available. (Fleming et al. unpublished)

Use in Nurseries.—Water containing 0.038 gram of emulsified heptachlor per liter was authorized in 1960 for the treatment of balled and potted plants. Plant balls not more than 16 inches in diameter or more than 14 inches in depth were immersed in the dip until saturated and then held in a compact mass for 2 weeks at not less than 60° F. before being certified for shipment. The treatment could be applied at any time of the year. The treated plants in pots could be in a certified status for 4 years.

Lindane

Lindane contains more than 99 percent of the gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane. It is a white crystalline solid, soluble in most of the common organic solvents but insoluble in water. (Bowen and Hall *1952*)

Formulation.—Chisholm (unpublished) prepared a laboratory formulation of the following composition:

Lindane	3 grams
Triton X–100	12 grams
Acetone to make	100 ml.

It was a clear liquid that emulsified readily when poured into water.

Toxicity to Grubs.—When the soil of potted plants was immersed until saturated at 60° F. in water containing 0.15 gram of emulsified lindane per liter, the highest dosage tested, all the third-instar grubs did not die during the 5 weeks following the treatment. In view of this result, lindane did not appear to be promising for eliminating the grubs in the soil of potted plants. (Fleming et al. unpublished)

Insecticide Emulsions and Solutions Applied to Soil Surface

Beginning in 1924 many insecticide emulsions and solutions were applied to the soil surface of potted plants, nursery beds before planting, and established nursery plots before digging the plants to destroy the grubs in the soil. Many of these treatments did not kill all the grubs or they severely injured the plants. The application of dilute carbon disulfide emulsion was the first authorized field treatment. It was for many years the only method for treating balled nursery stock before digging the plants.

Carbon Disulfide

Formulations.—The formulations of concentrated carbon disulfide emulsions used in these experiments are given on pages 66–69.

Potted Plants.—Fleming and Baker (*1935*) found that third-instar grubs in the soil of potted plants were killed without seriously injuring the plants by pouring a dilute emulsion of carbon disulfide on the soil surface and allowing it to percolate through the soil. A consistent mortality of the grubs was obtained only when a volume of the dilute emulsion equivalent to the volume of soil in the pots was used.

The dosage of carbon disulfide to kill the grubs in 8-inch pots of clay loam within 7 days was modified by the temperature. The dosages per liter of water and the temperature required were as follows:

Grams	° *F.*
1.1	32
.95	40
.75	50
.62	60
.46	70

The dosage for clay loam at 60° F. was effective in a mixture of clay loam and peat containing 25 percent of peat by volume, but a further increment in the proportion of peat required an increase in the dosage. The dosage had to be increased to 0.75 gram per liter to kill grubs in a 1:1 mixture of soil and peat, 0.95 gram in a 1:3 mixture, and 1.1 grams in peat alone. It appeared that the treatment should be limited to soil containing not more than 25 percent of organic matter at not less than 60°.

The treatment was effective in the soil of potted plants where the natural drainage was well established. It was not satisfactory in the soil of recently potted plants because the penetration of the insecticide liquid into the soil was slow and the soil usually remained waterlogged for several days. Plants were injured when the drainage was inadequate. The treatment was also unsatisfactory when the roots of the plants were potbound because that condition made the soil almost impervious to the proper penetration of liquid.

In 1924 a commercial nursery had 1,500 *Cibotium schiedeii* and 5,000 *Hydrangea macrophylla* plants in pots and tubs that could not be certified because the soil had been exposed to infestation. Preliminary tests indicated that the 0.62-gram dosage at 60° F. was not injurious to either species. A dosage of 0.95 gram killed the ferns, but 1.25 grams did not injure the hydrangeas seriously. Temporary authorization was given to treat these plants with dilute carbon disulfide emulsion.

The ferns and hydrangeas were treated with 0.62 gram at 60° F., using a volume of the insecticide liquid equivalent to the volume of soil in the pots and tubs. The average time to apply the liquid was 6 hours, but the drainage was so poor in a few pots and tubs that all the dilute emulsion could not be applied within 24 hours. The treatment was considered to be satisfactory in 95 percent of the pots and tubs where the required volume of the emulsion was taken up by the soil in not less than 3 hours or

more than 12 hours. After holding for 48 hours at 60° to assure destruction of the grubs, 95 percent of the plants were certified for shipment.

The application of dilute carbon disulfide emulsion to the soil surface of plants in pots and tubs was not approved for general use in greenhouses. The treatment was considered to be too slow and expensive to be practical.

Treating Plants Before Digging in Field.—Preliminary experiments in 1923 (Leach et al. *1924*) demonstrated that the application of a dilute emulsion of carbon disulfide to the soil around the plants before digging was effective in killing grubs in the soil among the roots without seriously injuring the plants. Leach and Fleming (*1927*) in 1924 developed a procedure for applying the dilute emulsion.

In preparing a plant for treatment, the trash and the weeds were removed and the surface of the ground was leveled to permit uniform coverage with the dilute emulsion. The lower branches were tied up to prevent the foliage from coming into contact with the insecticide. Then a strip of galvanized iron, 9 inches wide and the necessary length, was bent around the base of the plant, with the ends overlapping, to enclose an area 6 to 12 inches greater in diameter than the mass of soil to be dug with the plant. The strip was forced into the ground to a depth of 3 inches, and the soil was compacted on both sides of the metal to confine the emulsion.

The dilute emulsion was poured at 1.8 gallons (6.8 liters) per square foot into the enclosed area. The dosage was modified according to the temperature of the soil at a depth of 6 inches as follows:

Temperature (° F.) of soil	*Carbon disulfide (grams) per liter of water*
40	1.00
45	.94
50	.86
55	.80
60	.72
65	.57

The quantity of carbon disulfide applied per square foot of soil surface ranged from 6.9 grams at 40° to 3.9 grams at 65°. The collar and the treated soil were not disturbed for 48 hours. Then the plant could be dug and the soil mass wrapped with burlap or similar material for shipment.

The procedure was authorized in 1924. The effectiveness of the treatment on each batch of plants was determined by the

mortality of the grubs in a certain proportion of the plants that were infested artificially before applying the insecticide liquid. The treatment was not satisfactory under the following conditions: (1) A high water table and inadequate drainage, (2) hillsides where a level basin about a plant could not be obtained, (3) fabricated soils with a high content of peat, (4) ground tunneled by moles and rodents, and (5) recently transplanted plants. When the soil conditions in the nurseries appeared to be favorable, the treatment was effective in killing all the grubs within the upper 6 inches of soil around the roots of about 95 percent of the plants.

Fleming and Baker (*1935*) improved the effectiveness of the treatment to practically 100 percent under favorable conditions by increasing the volume and the dosage of the dilute emulsion applied to the soil. The volume was increased to 2.4 gallons (9 liters) per square foot and the dosage was increased as follows:

Temperature (° F.) of soil	*Carbon disulfide (grams) per liter of water*
40	1.15
45	1.05
50	.95
55	.85
60	.75
65	.65

The quantity of carbon disulfide applied per square foot of soil surface with this modified treatment ranged from 10.4 grams at 40° to 5.9 grams at 65°.

The modified treatment was authorized in 1929. The volume of water, however, was increased to 2.5 gallons per square foot of soil surface to facilitate measuring in the field. The application of the insecticide liquid was considered satisfactory when not less than 10 minutes or more than 5 hours elapsed before all the liquid disappeared from the surface of the soil. Fleming and Baker (*1935*) found that there was no change in the insecticide action when the insecticide liquid was taken up by the soil within that period, but as the time that the emulsion remained on the surface of the soil was prolonged for more than 5 hours, the mortality of the grubs was progressively reduced. To standardize the treatment in the commercial nurseries, the plants had to be dug within 2 to 5 days after applying the emulsion.

The treatment with carbon disulfide in the field was applied in commercial nurseries to many varieties of plants with apparently no more injury to the plants than is normally caused by transplanting. It was the general experience that healthy established

plants, treated in the early spring or in the fall while dormant or semidormant, tolerated the treatment. Injury was caused when the plants were growing actively.

In preliminary tests in the spring and in the fall when the plants were not growing vigorously, Fleming and Baker (*1935*) treated the following species successfully:

Abies concolor
Buxus sempervirens
Chamaecyparis pisifera
Juniperus chinensis
Juniperus communis
Juniperus excelsa
Hydrangea macrophylla
Lonicera sp.
Picea abies
Picea pungens
Pinus strobus
Pinus sylvestris
Rhododendron catawbiense
Rhododendron obtusum
Rosa wichuraiana
Syringa vulgaris
Taxus canadensis
Taxus cuspidata
Thuja occidentalis
Thuja orientalis
Tsuga canadensis

Johnson (unpublished report cited by Fleming and Baker *1935*) stated that the following plants had been treated in the commercial nurseries as a basis for certification:

Abelia chinense
Abelia grandiflora
Abies alba
Abies cephalonica
Abies cilicica
Abies concolor
Abies homolepis
Abies lasiocarpa
Abies nordmanniana
Abies veitchi
Acer ginnala
Acer japonicum
Acer palmatum
Acer platanoides
Acer saccharinum
Aesculus hippocastanum
Amelanchier sp.
Betula populifolia
Buxus microphylla
Buxus sempervirens
Calluna vulgaris
Carpinus betulus
Carpinus caroliniana
Cedrus atlantica
Cedrus libanensis
Cercis canadensis
Cercis chinensis
Chaenomeles japonica
Chamaecyparis nootkatensis
Chamaecyparis obtusa
Chamaecyparis pisifera
Chamaecyparis thyoides
Chionanthus virginicus
Cornus alba
Cornus florida
Cornus kousa
Cornus mas
Cornus racemosa
Cornus stolonifera
Corylopsis pauciflora
Corylus americana
Cotoneaster francheti
Cotoneaster horizontalis
Cotoneaster rotundifolia
Crataegus crus-galli
Crataegus intricata
Crataegus mollis
Crataegus oxyacantha
Crataegus phaenopyrum
Crataegus punctata
Cryptomeria japonica
Daphne cneorum
Enkianthus campanulatus
Enkianthus perulatus
Euonymus alatus
Euonymus americanus
Euonymus japonicus
Fagus grandifolia

Fagus sylvatica
Fatsia japonica
Forsythia intermedia
Forsythia suspensa
Forsythia viridissima
Ginkgo biloba
Hibiscus syriacus
Ilex aquifolium
Ilex crenata
Ilex glabra
Ilex opaca
Ilex serrata
Juglans sp.
Juniperus chinensis
Juniperus excelsa
Juniperus horizontalis
Juniperus procumbens
Juniperus sabina
Juniperus scopulorum
Juniperus squamata
Juniperus virginiana
Kalmia latifolia
Kerria japonica
Koelreuteria paniculata
Larix decidua
Larix leptolepis
Leucothoe catesbaei
Ligustrum obtusifolium
Ligustrum ovalifolium
Lindera benzoin
Liquidambar styraciflua
Magnolia acuminata
Magnolia cordata
Magnolia kobus
Magnolia liliflora
Magnolia macrophylla
Magnolia soulangeana
Magnolia stellata
Magnolia tripetala
Magnolia virginiana
Mahonia aquifolium
Mahonia bealei
Malus baccata
Malus coronaria
Malus dawsoniana
Malus floribunda
Malus ioensis
Malus pumila
Malus sargenti
Malus scheideckeri
Malus spectabilis
Myrica cerifera
Nyssa sylvatica
Oxydendrum arboreum
Paulownia tomentosa
Philadelphus coronarius
Phillyrea decora
Photinia villosa
Picea abies
Picea bicolor
Picea engelmanni
Picea glauca
Picea omorika
Picea orientalis
Picea polita
Picea pungens
Pieris floribunda
Pieris japonica
Pinus banksiana
Pinus cembra
Pinus densiflora
Pinus flexilis
Pinus mugo
Pinus nigra
Pinus parviflora
Pinus peuce
Pinus ponderosa
Pinus resinosa
Pinus strobus
Pinus sylvestris
Pinus thunbergi
Platanus orientalis
Prunus avium
Prunus cerasifera
Prunus persica
Prunus sargentii
Prunus serrulata
Prunus sieboldii
Pseudosasa japonica
Pseudotsuga taxifolia
Pyracantha coccinea
Quercus falcata
Quercus palustris
Quercus robur
Rhododendron arborescens
Rhododendron calendulaceum
Rhododendron carolinianum
Rhododendron catawbiense
Rhododendron indicum
Rhododendron japonicum
Rhododendron luteum
Rhododendron maximum

Rhododendron molle
Rhododendron mucronatum
Rhododendron nudiflorum
Rhododendron obtusum
Rhododendron schippenbachi
Rhododendron vaseyi
Rhododendron viscosum
Rhododendron yedoense
Rhus sp.
Sciadopitys verticillata
Styrax japonica
Symphoricarpos orbiculatus
Syringa amurensis
Syringa chinensis
Syringa vulgaris
Taxodium distichum
Taxus baccata
Taxus canadensis
Taxus cuspidata
Thuja occidentalis
Thuja orientalis
Thuja standishi
Tilia europaea
Tsuga canadensis
Tsuga caroliniana
Tsuga diversifolia
Tsuga sieboldii
Ulmus americana
Ulmus procera
Viburnum carlesii
Viburnum cassinoides
Viburnum dilatatum
Viburnum lentago
Viburnum molle
Viburnum opulus
Viburnum prunifolium
Viburnum sieboldii
Wistaria frutescens
Wistaria sinensis

Use in Nurseries.—The carbon disulfide field treatment was not well adapted to the requirements of wholesale nurseries because it entailed largely the application of the insecticide to individual plants. In spite of this limitation, 71,721 plants were treated for certification during 1925–30 (Johnson unpublished). During the next 10 or 15 years an additional 190,918 plants were treated (Middleton and Cronin *1952*).

Aldrin

The preparation of an emulsifiable mixture containing 3.6 percent of technical aldrin by weight is given on page 85.

Potted Plants.—Third-instar grubs in friable soil of potted plants at 60° F. were killed by pouring on the soil surface a volume of dilute aldrin emulsion equivalent to one-fifth the volume of the soil. Water containing 0.075 gram of aldrin per liter killed all the grubs within 2 weeks. The 0.038- and 0.019-gram dosages killed them within 3 and 4 weeks, respectively. The treatment was effective in masses of soil up to 14 inches in depth.

The average deposit of aldrin per cubic foot of soil was 0.4, 0.2, and 0.1 grams with the 0.075-, 0.038-, and 0.019-gram dosages, respectively. Aldrin mixed with soil at the rate of 0.12 gram per cubic foot was effective in killing newly hatched grubs after the soil had weathered for 4 years. It would be expected that the 0.038-gram dosage would be effective against first-instar grubs for at least that period of time.

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PREVENTING JAPANESE BEETLE DISPERSION BY FARM PRODUCTS AND NURSERY STOCK
FLEMING, W.E. 2 OF 3

In preliminary tests the application of 0.15 gram of aldrin to the soil of potted *Pelargonium* sp. had no effect on the subsequent growth of the plants. Tests in nurseries and greenhouses showed that *Hedera helix, Hydrangea arborescens, H. paniculata, Nephrolepis exaltata, Rhododendron indicum,* and *R. obtusum* were not injured by the 0.038-gram dosage. Other plants were treated successfully with the 0.038-gram dosage in commercial nurseries and greenhouses, but information on the varieties used is not now available. (Fleming et al. unpublished)

The treatment of potted plants at not less than 60° F. by pouring water containing 0.038 gram of emulsified aldrin on the soil surface was authorized in 1959. A volume of the insecticide liquid equivalent to one-fifth the volume of the soil was required. The treatment could be used on friable soil not more than 14 inches in depth. The plants could be certified for shipment 14 days after the application of the dilute emulsion. There was a possibility that the plants in the treated soil could be in a certified status for 4 years, but the longevity of the certified period would be determined by bioassays.

Bedrench

Bedrench is an emulsifiable commercial mixture containing 81 percent of allyl alcohol and 11.5 percent of ethylene dibromide. As a preplanting treatment to control weed seeds, nematodes, soil insects, and fungi, the manufacturer recommended mixing 1.5 gallons of the formulation with 100 gallons of water and applying 1 gallon of the diluted formulation per square yard of fallow soil when the soil temperature was not lower than 40° F.

Fleming and Tashiro (unpublished) found that the treatment recommended by the manufacturer killed third-instar grubs within 2 weeks at 40° F. A dilution of 0.75 gallon with 100 gallons of water applied in the same manner killed the grubs within 1 week at 70°.

The preplanting treatment with Bedrench as recommended by the manufacturer was authorized in 1958 as a basis for certifying plants grown in the treated soil. When Bedrench was applied, the soil had to be at least 40° F.; it had to be in good tilth and prepared for planting. It could not be disturbed for at least 2 weeks. The nurseryman was to follow the recommendation of the manufacturer in planting the treated area. The plants in the treated soil could remain in a certified status until the soil was exposed to oviposition by the adult Japanese beetle.

Cresol

Mason (unpublished) diluted an emulsifiable formulation containing 4 pounds of cresol per gallon 1:50 with water and applied 500 ml. of this dilution per square foot to the soil of potted azaleas at approximately 70° F. The cresol was applied at 4.67 grams per square foot. The treatment killed only 44 percent of the third-instar grubs within 3 weeks.

D-D

D-D contains approximately two parts of 1,3-dichloropropene, one part of 1,2-dichloropropane, and one part of associated chlorinated hydrocarbons (Mason and Chisholm unpublished).

Formulations.—Chisholm (unpublished) prepared emulsifiable formulations by dissolving Tween 20, a polyoxyalkylene derivative of sorbitan monolaurate, in D-D. One formulation contained 5 pounds of D-D and 0.25 pound of Tween 20 per gallon and the other 9.5 pounds of D-D and 0.5 pound of Tween 20. The first formulation contained 60 grams of the mixture in 100 ml. and the second 113 grams. Both formulations were clear liquids. They emulsified by shaking with an equal volume of water. These emulsions were diluted readily with water.

Potted Plants.— A dosage of 1.5 grams of emulsified D-D in 500 ml. of water per square foot of soil surface killed 98 percent of the third-instar grubs in the soil of the potted plants within 3 weeks. A 3-gram dosage killed all of them. Eggs did not hatch in soil to which the 1.5 grams were applied. Potted plants did not tolerate the application of D-D to the soil. The 1.5-gram dosage caused some injury to *Rhododendron* sp. and it injured severely or killed *Pandanus* sp. and *Vitis* sp. (Mason and Chisholm unpublished)

Fallow Land.—The application of 0.1 pound of emulsified D-D in 2 gallons of water per square yard, or 484 pounds in 9,680 gallons of water per acre, to fallow land in September eliminated a grub population of 35 per square foot within 3 weeks. Most of the grubs were within 4 inches of the soil surface, but some were 9 inches deep. (Mason and Chisholm unpublished)

When D-D was applied to fallow soil in mid-May and the third-instar grubs were removed from the soil 5 days later for observation, 7.5 pounds of emulsified D-D in 200 gallons of water per 1,000 square feet, or 327 pounds in 8,712 gallons of water per acre, killed 97 percent of the grubs within the upper 2 inches of soil and all of them 6 to 16 inches below the surface.

A month later 2.5 pounds of the mixture in 100 gallons of water, or 109 pounds in 4,356 gallons of water per acre, killed all grubs to a depth of 9 inches, and that dosage in 200 gallons of water was effective to a depth of 16 inches. (Mason et al. unpublished)

Pupae were more resistant than third-instar grubs. The application of 7.5 pounds of the emulsified mixture in 200 gallons of water per 1,000 square feet killed 86 percent of the pupae removed from the soil 5 days after treatment and 98 percent of them left in the soil to complete their transformation to adult beetles (Mason et al. unpublished).

The possibilities of using emulsified D-D to treat fallow land were not explored further.

o-Dichlorobenzene

Burgess et al. (unpublished) prepared an emulsifiable *o*-dichlorobenzene ($C_6H_4Cl_2$) of the following composition:

	Percent by weight
o-Dichlorobenzene	60.0
Oleic acid	17.5
Potassium hydroxide	2.5
Ethyl alcohol (95 percent)	14.0
Water	6.0

It was a clear liquid with a specific gravity of 1.1111 at 25° C. It formed an emulsion upon dilution with water. Each 100 ml. of the formulation contained 66.7 grams of *o*-dichlorobenzene.

When one-fifth gallon (775 ml.) of dilute emulsion per square foot was applied to the soil of potted plants at 57° F. and the grubs were removed from the soil 1 week later for observation, 25.8 grams of emulsified *o*-dichlorobenzene per square foot killed all third-instar grubs during the following 2 days, and 12.9 grams killed them during the following 2 weeks. A dosage of 6.45 grams was not completely effective. The quantity of *o*-dichlorobenzene needed to kill the grubs made the treatment impractical. The 12.9 dosage is equivalent to 1,240 pounds of *o*-dichlorobenzene per acre.

p-Dichlorobenzene Plus Benzene

Burgess et al. (unpublished) dissolved *p*-dichlorobenzene ($C_6H_4Cl_2$) in benzene and prepared an emulsifiable formulation of the following composition:

	Percent by weight
p-Dichlorobenzene	25.0
Benzene	35.0
Oleic acid	17.5
Potassium hydroxide	2.5
Ethyl alcohol (95 percent)	14.0
Water	6.0

It was a clear liquid with a specific gravity of 0.942 at 25° C. It formed an emulsion upon dilution with water. Each 100 ml. of the formulation contained 23.6 grams of *p*-dichlorobenzene and 33 grams of benzene.

When one-fifth gallon (775 ml.) of dilute emulsion per square foot was applied to the soil of potted plants at 57° F. and the third-instar grubs were removed 1 week later for observation, the 18.3 grams of *p*-dichlorobenzene and 25.6 grams of benzene per square foot killed all the grubs during the following 2 weeks. The mortality was not complete when the dosages were reduced to 9.2 and 12.8 grams, respectively. When two-fifths gallon of dilute emulsion was applied, the mortality with the 18.3–25.6 grams was accelerated; all grubs were dead within 1 week after removal from the treated soil.

p-Dichlorobenzene Plus Ethylene Dichloride

Burgess et al. (unpublished) also dissolved *p*-dichlorobenzene in ethylene dichloride and prepared an emulsifiable formulation of the following composition:

	Percent by weight
p-Dichlorobenzene	12.50
Ethylene dichloride	60.00
Oleic acid	12.00
Potassium hydroxide	1.75
Ethyl alcohol (95 percent)	9.75
Water	4.00

It was a clear liquid with a specific gravity of 1.130 at 25° C. It formed an emulsion upon dilution with water. Each 100 ml. of the formulation contained 14.1 grams of *p*-dichlorobenzene and 67.8 grams of ethylene dichloride.

When one-fifth gallon (775 ml.) of dilute emulsion per square foot was applied to the soil of potted plants at 55° F. and the third-instar grubs were removed 1 week later for observation, the 10.9 grams of *p*-dichlorobenzene and 52.5 grams of ethylene dichloride per square foot killed all the grubs during the follow-

ing 2 weeks, but the mortality was not complete with 5.4 grams of *p*-dichlorobenzene and 26.2 grams of ethylene dichloride per square foot. All grubs were killed within 2 days after removal from soil by the 5.4–26.2 grams when the dilute emulsion was applied at one-fifth gallon per cubic foot of soil.

Dichloroethyl Ether

Chisholm (unpublished) prepared an emulsifiable formulation containing about 2 pounds of dichloroethyl ether per gallon. Each 100 ml. of the formulation contained 25 grams of the chemical. The formulation emulsified when mixed with water.

Mason (unpublished) applied several dilutions of the formulation to the soil of potted plants at 500 ml. per square foot of soil surface. Five grams of emulsified dichloroethyl ether per square foot killed all third-instar grubs and 2.5 grams killed 92 percent. However, 6.2 grams killed only 85 percent of the pupae.

The application of 2 gallons of water containing 37.8 grams of emulsified dichloroethyl ether per square yard of fallow land in May killed 96 percent of the third-instar grubs in the upper 4 inches of soil within 3 weeks. The mortality was 46, 21, and 6 percent at a depth of 9, 12, and 16 inches, respectively. (Mason unpublished)

The 37.8-gram treatment used 3 days before planting prevented the germination of lettuce and caused poor stands of beets, corn, lima beans, okra, string beans, and Swiss chard. Carrots, squash, and turnips germinated normally. The preplanting treatment had no effect on young plants of broccoli, cabbage, cauliflower, eggplant, sweetpotato, and tomato. The treatment used 1 month after planting caused some injury to most of the plants. (Mason unpublished)

Dichloroethyl Formal

Chisholm (unpublished) prepared an emulsifiable formulation containing about 2 pounds of dichloroethyl formal (bis(2-chloroethoxy)methane) per gallon. Each 100 ml. of the formulation contained 25 grams of the chemical. It emulsified when mixed with water.

The application of 500 ml. of water containing 5 grams of emulsified dichloroethyl formal per square foot to the soil of potted plants killed all third-instar grubs within 3 weeks. A 2.5- and a 1.25-gram dosage killed 68 and 48 percent, respectively, of the grubs. (Mason unpublished)

Ethylene Dibromide

Formulations.—The preparation of miscible formulations of ethylene dibromide is discussed on pages 77–78.

Potting Soil.—Mason (unpublished) applied 3 grams of emulsified ethylene dibromide in 1 gallon of water per square yard to the surface of friable soil in a bin at between 40° and 75° F. The rate of insecticide action increased as the temperature was raised. An exposure of 24 hours killed all third-instar grubs to a depth of 13 inches in a loam and 12 inches in a 3:1 mixture of loam and peat, but it did not kill all the grubs in the upper 12 inches of mixtures containing higher proportions of peat. Prolonging the exposure for 48 hours killed all grubs to a depth of 19 inches in the loam and 17 inches in the 3:1 mixture. The mortality in mixtures containing higher porportions of peat was variable. All the grubs were not dead at the end of these exposures, but those alive at that time died during the following 3 weeks.

An exposure of 24 hours in soil treated with 4 grams of emulsified ethylene dibromide in 2 gallons of water per square yard killed grubs to a depth of 13 inches in a 1:1 loam-peat mixture, 12 inches in a 1:3 mixture, and 9 inches in peat. When the exposure was prolonged for 48 hours, all grubs were killed to a depth of 21 inches in the 1:1 mixture and 19 inches in the 1:3 mixture and peat.

These treatments were effective in killing eggs and pupae in the soil.

The treatment of friable potting soil with emulsified ethylene dibromide was authorized in 1947 for use throughout the year at between 40° and 75° F. The treatment was used on 1-foot layers of soil as the bin was filled, applying 3 grams of emulsified ethylene dibromide in 1 gallon of water per square yard of surface for soil containing not more than 25 percent of organic matter and 4 grams in 2 gallons of water per square yard for soils with a higher content of organic matter. The soil could be certified 48 hours after the treatment was used. The soil should be well aerated before using it for potting plants. Certification of the soil continued until it was exposed to reinfestation.

During 1947–50 the commercial nurseries treated 242 cubic yards of soil with emulsified ethylene dibromide (Middleton and Cronin *1952*).

Potted Plants.—Mason (unpublished) applied 500 ml. of water containing 0.052 gram of emulsified ethylene dibromide per square foot to the soil surface of potted plants at between 40°

and 75° F. and killed all the third-instar grubs within 2 weeks. The 0.026-gram dosage was effective against the grubs within 3 weeks at the higher temperatures. In preliminary tests *Gardenia* sp., *Geranium* sp., *Pandanus australis*, and *Rhododendron obtusum* were not affected by the 0.052-gram dosage.

The treatment of potted plants by pouring on the soil surface water containing 0.5 gram of emulsified ethylene dibromide per gallon was authorized in 1947. Initially the dilute emulsion was applied at 500 ml. per square foot of soil surface, but later a volume of the emulsion equivalent to one-fifth the volume of the soil was used in the treatment of pots and tubs with soil not more than 14 inches in depth. The treatment was restricted to the period of the year when only grubs were in the soil. The plants could be certified for shipment 24 hours after application of the emulsion. The certification of the plants continued until the soil was exposed to reinfestation by the adult Japanese beetle. Information is not available on the number of potted plants in the commercial nurseries treated by pouring a dilute emulsion of ethylene dibromide on the soil surface.

Nursery Beds and Plots.—Two grams of emulsified ethylene dibromide applied in 1 gallon of water per square yard of soil surface (20 pounds in 4,840 gallons per acre) usually killed all third-instar grubs in planted or unplanted beds and plots when the soil was a sandy loam or a clay loam, but occasionally the treatment was not completely effective. The grubs were killed consistently in these soils at 40° to 75° F. by increasing the dosage to 3 grams per gallon. Four grams per gallon per square yard were not completely effective in killing grubs in peat and in soils high in organic matter, but this dosage in 2 gallons of water per square yard did kill all the grubs. (Chisholm and Mason *1948a*; Mason et al. unpublished)

The treatment of planted and unplanted nursery beds and plots with a dilute emulsion of ethylene dibromide was authorized in 1947. The dilute emulsion could be applied in the spring or the fall when only grubs were in the soil and the soil temperature was between 40° and 75° F. Usually 3 grams of emulsified ethylene dibromide in 1 gallon of water were applied per square yard of soil surface, but soils high in organic matter and peat required 4 grams of the chemical in 2 gallons of water per square yard of surface. The treated beds and plots were continued in a certified status until exposed to reinfestation by the adult beetle. The plants could be certified for shipment 24 hours after the dilute emulsion was applied.

During 1947–50 emulsified ethylene dibromide was applied to 102,999 square feet of beds and plots in commercial nurseries (Middleton and Cronin *1952*).

Ethylene Dibromide-Aldrin

Chisholm (unpublished) prepared a miscible formulation of ethylene dibromide and aldrin of the following composition:

	Percent by weight
Ethylene dibromide	13.0
Aldrin (technical)	6.5
Cellosolve (ethylene glycol monoethyl ether)	6.5
Tween 20 (a polyoxyalkylene derivative of sorbitan monolaurate)	6.5
Isopropyl alcohol (99 percent)	67.5

The ingredients were mixed in the order given to form a clear, dark mixture. Each 100 ml. of the formulation contained 12 grams of ethylene dibromide and 6 grams of aldrin. The mixture was diluted by pouring into water. In most of the dilutions used, the ethylene dibromide was in solution and the aldrin was dispersed as an emulsion of very small particles.

Mason (unpublished) applied 2 grams of emulsified ethylene dibromide and 1 gram of emulsified aldrin in 1 gallon of water per square yard (20 and 10 pounds, respectively, in 4,840 gallons per acre) to the soil of fallow plots in October. The soil temperature during the following 21 days ranged from 48° to 61° F. The third-instar grubs were removed periodically from the treated soil for observation. An exposure of 2 days was sufficient for the grubs to obtain a toxic dosage. The mortality ranged from 91 to 99 percent 21 days after the treatment was used. This was the level of mortality with 2 grams of ethylene dibromide under these conditions, indicating that the aldrin had contributed little to the velocity of insecticide action. The ethylene dibromide would be dissipated within a few weeks, but the residue of aldrin would persist for several years. The longevity of the insecticide action of aldrin in these plots was not investigated.

This treatment was used on cultivated soil in which eggs had been deposited. When the eggs were removed from the treated soil 1 week after the application, 99 to 100 percent of them did not hatch. Eggs deposited subsequently in the treated soil during the remainder of the summer were killed within 2 weeks.

Ethylene Dibromide-Chlordane

Formulation.—The preparation of miscible mixtures containing ethylene dibromide and chlordane is discussed on pages 82–83.

Potting Soil.—The addition of chlordane did not accelerate the insecticide action of ethylene dibromide against third-instar grubs. When the ethylene dibromide-chlordane formulation was diluted with water and 1 gallon of the dilute emulsion was applied per square yard of surface to 1-foot layers of friable soil in a bin, 3 grams of ethylene dibromide and 1.5 grams of chlordane per square yard killed the grubs within 3 weeks at 40° to 75° F. in soils containing not more than 25 percent of organic matter. A dosage of 4 grams of ethylene dibromide and 2 grams of chlordane in 2 gallons of water per square yard was required to kill the grubs in soils containing more organic matter. An exposure of 48 hours was sufficient for the grubs to obtain a toxic dosage (Mason unpublished). The dosages of ethylene dibromide with the ethylene dibromide-chlordane formulation are the same as those with ethylene dibromide alone.

The ethylene dibromide-chlordane formulation applied at these rates was authorized in 1960 as a substitute for the ethylene dibromide treatment of potting soil, largely as a convenience to nurseries that had this formulation but not the ethylene dibromide formulation. The potential residual action of chlordane in the soil was not considered in the authorization.

Potted Plants.—Fleming and Maines (unpublished) applied 0.052 gram of emulsified ethylene dibromide and 0.026 gram of emulsified chlordane in 500 ml. of water per square foot to the soil of potted plants at 65° to 75° F. All the grubs were dead within 5 days in the 2- and 3-inch pots, but an exposure of 7 days was required to kill all of them in the 4-, 5-, and 6-inch pots, and an exposure of 14 days was needed to kill all of them in the 8- and 11-inch pots. Applying the dilute emulsion according to the area of the soil surface did not make proper adjustment in the dosage for the depth of the soil.

When water containing 0.5 gram of emulsified ethylene dibromide and 0.25 gram of emulsified chlordane per gallon was applied at a rate equivalent to one-fifth the volume of soil in the pots, the dosage per square foot of soil surface varied with the depth as follows:

Depth of soil (inches)	Amount per square foot of soil surface (grams)	
	Ethylene dibromide	Chlordane
1	0.059	0.030
2	.118	.059
3	.177	.089
4	.236	.118
6	.354	.177
8	.472	.236
10	.590	.295
12	.708	.354
14	.826	.413

With this modification in the application of the insecticide liquid, the mortality of the third-instar grubs progressed at about the same rate in the different masses of soil.

The ethylene dibromide-chlordane formulation was authorized in 1960 as a substitute for the ethylene dibromide formulation for the treatment of potted plants, largely as a convenience to nurseries that had this formulation and not the ethylene dibromide formulation. Only the fumigating action of the formulation was considered because no information was available on the persistence of chlordane in masses of soil varying in volume from 6 to over 2,000 cubic inches.

Nursery Plots.—In July Mason (unpublished) applied 2 grams of emulsified ethylene dibromide and 1 gram of emulsified chlordane in 1 gallon of water per square yard (20 and 10 pounds, respectively, in 4,840 gallons per acre) to cultivated soil in which eggs had been deposited. When the eggs were removed from the treated soil 1 week after the application, 99 percent did not hatch. Eggs deposited subsequently in the treated soil during the remainder of the summer were killed within 2 weeks.

This treatment was used on cultivated plots of sandy loam in October. The soil temperature during the following 3 weeks at a depth of 3 inches ranged from 48° to 61° F. At the end of that period 98 percent of the third-instar grubs in the soil were dead or moribund. All the grubs were dead when 3 grams of emulsified ethylene dibromide and 1.5 grams of emulsified chlordane were applied per square yard.

A dosage of 3 grams of ethylene dibromide and 1.5 grams of chlordane in a gallon of water per square yard killed third-instar grubs within 3 weeks at 40° to 75° F. in soils containing not more than 25 percent of organic matter. A dosage of 4 grams of ethylene dibromide and 2 grams of chlordane in 2 gallons of water per square yard was required to kill the grubs in soils

containing more organic matter.

The application of water containing emulsified ethylene dibromide and emulsified chlordane to nursery plots was authorized in 1956. The dosage was 3 grams of ethylene dibromide and 1.5 grams of chlordane in 1 gallon of water per square yard for soils with less than 25 percent organic matter and 4 grams of ethylene dibromide and 2 grams of chlordane in 2 gallons of water per square yard for soils containing more organic matter. There was no seasonal limitation on this treatment. The soil had to be friable and between 40° and 75° F. The treated plots could be in a certified status until exposed to reinfestation by the adult beetle, unless as soon as the soil was workable after a 24-hour exposure period, the insecticide was mixed thoroughly with the upper 3 inches of soil. This operation was to assure uniform distribution of the chlordane in this layer of soil. Then the treated plot could be in a certified status for possibly 4 years.

Ethylene Dibromide-Dieldrin

Chisholm (unpublished) prepared a miscible formulation of ethylene dibromide and dieldrin of the following composition:

	Percent by weight
Ethylene dibromide	13.0
Dieldrin (technical)	6.5
Cellosolve (ethylene glycol monoethyl ether)	6.5
Tween 20 (a polyoxyalkylene derivative of sorbitan monolaurate)	6.5
Isopropyl alcohol (99 percent)	67.5

The ingredients were mixed in the order given to form a clear, dark mixture. Each 100 ml. of the formulation contained 12 grams of ethylene dibromide and 6 grams of dieldrin. The mixture was diluted by pouring into water. In most of the dilutions used, the ethylene dibromide was in solution and the dieldrin was dispersed as an emulsion of very small particles.

Mason (unpublished) applied 2 grams of emulsified ethylene dibromide and 1 gram of emulsified dieldrin in 1 gallon of water per square yard (20 and 10 pounds, respectively, in 4,840 gallons per acre) to the soil of fallow plots in October. The soil temperature during the following 21 days ranged from 48° to 61° F. The third-instar grubs were removed periodically from the treated soil for observation. An exposure of 2 days was sufficient for the grubs to obtain a toxic dosage. The mortality ranged from 98 to 100 percent 21 days after the application. This was the level of mortality with 2 grams of ethylene dibromide under these conditions, indicating that the dieldrin had contributed little to

the velocity of the insecticide action. The longevity of the insecticide action of dieldrin in these plots was not investigated.

This treatment was used on cultivated soil in which eggs had been deposited. When the eggs were removed from the treated soil 1 week after the treatment, 98 to 100 percent did not hatch. Eggs deposited subsequently in the treated soil during the remainder of the summer were killed within 2 weeks.

Ethylene Dichloride

Formulation.—The preparation of miscible formulations of ethylene dichloride is discussed on page 73.

Potting Soil.—Applying 45 grams of emulsified ethylene dichloride in 2 gallons of water per square yard to potting soil in a bin killed all third-instar grubs within the upper 12 inches of soil in 2 weeks at 70° F., but the mortality decreased progressively with increased depth. It was only 15 percent 36 inches below the surface. (Mason unpublished)

Potted Plants.—A dosage of 3 grams of emulsified ethylene dichloride in 500 or 1,000 ml. of water per square foot of soil surface killed third-instar grubs in the soil of potted plants. The depth of the soil in the pots and tubs ranged from 4 to 14 inches, but within these limits the depth did not modify the rate of insecticide action. The grubs were all dead within 1 week at 80° F. and within 2 weeks at 60°. The mortality at 40° was 99 percent in 3 weeks when they were last observed. An exposure of 48 hours in the treated soil at 60° was sufficient to kill all the grubs within 2 weeks. The penetration of the emulsion was slower in soil saturated with water than in a moist, friable soil and also in soil with a high organic content than in soil with little organic matter, but these variations did not modify the insecticide action. (Mason and Coles unpublished)

Three grams of emulsified ethylene dichloride in 500 ml. of water per square foot of soil surface did not injure several species of potted plants in commercial greenhouses, including—

Aglaonema simplex
Begonia sp.
Cibotium schiedeii
Cissus sp.
Citrus aurantium
Cyclamen sp.
Dieffenbachia picta
Dracaena fragrans
Gardenia sp.
Genista sp.
Geranium sp.
Howea sp.
Hydrangea sp.
Kalanchoe sp.
Nephrolepis exaltata
Pandanus veitchii
Peperomia sp.
Philodendron cordatum
Rhododendron indicum
Rhododendron obtusum
Solanum sp.

Other species of plants were treated successfully at the commercial greenhouse and nurseries, but information on these species is not now available (Mason and Coles unpublished).

Three grams of emulsified ethylene dichloride in 500 ml. of water per square foot of soil surface was authorized in 1942 as a basis for certifying plants growing in pots and tubs. The treatment was restricted to the period of the year when only grubs were in the soil. The treatment could be used on masses of soil up to 14 inches deep at 45° to 75° F.

Plants in Beds.—Forty five grams of emulsified ethylene dichloride in 2 gallons of water per square yard of soil surface killed third-instar grubs to a depth of 9 inches in planted plots of sandy loam, clay loam, and a mixture of loam and organic matter within 3 weeks, but the treatment killed only 50 percent in the upper 6 inches of a muck. A dosage of 78 grams of ethylene dichloride in 3 gallons of water per square yard killed the grubs to a depth of 12 inches in the muck within 3 weeks. These treatments caused no injury to the plants in the plots. (Mason unpublished; Mason et al. *1943*)

Ethylene Dichloride-*p*-Dichlorobenzene

Chisholm (unpublished) prepared an emulsifiable formulation of ethylene dichloride and *p*-dichlorobenzene of the following composition:

	Percent by weight
Ethylene dichloride	60.00
p-Dichlorobenzene	12.50
Oleic acid	12.00
Potassium hydroxide	1.75
Ethyl alcohol (95 percent)	9.75
Water	4.00

It was a clear liquid with a specific gravity of 1.130 at 25° C. It formed an emulsion when diluted with water. Each 100 ml. of the formulation contained 67.8 grams of ethylene dichloride and 14.1 grams of *p*-dichlorobenzene.

Burgess et al. (unpublished) applied one-fifth gallon (757 ml.) of water containing 25.6 grams of emulsified ethylene dichloride and 5.3 grams of emulsified *p*-dichlorobenzene per square foot of soil surface to potted azaleas. When the third-instar grubs were removed for observation 1 week after treating the soil, the mortality ranged from 31 to 87 percent, but 2 weeks later it ranged from 98 to 100 percent. Increasing the dosage to 51.3 grams of ethylene dichloride and 10.6 grams of *p*-dichlorobenzene per square foot killed all the grubs within 3 weeks.

Ethylene Oxide

Chisholm (unpublished) prepared an emulsifiable formulation containing 4 grams of ethylene oxide in 100 ml. of the mixture. The composition of this formulation is not now available.

Fleming and Maines (unpublished) applied 0.75 gallon of dilute ethylene oxide emulsion per cubic foot of soil at 50° F. to the soil surface of potted *Pelargonium* sp. A week later the third-instar grubs were removed from the treated soil for observation. A dosage of 6.4 grams of emulsified ethylene oxide per cubic foot of soil, the highest dosage tested, killed 71 percent of the grubs and injured 28 percent of the plants of one variety of *Pelargonium* and 50 percent of the plants of another variety.

Heptachlor

The preparation of an emulsifiable mixture containing 3.5 percent of technical heptachlor by weight is given on page 87.

Potted Plants.—Third-instar grubs in friable soil of potted plants at 60° F. were killed by pouring on the soil surface a volume of dilute heptachlor emulsion equivalent to one-fifth the volume of the soil. Water containing 0.15 gram of heptachlor per liter killed all the grubs within 2 weeks. The 0.075- and 0.038-gram dosages killed them within 3 and 4 weeks, respectively. The treatment was effective on masses of soil up to 14 inches deep.

The average deposit of heptachlor per cubic foot was 0.8, 0.4, and 0.2 grams with the 0.15-, 0.075-, and 0.038-gram dosages, respectively. Heptachlor mixed with soil at 0.12 gram per cubic foot was effective in killing newly hatched grubs after the soil had weathered for 4 years. It would be expected that 0.038 gram of heptachlor would be effective against first-instar grubs for at least that period of time.

In preliminary tests the application of the 0.15-gram dosage to the soil of potted *Pelargonium* sp. had no effect on the subsequent growth of the plants. Tests in nurseries and greenhouses showed that *Hedera helix*, *Hydrangea arborescens*, *H. paniculata*, *Nephrolepis exaltata*, *Rhododendron indicum*, and *R. obtusum* were not injured by the 0.038-gram dosage. (Fleming et al. unpublished)

The treatment of potted plants at not less than 60° F. by pouring water containing 0.038 gram of emulsified heptachlor per liter on the soil surface was authorized in 1960. A volume of the insecticide liquid equivalent to one-fifth of the soil volume was required. The treatment could be used on friable soil not more than 14

inches deep. The plants could be certified for shipment 14 days after the application of the dilute emulsion. There was a possibility that the plants in the treated soil could be in a certified status for 4 years, but the longevity of the certified period would be determined by bioassays.

Methyl Bromide

Interest in an aqueous solution of methyl bromide to kill Japanese beetle grubs in soil was stimulated by the favorable results obtained by Livingstone et al. (*1940*) in destroying grubs of *Graphognathus* spp. in the soil of balled nursery stock that had been plunged into sandboxes. They used a 0.3-percent solution by volume and applied it at 40 gallons per 100 square feet of surface area.

Preparation.—In the initial experiments an aqueous solution of methyl bromide was prepared by mixing methyl bromide with twice its volume of 95-percent ethyl alcohol and adding the mixture to water in an open drum. Chisholm and Koblitsky (*1942*) prepared a 0.15-percent solution in this manner, using 1 pound (262 ml.) of methyl bromide, 524 ml. of 95-percent ethyl alcohol, and 46 gallons (174 liters) of water. They analyzed the water immediately after preparation to determine the concentration of methyl bromide. When the solution was prepared at 25° C, 40 to 50 percent of the methyl bromide was lost. The loss of methyl bromide decreased as the temperature was lowered until at 1° substantially all the chemical was in solution. This method of preparing the solution was not satisfactory.

Chisholm and Koblitsky (*1942*) prepared an aqueous solution of methyl bromide in a closed drum, which was equipped with a pressure gage, a ⅛-inch copper tube extending from a hole drilled in the bung to the bottom of the drum for introducing methyl bromide, and a spigot for withdrawing the solution. The water was placed in the drum and the drum was sealed. A can of methyl bromide was inserted in the applicator and its contents were discharged through the copper tube into the water. When the drum was nearly full and sufficient time was allowed for the methyl bromide to go into solution, as indicated by the pressure being practically normal, substantially all the methyl bromide was in solution at 11.5° and 25° C. When the solution was prepared in this manner, the addition of the alcohol was not necessary.

Potting Soil and Unplanted Beds and Plots.—Donohoe (unpublished) killed third-instar grubs to a depth of 12 inches or

more in potting soil and in unplanted beds and plots by applying 3 gallons of methyl bromide solution per square yard, using 0.15-, 0.1-, and 0.05-percent solutions at soil temperatures of 47°, 57°, and 68° F., respectively. An exposure of 48 hours in the treated soil was sufficient to kill the grubs, although at that time many of them appeared to be normal.

The procedure was authorized in 1942 for use when only grubs were in the soil. The soil could be certified 48 hours after the application of the solution. It could be in a certified status until exposed to reinfestation by the adult beetle. During 1942–50 methyl bromide solution was applied to 383 cubic yards of potting soil in commercial nurseries and greenhouses (Middleton and Cronin *1952*).

Plants Before Digging in Field.—Donohoe (unpublished) killed third-instar grubs in the soil among the roots of woody plants before digging without injuring the plants by applying 3 gallons of methyl bromide solution per square yard to the soil. A metal collar, larger in diameter than the soil ball to be dug with the plant, was pressed into the soil and the soil was compacted against the outer and inner sides of the collar to confine the solution to the area to be treated. The concentrations of the methyl bromide solution and the soil temperatures were as follows:

Methyl bromide (percent)	*Soil temperature (° F.)*
0.15	40
.125	44
.1	47
.075	52
.05	57
.04	63
.025	68
.015	73

Any type of soil could be treated provided it was friable and all the solution penetrated into the soil within 30 minutes after application. Under these conditions the grubs obtained a toxic dosage within 20 hours.

The treatment of plants in the field with methyl bromide solution was authorized in 1942 for use during the period of the year when only grubs were in the soil. The plants could be dug in not less than 20 hours or more than 5 days after the application of the solution. Information on the number of plants treated in the commercial nurseries is not now available.

Mylone

Mylone is a wettable powder containing 85 percent of tetrahydro-3,5-dimethyl-2*H*-1,3,5-thiodiazine-2-thione. It was recommended by the manufacturer as a preplanting treatment to eliminate weeds, nematodes, fungi, and insects in the soil. According to the manufacturer, Mylone undergoes chemical degradation in moist soil. The breakdown in the soil was presumed to be initially to formaldehyde and (methylamino) methyl methyl dithiocarbamate, then to monomethylamine, hydrogen sulfide, and methyl isothiocyanate, and finally to carbon dioxide, ammonia, hydrogen sulfide, and sulfur dioxide.

The manufacturer recommended that the powder be applied at the rate of 300 pounds per acre with a fertilizer spreader or suspended in water and sprayed over the area. After Mylone had been applied, it was mixed with the upper 5 or 6 inches of soil with a rotary cultivator or disk harrow. The treated area was then soaked immediately with water to a depth of 2 inches.

Fleming et al. (unpublished) mixed the wettable powder with soil and applied it as a suspension in water to the soil. One hundred pounds per acre mixed to a depth of 6 inches killed all third-instar grubs within 1 week at 70° F. and 300 pounds within 1 week at 40°. Most of the toxicity to the grubs dissipated in about 2 weeks.

The preplanting treatment with Mylone at the rate of 300 pounds per acre was authorized in 1957 for use when only grubs were in the soil and the soil temperature was at least 40° F. The treated soil could be in a certified status until exposed to reinfestation by the adult Japanese beetle.

Nicotine

Burgess et al. (unpublished) applied one-fifth gallon (757 ml.) of water containing 7.6 ml. of 95-percent free nicotine per square foot of soil surface to potted *Gardenia jasminoides* and *Piper* sp. Three weeks later 57 percent of the third-instar grubs were dead. The treatment had no effect on the plants. The tests with nicotine were not encouraging.

Vapam

Vapam, a water-miscible solution containing 32.7 percent of sodium *N*-methyldithiocarbamate, was recommended by the manufacturer as a preplanting treatment at the rate of 1 quart to 11 gallons of water per 12 square yards at temperatures above

60° F. to control annual and perennial weeds, soil fungi, and nematodes.

Fleming et al. (unpublished) found that this treatment killed all the third-instar grubs within 1 week at 40° F. and one-half the dosage of Vapam killed them within 1 week at 70°.

Vapam at 1 quart to 11 gallons of water per 12 square yards was authorized as a preplanting treatment in 1958 for use when only grubs were in the soil and the soil temperature was at least 40° F. The treated soil could remain in a certified status until exposed to reinfestation by the adult beetle.

Insecticide Liquids Injected Into Soil

Carbon Disulfide

Potting Soil.—In a preliminary study of the diffusion of carbon disulfide in moist, friable soil in a covered fumigation box at 55° F., 240 ml. of the chemical were injected to a depth of 6 inches at the central point on the surface of the soil. All third-instar grubs were killed within 24 hours to a depth of 12 inches within a horizontal radius of 5 inches from injection point and within a horizontal radius of 10 inches at depths of 18 and 24 inches. When the exposure was prolonged for 48 hours, all grubs were killed to a depth of 24 inches within a radius of 15 inches from the injection point. (Fleming *1923*; Fleming and Baker *1935*)

To assure adequate diffusion of the vapor, the carbon disulfide was injected into 18-inch layers of soil to a depth of 2 inches at points 18 inches apart. The chemical was usually applied to each 18-inch layer of soil as the fumigation box was filled, but when the soil was in the box, the chemical could be poured through a tube to the proper depths. A dosage of 240 ml. per cubic yard, 30 ml. per injection hole, was effective in killing eggs, grubs, and pupae in friable sandy loam within 48 hours at temperatures above 50° F., but it was not completely effective in killing the immature stages in the heavier soils and in decomposed manure and peat. A dosage of 352 ml. (about 1 pound) per cubic yard, 44 ml. per injection, was completely effective in killing all stages within 48 hours at temperatures not lower than 45° in sands, sandy loams, loams, clay loams, peat, compost, and rotted manure. The treatment was not satisfactory in wet, slightly permeable soils because the vapor did not diffuse in insecticide concentration even during an exposure of 96 hours. (Fleming *1923, 1930a*; Fleming and Baker *1935*)

Carbon disulfide at 352 ml. per cubic yard with an exposure of 48 hours partially sterilized the soil. The number of bacteria in the soil was not modified significantly, but the fungus population was simplified by the treatment and consisted largely of species of *Penicillium* and *Zygorhynchus*. These species were three times as abundant in the treated soil as all fungi in the soil before it was fumigated. The nitrates in the soil were not changed appreciably, but the ammonia increased from 0.6 to 2 mg. per 100 grams of soil within 112 days. (Fleming *1929*)

After the treated soil was aerated, most plants grew normally. Fleming and Baker (*1935*) reported that *Cyclamen persicum* was slightly retarded in the treated soil, but other plant species grew normally or were stimulated in growth, including—

Abies concolor
Actinophlocus sanderianus
Adiantum sp.
Agave americana
Aspidistra elatior
Begonia maculata
Butia capitata
Buxus sempervirens
Calluna vulgaris
Chamaecyparis pisifera
Cibotium schiedei
Codiaeum variegatum
Cotoneaster horizontalis
Cyrtomium falcatum
Dracaena sp.
Euphorbia pulcherrima
Ficus elastica
Hedera helix
Hydrangea macrophylla
Pandanus utilis
Picea abies
Polypodium sp.
Pseudotsuga taxifolia
Quercus falcata
Rhododendron indicum
Rhododendron obtusum
Rosa sp.
Sansevieria zeylanica
Syringa vulgaris
Thuja occidentalis
Thuja orientalis

Fumigation of potting soil by injecting 352 ml. (about 1 pound) of carbon disulfide per cubic yard was authorized in 1924 for use at any time of the year to destroy the immature stages of the beetle. The exposure was 48 hours in a covered box or bin at not lower than 45° F. The treated soil should be aerated before using it to pot plants. This was the first chemical treatment for certifying soil. The soil could be in a certified status until exposed to reinfestation by the adult beetle. Middleton and Cronin (*1952*) reported that during 1924–50 carbon disulfide had been used to fumigate 52,714 cubic yards of potting soil in the commercial nurseries and greenhouses.

Unplanted Plots.—Fleming and Baker (*1935*) injected carbon disulfide into the soil of unplanted plots at 45° F., covered the treated area with a tarpaulin after each injection, and left the soil undisturbed for 48 hours. All injections were made to a depth of 2 inches because grubs within the upper 2 inches of

soil were the most likely to survive the treatment. The injection of 15 ml. of carbon disulfide killed all grubs within a radius of 9 inches from the point of injection. A dosage of 21 ml. was effective within a radius of 14 inches, 30 ml. within a radius of 18 inches, and 45 and 90 ml. within a radius of 24 inches. The minimum practical dosage appeared to be 21 ml. per injection. It killed eggs, grubs, and pupae in the soil. To assure the destruction of the immature stages, 21 ml. of carbon disulfide were injected at 1-foot intervals to a depth of 2 inches over the area of the plots.

The injection of carbon disulfide in this manner was authorized some time prior to 1929 for the treatment of friable soil in unplanted beds, plots, and cold frames at not lower than 45° F. The soil could be in a certified status until exposed to reinfestation by the adult beetle. Middleton and Cronin (*1952*) reported that by 1950 the carbon disulfide injection treatment had been applied to 457,213 square feet of unplanted land in commercial nurseries.

Potted Plants.—Fleming and Baker (*1935*) injected carbon disulfide at the rate of 21 ml. per square foot of soil surface into the soil of potted *Cibotium schiedeii* and *Rhododendron obtusum*. After an exposure of 2 weeks at 60° F. the treatment had not killed all the third-instar grubs in the pots. An occasional grub close to the soil surface or in contact with the clay pot was not killed. The treatment seriously injured both plant species. However, when 7 ml. were injected three times at 48-hour intervals, all the grubs in the pots were killed and the plants received only minor injury. The injection of small dosages over several days was not viewed with favor by the nurserymen because of the manual labor involved.

Lipp (unpublished) injected water containing 3 ml. of emulsified carbon disulfide per liter at a pressure of 5 p.s.i. into the soil of potted plants, introducing a volume of the insecticide liquid equivalent to the volume of the soil. The treatment killed all the grubs within 1 week, but it also killed the plants, including *Hydrangea macrophylla, Lonicera* sp., *Petunia* sp., and *Rhododendron obtusum*.

Plants in Field.—In the spring Fleming and Baker (*1935*) injected carbon disulfide at 21 ml. per square foot into the soil around *Picea abies, Thuja occidentalis,* and *Tsuga canadensis,* which were growing in the nursery. Three weeks after treatment all the *Picea* plants were dead and the growing tips of *Thuja* and *Tsuga* were wilted. All the *Thuja* and *Tsuga* plants

eventually died. The treatment killed all roots within 4 inches of the points of injection and sometimes killed the rootlets at greater distances.

Chloropicrin

Chloropicrin (CCl_3NO_2) is a colorless liquid at room temperature. Its boiling point is 112.4° C., melting point −64°, specific gravity 1.651 at 20°, and vapor pressure 18.3 ml. at 20°. Low concentrations of the vapor cause vomiting and intense irritation of the eyes and throat. (Chisholm *1952*) It was used extensively by nurserymen in 1943 to control soil insects, weeds, certain species of fungi, and nematodes in soil to be used for potting plants.

Donohoe (*1944*) killed all *Popillia* grubs within 5 days in potting soil in bins by injecting 4.5 ml. of chloropicrin per cubic foot of soil at 50° F., 3.5 ml. at 60°, and 3 ml. at 70°. The fumigant was applied to 1-foot layers of soil by injecting it to a depth of 2 to 6 inches in the center of each square foot of soil surface. After each layer was treated, the soil was wet with water to a depth of about 1 inch. In 1948 Fleming (unpublished) found that this treatment was effective in killing the eggs.

The injection of chloropicrin in this manner was authorized in 1943 to kill grubs in potting soil. The soil could be in a certified status until exposed to reinfestation by the adult beetle. Middleton and Cronin (*1952*) reported that during 1943–50 the commercial nurseries had fumigated 446 cubic yards of soil with chloropicrin.

D-D

D-D was recommended by the manufacturer as a preplanting treatment to control nematodes in the soil. It was applied at from 20 to 200 gallons per acre at 40° to 80° F. The fumigant was applied in streams 10 to 12 inches apart at a depth of 6 to 8 inches by means of chisel-type applicators. Immediately after application the land was rolled or soaked with water to a depth of 2 inches to seal the fumigant in the soil.

D-D injected into soil at 20 gallons per acre killed all grubs in the soil within 1 week at 70° F., but the treatment was not completely effective at 40°. Higher dosages were not tested at the lower temperature. (Fleming et al. unpublished)

The injection of D-D at 20 gallons per acre was authorized in 1958 as a preplanting treatment, but its use was restricted to the period of the year when only grubs were in the soil and the soil

was at least 70° F. The treated soil could be certified 2 weeks after the application of the fumigant. Certification could continue until the soil was subject to reinfestation by the adult beetle.

o-Dichlorobenzene

Chisholm (unpublished) prepared a miscible formulation of *o*-dichlorobenzene of the following composition:

	Percent by weight
o-Dichlorobenzene	2.5
Tween 20 (a polyoxyalkylene derivative of sorbitan monolaurate)	2.5
Isopropyl alcohol (99 percent)	95.0

It was a clear liquid that emulsified readily when mixed with water. Each 100 ml. contained 2 grams of *o*-dichlorobenzene.

The formulation was diluted with water and injected into the soil of potted *Pelargonium* sp. at rates up to 3.2 grams of *o*-dichlorobenzene per cubic foot of soil. The treated plants were held in the greenhouse where the temperature varied between 60° and 65° F. Three weeks after treatment the 3.2-gram dosage had killed 64 percent of the third-instar grubs and had injured 77 percent of the plants. (Fleming et al. unpublished)

p-Dichlorobenzene

Fleming et al. (unpublished) dissolved *p*-dichlorobenzene in isopropyl alcohol and injected the solution into the soil of potted *Pelargonium* sp. The maximum dosage tested was 2.6 grams of *p*-dichlorobenzene per cubic foot of soil. Three weeks after treatment 39 percent of the third-instar grubs were dead and 12 percent of the plants showed injury.

Burgess et al. (unpublished) injected *p*-dichlorobenzene dissolved in acetone and in a 3:1 mixture of ethylene dichloride and carbon tetrachloride into the soil of balled and burlapped *Rhododendron* sp., injecting 256 ml. of the solutions per cubic foot of soil. A dosage of 64 grams of *p*-dichlorobenzene dissolved in acetone killed 78 percent of the third-instar grubs within 3 weeks, whereas this dosage dissolved in the ethylene dichloride-carbon tetrachloride mixture killed all of them within 3 weeks. However, the mixture of ethylene dichloride and carbon tetrachloride killed all the grubs within this period, indicating that the solvent was largely responsible for the mortality. No information was given on the reactions of the plants to these treatments.

Dichloroethyl Ether

Fleming et al. dissolved dichloroethyl ether in isopropyl alcohol and injected the solution into the soil of potted *Pelargonium* sp. Three weeks after the treatment, 1.6 grams of dichloroethyl ether per cubic foot of soil had killed 20 percent of the third-instar grubs and had injured 3 percent of the plants.

Dorlone

Dorlone, containing 75.2 percent of mixed dichloropropenes and 18.7 percent of ethylene dibromide, was recommended by the manufacturer at the rates of 6 to 12 gallons per acre as a preplanting treatment at temperatures above 50° F. to control nematodes in the soil. It was applied in streams 10 to 12 inches apart and 6 to 8 inches deep by a chisel-type applicator. Immediately after the application, the land was rolled or soaked to a depth of 2 inches with water to confine the fumigant.

Dorlone injected into soil at 12 gallons per acre killed all third-instar grubs within 1 week at 40° F., but 6 gallons per acre did not kill all the grubs within 2 weeks at 70° (Fleming et al. unpublished).

The injection of Dorlone at 12 gallons per acre was authorized in 1958 as a preplanting treatment for use when only grubs were in the soil and the soil was at least 40° F. The soil could be certified 2 weeks after application of the fumigant. It could be in a certified status until exposed to reinfestation by the adult beetle.

Epichlorohydrin

Fleming et al. dissolved epichlorohydrin (1-chloro-2,3-epoxypropane) in isopropyl alcohol and injected the solution into the soil of potted *Pelargonium* sp. Three weeks after the treatment, a dosage of 1.6 grams of epichlorohydrin per cubic foot of soil had killed no grubs, but it injured 42 percent of the plants.

Ethylene Dibromide

Formulations.—Chisholm et al. (*1954*) prepared a clear solution of ethylene dibromide that was miscible with water in most proportions. The composition of this formulation was as follows:

	Percent by weight
Ethylene dibromide	2.5
Tween 20 (a polyoxyalkylene derivative of sorbitan monolaurate)	2.5
Isopropyl alcohol (99 percent)	95.0

Circumstances involving patents and other restrictions made it necessary in 1956 to substitute Dowfume W–85, a mixture containing 83 percent of ethylene dibromide and 17 percent of a light petroleum fraction, and to modify the formulation as follows (Fleming et al. *1958*):

	Percent by weight
Dowfume W–85	3.1
Tween 20	2.9
Isopropyl alcohol	94.0

Both of these formulations contained 2 grams of ethylene dibromide in 100 ml. and were equally effective against the grubs.

Injector.—Fleming et al. (*1958*) used a hypodermic syringe equipped with a 3½-inch needle in the early tests. It had to be operated manually each time the liquid was discharged into the soil and the open end of the needle often became clogged. Fest (*1954*) modified a commercial calking gun to serve as an injector. This injector was invaluable in the exploratory work, but it was rather large and cumbersome for injecting small dosages and there was no visual check on the volume of liquid ejected.

In 1954, a veterinary hypodermic syringe was found to be more suitable. It had a capacity of 2 ml. and was graduated in units of 0.1 ml. Since it was made of glass, the operator could observe the amount of liquid drawn into the chamber and ascertain when all of it had been discharged. The liquid was ejected with one downstroke of the plunger, and the syringe was refilled automatically as the plunger returned to its resting position. The stainless steel needles, 3½ and 6 inches long, were modified by sealing the end with solder and boring two holes in the side just above this plug with a No. 60 drill. This modification prevented soil from being pushed into the needle when it was inserted into the ground.

Factors Modifying Effectiveness.—Fleming et al. (*1958*) determined the effect of dosage, temperature, moisture, soil type, and other factors on the effectiveness of miscible ethylene dibromide in killing third-instar grubs in soil.

In the study of dosage, miscible ethylene dibromide was injected to a depth of 3 inches into moist sandy loam at 10, 20, 40, and 80 ml. per square foot of soil surface. Each dosage was distributed among five injections per square foot. This introduced 0.2, 0.4, 0.8, and 1.6 grams of ethylene dibromide per square foot. When the grubs were removed 5 days later, many of them at that time appeared to be normal. The 0.2-gram dosage was inadequate; many of the grubs appeared to be normal after 38

days. The 0.4-gram dosage killed all grubs within the upper 6 inches of soil within 21 days and the 0.8 and 1.6 grams within 14 days. The 0.4 gram of injected ethylene dibromide per square foot appeared to be about the minimum needed to kill grubs within the upper 6 inches of soil within a reasonable time.

To study the horizontal diffusion within the upper 6 inches of soil, 0.08 gram of ethylene dibromide, the amount introduced per injection in the 0.4-gram treatment, was injected 3 inches into a *wet* silt loam at 40° F., and after 3, 5, and 7 days the grubs were transferred to untreated soil for observation. When the soil was lightly compacted, an exposure of 3 days was sufficient to kill all grubs within 5 inches of the injection, but when the soil was very compact, 7 days were needed. At greater distances the results were variable. The ability of miscible ethylene dibromide to diffuse through a waterlogged soil was very important. To assure that all points within the upper 6 inches have an adequate amount of fumigant, the injections should be not more than 7 inches apart.

To study the downward diffusion, ethylene dibromide was injected to a depth of 3 inches at 0.4 gram per square foot into *wet* soils, including a muck, a sandy loam, a loam, two clay loams, and a silt loam, at 40° F. Five injections of miscible ethylene dibromide were made per square foot. When the muck was lightly compacted, all grubs to a depth of 8 inches were dead within 11 days, but when the muck was moderately compacted, 21 days were required before all grubs to a depth of 6 inches succumbed and 42 days for those 6–8 inches deep. The type of loam seemed to have little effect on the speed of insecticide action. When these soils were lightly compacted, all grubs to a depth of 8 inches were dead within 14 days; when very compact, the grubs in the upper 6 inches died within 21 days, and most of those in the 6- to 8-inch layer within 42 days.

To study the effect of temperature on the insecticide action within the upper 6 inches, ethylene dibromide was injected into wet compact sandy loam at 0.4 gram per square foot. Seven days later when the grubs were transferred to untreated soil, 19 percent of those in soil at 35° F. were dead, 44 percent at 45°, 89 percent at 55°, and 98 percent at 65°. However, all grubs alive at that time had obtained a toxic dosage. Complete mortality was obtained at all temperatures within 42 days.

Plants Before Digging.—Ethylene dibromide was injected to a depth of 3 inches at 0.4 gram per square foot into the soil about the base of plants growing in the nursery row. The diameter

of the area treated was 6 inches larger than that of the soil ball to be dug. Four injections evenly distributed were required for a circular area 1 foot in diameter, nine injections for one 1.5 feet in diameter, 16 for one 2 feet in diameter, 35 for one 3 feet in diameter, and 63 for an area 4 feet in diameter. During the 7 days before the plants were dug the average soil temperatures ranged from 39° to 56° F. and the rainfall from 0.04 to 2.5 inches. The treatment killed all grubs in the soil to a depth of 10 inches. There was no indication that this dosage of ethylene dibromide had injured the plants. (Fleming et al. *1958*)

Although the treatment was successful, it was not considered to be practical because of the time and the labor involved in injecting the miscible ethylene dibromide into the soil about the base of individual plants in the nursery row.

Balled Nursery Stock.—Since the horizontal diameter and the depth of the soil of balled nursery stock varied with the species and the size of the plants, miscible ethylene dibromide was injected at 40 ml. of formulation (0.8 gram of ethylene dibromide) per cubic foot of soil. By adjusting the amount of formulation according to the volume of the soil, overdosing of shallow soil balls was avoided and an adequate dosage for the deeper ones was assured.

Injections each of 2 ml. of the formulation were made to a depth of 3 inches into the upper surface of soil balls less than 10 inches in horizontal diameter and not more than 8 inches in depth; 4-ml. injections were made into the upper surface of soil balls larger than 10 inches in horizontal diameter and not more than 8 inches deep and into the top and bottom of soil balls more than 10 inches in horizontal diameter and 8–12 inches deep. The injections ranged from two into the top of soil balls 6 inches in horizontal diameter and 6 inches deep to 16 into the top and 16 into the bottom of soil balls 24 inches in horizontal diameter and 16 inches deep.

The formulation was applied to balled nursery stock in Maryland, New Jersey, North Carolina, Ohio, and Virginia to obtain information on its effectiveness under different conditions. The insecticide action progressed at about the same rate in all these areas. Only 0.4 percent of the third-instar grubs removed from the soil balls 1 week after treatment had not succumbed at the end of 6 weeks. All the grubs most probably would have died sooner if they had been left in the treated soil. (Fleming et al. *1958*)

The speed of insecticide action was modified by the temperature, the extent to which the soil was saturated by water, and

the organic matter in the soil. When the grubs were removed from the treated soil 1 week after injection, the mortality ranged from 22 percent at 33°–39° F. to 81 percent at 70°–79°, but all the grubs alive at that time had obtained a toxic dosage. A week later the mortality ranged from 85 percent at the low temperatures to 92 percent at the high temperatures. The mortality was 100 percent in 3 weeks at 70°–79° and 99 percent or more at the lower temperatures. One week after treatment the mortality ranged from 67 percent in soils less than 20 percent saturated by water to 40 percent in soils 80–100 percent saturated, but it was practically 100 percent in all the soils in 6 weeks. One week after treatment the mortality ranged from 59 percent in soils low in organic matter to 44 percent in soils high in organic matter, but in 6 weeks the mortality was 99 percent or more in all the soils.

Many species of balled and burlapped plants were treated at commercial nurseries during the spring and the fall shipping seasons to determine their reaction to the injection of 0.8 gram of ethylene dibromide per cubic foot into the soil. The plants were usually tolerant of the treatment, except when growing vigorously in the late spring or when in poor condition. The plant species treated were as follows (Fleming et al. *1958*, unpublished):

Abelia grandiflora
Abies concolor
Abies homolepis
Acer japonicum
Acer platanoides
Acer rubrum
Aesculus parviflora
Albizzia julibrissin
Berberis julianae
Betula lutea
Betula papyrifera
Betula populifolia
Buxus sempervirens
Carpinus betulus
Carpinus caroliniana
Castanea sp.
Cedrus deodara
Cedrus libanensis
Cercidiphyllum japonicum
Cercis canadensis
Chaenomeles japonica
Chamaecyparis nootkatensis
Chamaecyparis obtusa
Chamaecyparis pisifera
Chionanthus retusus
Chionanthus virginicus
Chrysanthemum sp.
Cladrastis lutea
Clethra sp.
Cornus alba
Cornus alternifolia
Cornus florida
Cornus kousa
Cotinus coggygria
Cotoneaster divaricata
Cotoneaster lucida
Crataegus crus-galli
Crataegus intricata
Crataegus mollis
Crataegus oxyacantha
Crataegus phaenopyrum
Crataegus punctata
Cryptomeria japonica
Daphne cneorum
Deutzia scabra
Elaeagnus angustifolia
Enkianthus sp.
Euonymus alatus

Euonymus europaeus
Euonymus kiautschovicus
Fagus grandifolia
Fagus sylvatica
Forsythia intermedia
Franklinia alatamaha
Fraxinus pennsylvanica
Gaillardia aristata
Ginkgo biloba
Gleditsia triacanthos
Halesia carolina
Hamamelis japonica
Hibiscus syriacus
Hydrangea macrophylla
Ilex crenata
Ilex opaca
Ilex rotunda
Juniperus chinensis
Juniperus communis
Juniperus excelsa
Juniperus horizontalis
Juniperus procumbens
Juniperus sabina
Juniperus virginiana
Kalmia latifolia
Laburnum alpinum
Laburnum watereri
Larix leptolepis
Ledum sp.
Leucothoe catesbaei
Ligustrum lucidum
Lindera benzoin
Liquidambar formosana
Liquidambar styraciflua
Liriodendron tulipfera
Lonicera bella
Lonicera fragrantissima
Lonicera tatarica
Magnolia denudata
Magnolia kobus
Magnolia liliflora
Magnolia salicifolia
Magnolia sieboldi
Magnolia soulangeana
Magnolia stellata
Magnolia tripetala
Magnolia virginiana
Mahonia aquifolium
Malus atrosanguinea
Malus baccata
Malus halliana
Malus hupehensis
Malus ioensis
Malus purpurea
Malus robusta
Malus sieboldii
Myrica cerifera
Nandina domestica
Nyssa sylvatica
Osmanthus sp.
Oxydendrum arboreum
Philadelphus grandiflorus
Philadelphus virginalis
Phlox sp.
Picea abies
Picea engelmannii
Picea omorika
Picea pungens
Pieris japonica
Pinus bungeana
Pinus cembra
Pinus densiflora
Pinus mugo
Pinus nigra
Pinus parviflora
Pinus strobus
Pinus sylvestris
Populus sp.
Prunus americana
Prunus kansuensis
Prunus persica
Prunus sargentii
Prunus subhirtella
Prunus yedoensis
Pseudotsuga taxifolia
Pterostyrax sp.
Pyracantha coccinea
Quercus borealis
Quercus falcata
Quercus palustris
Quercus phellos
Quercus robur
Rhododendron calendulaceum
Rhododendron carolinianum
Rhododendron catawbiense
Rhododendron indicum
Rhododendron maximum
Rhododendron molle
Rhododendron mucronulatum
Rhododendron nudiflorum
Rhododendron obtusum
Rhododendron roseum

Rhododendron schlippenbachi
Rhododendron vaseyi
Rhododendron viscosum
Rhododendron yedoense
Rhodotypos scandens
Salix babylonica
Sophora japonica
Sorbus aucuparia
Spiraea vanhouttei
Styrax obassia
Syringa vulgaris
Tamarix odessana
Taxodium distichum
Taxus baccata
Taxus brevifolia
Taxus canadensis
Taxus cuspidata
Taxus hunnewelliana
Taxus media
Thuja occidentalis
Thuja orientalis
Thuja plicata
Tilia tomentosa
Tsuga canadensis
Ulmus procera
Vaccinium spp.
Viburnum burkwoodii
Viburnum carlesii
Viburnum dentatum
Viburnum fragrans
Viburnum lantana
Viburnum setigerum
Viburnum sieboldii
Viburnum tomentosum
Vitex negundo
Weigela floribunda
Weigela florida
Weigela japonica

The injection of ethylene dibromide at 0.8 gram per cubic foot of soil in balled and burlapped nursery stock was authorized in 1954 as a basis for certification. The treatment could be used when only grubs were in the soil and the soil was not less than 40° F. The plants could be certified for shipment 3 days after the treatment. Certification could continue until the plants were exposed to reinfestation by the adult beetle.

Potted Plants.—Miscible ethylene dibromide injected into the soil of potted plants at 0.4 gram per cubic foot of soil killed third-instar grubs within 4 weeks in sandy loam, silt loam, and muck at 33° to 68° F. The mortality with the 0.2-gram dosage approached 100 percent in 4 weeks. (Fleming et al. *1958*)

The ethylene dibromide injected at 0.4 gram ranged from 0.8 mg. in small pots containing 59 ml. (2 fluid ounces) of soil to 267 mg. in large pots and tubs containing 18.9 liters (5 gallons) of soil. To facilitate the application, miscible ethylene dibromide was diluted with water so that the required amount of ethylene dibromide could be applied as follows:

Injections (number)	*Diluted miscible ethylene dibromide (ml.)*	*Volume of soil (liter)*[1]
1	1	Up to 650 ml.
2	1	650 ml.–1.3
4	1	1.3–2.7
4	2	2.7–8.3
6	2	8.3–14
8	2	14–18.9

[1] Unless otherwise indicated.

The injections were made to one-half the depth of the soil. The insecticide action progressed at about the same rate in the different volumes of soil, indicating that the volume of the soil was not a limiting factor. (Fleming et al. *1958*, unpublished)

The injection of 0.4 gram into the soil of potted plants did not injure *Ageratum houstonianum, Begonia rex-cultorum, Gardenia jasminoides, Hydrangea macrophylla, Ilex opaca, Pelargonium hortorum, Rhododendron obtusum,* and *Rosa* sp., but it retarded or killed *Daphne cneorum* and *Viola cornuta.* Some horticultural varieties of *Pelargonium* tolerated the treatment, whereas others were seriously retarded or killed. (Fleming et al. *1958*, unpublished)

The injection of ethylene dibromide at 0.4 gram per cubic foot of soil was authorized in 1955 as a basis for certifying potted plants. The treatment could be used when only grubs were in the soil and the soil was not less than 40° F. The plants could be certified for shipment 3 days after treatment. Certification could be continued until the plants were exposed to reinfestation by the adult beetle.

Ethylene Dibromide-Epichlorohydrin

A 1:1 mixture of ethylene dibromide and epichlorohydrin dissolved in isopropyl alcohol and injected at 0.4 gram per cubic foot of soil killed all third-instar grubs within 3 weeks in the soil of potted *Pelargonium* sp. and injured 43 percent of the plants (Fleming et al. unpublished).

Ethylene Dibromide-Glycidyl Phenyl Ether

A 1:1 mixture of ethylene dibromide and glycidyl phenyl ether (1,2-epoxy-3-phenoxypropane) dissolved in isopropyl alcohol and injected at 0.4 gram per cubic foot of soil killed 26 percent of the grubs within 3 weeks in the soil of potted *Pelargonium* sp. and injured 43 percent of the plants (Fleming et al. unpublished).

Ethylene Dichloride

Ethylene dichloride dissolved in isopropyl alcohol was injected into the soil of potted plants and of balled nursery stock. A dosage of 3.2 grams per cubic foot of soil was required to kill all third-instar grubs within 3 weeks in the soil of potted plants, and 6.4 grams killed them within 2 weeks. The dosage had to be increased to 8 and 9.6 grams per cubic foot to kill the grubs in soil balls of a silt loam and of muck, respectively. These dosages

did not injure beans growing in the pots or the balled and burlapped *Ligustrum ovalifolium.* (Fleming et al. unpublished)

Ethylene Oxide

Ethylene oxide dissolved in isopropyl alcohol and injected into the soil of potted *Pelargonium* sp. at 3.2 grams per cubic foot of soil killed 54 percent of the third-instar grubs within 3 weeks and injured 27 percent of the plants (Fleming et al. unpublished).

Cyclic Ethylene Trithiocarbonate

Chisholm (unpublished) prepared a miscible formulation containing 4 grams of cyclic ethylene trithiocarbonate ($C_4H_8CS_3$), 4 grams of Triton-X-100, and sufficient acetone to make 100 ml. It was a clear solution that formed an emulsion when water was added. Clear solutions were not obtained when the amount of cyclic ethylene trithiocarbonate was increased.

The injection of cyclic ethylene trithiocarbonate at 0.8 gram per cubic foot into the soil of balled and burlapped *Ligustrum ovalifolium* killed only 17 percent of the third-instar grubs within 3 weeks. The treatment did not injure the plants. (Fleming et al. unpublished)

Glycidyl Phenyl Ether

Glycidyl phenyl ether (1,2-epoxy-3-phenoxypropane) dissolved in isopropyl alcohol and injected into the soil of potted *Pelargonium* sp. at 1.6 grams per cubic foot killed 35 percent of the third-instar grubs within 3 weeks and injured 7 percent of the plants (Fleming et al. unpublished).

Hexachlorocyclopentadiene

Hexachlorocyclopentadiene ($C_5H_2Cl_6$) dissolved in isopropyl alcohol and injected into the soil of potted *Pelargonium* sp. at 3.2 grams per cubic foot killed all the third-instar grubs within 3 weeks. A dosage of 0.8 gram was very injurious to the plants. (Fleming et al. unpublished)

Isobenzan

Isobenzan, a mixture of dichloropropenes, was recommended by the manufacturer at 20 gallons per acre at 40° to 80° F. as a preplanting treatment to control nematodes in the soil. The fumigant was applied in streams 10 to 12 inches apart to a depth of 6 to 8 inches by a chisel-type applicator. To seal the fumigant after application the land was rolled or soaked to a depth of 2 inches with water.

Isobenzan injected into soil at 10 gallons per acre killed third-instar grubs within 1 week at 70° F., but a dosage of 20 gallons per acre was not completely effective in killing the grubs at 40° (Fleming et al. unpublished).

The injection of isobenzan at 20 gallons per acre was authorized in 1958 as a preplanting treatment for use when only grubs were in the soil and the temperature was at least 70° F. The nursery plot could be certified 2 weeks after application and could be in a certified status until exposed to reinfestation by the adult beetle.

Lindane

A miscible formulation of lindane injected into the soil of balled and burlapped *Ligustrum ovalifolium* at 0.32 gram per cubic foot of soil had no effect on the third-instar grubs or the plants (Fleming et al. unpublished).

Methyl Bromide

Potting Soil.—Eggs, grubs, and pupae in potting soil in a bin were killed to a depth of 12 inches within 72 hours at 40° F. by injecting 1 ml. of methyl bromide to a depth of 4 inches into the center of each 10-inch square of soil surface and covering immediately to confine the fumigant (Donohoe unpublished; Mason unpublished).

Fumigation of potting soil in this manner was authorized in 1946. Successive 1-foot layers of soil could be treated as the bin was being filled. Immediately after the treatment of the top layer the soil was wetted to a depth of about one-fourth inch and covered to confine the fumigant. The soil could be certified 72 hours after applying the methyl bromide. Middleton and Cronin (*1952*) reported that during 1946–50 methyl bromide had been used to fumigate 383 cubic yards of potting soil in commercial nurseries.

Unplanted Plots.—Methyl bromide was applied as a preplanting treatment to nursery plots in the same manner as in the treatment of potting soil at not less than 40° F. The area treated in not more than 15 minutes was sprinkled with water to wet the soil to a depth of about one-fourth inch to confine the fumigant. The treatment was effective in killing eggs, grubs, and pupae within the upper 12 inches of the soil surface. (Donohoe unpublished)

The injection of methyl bromide as a preplanting treatment was authorized in 1946. The plot could be in a certified status

until exposed to reinfestation by the adult beetle. There is no record that this treatment was used as a basis for certification in commercial nurseries.

A more practical method was to apply methyl bromide in streams 10 to 12 inches apart to a depth of 6 to 8 inches by means of a chisel-type applicator. The manufacturer recommended applying 4.5 gallons per acre of a formulation containing 83 percent of methyl bromide in this manner as a preplanting treatment to control nematodes, soil insects, and other pests. After the treatment the land was rolled or soaked to a depth of 2 inches with water to confine the fumigant. Fleming et al. (unpublished) found that this treatment was effective in killing all third-instar grubs within 1 week at 70° F., but it was not completely effective within 2 weeks at 40°.

The application of 4.5 gallons of the 83-percent formulation per acre in this manner at not less than 70° F. was authorized in 1958 as a preplanting treatment when only grubs were in the soil. The plot could be certified 2 weeks after the application. It could be in a certified status until exposed to reinfestation by the adult beetle.

Nemagon

Nemagon contains 97 percent by weight of 1,2-dibromo-3-chloropropane. It was recommended by the manufacturer to be injected at 1–2 gallons per acre to control nematodes.

Unplanted Plots.—The injection of Nemagon at 2.5 gallons per acre, followed by an application of water to seal the fumigant in the soil, had little effect on third-instar grubs in the soil (Fleming et al. unpublished).

Potted Plants.—Chisholm (unpublished) prepared several miscible formulations containing Nemagon, Tween 20, and isopropyl alcohol. The most concentrated of these formulations contained 32 grams of Nemagon and 32 grams of Tween 20 in 100 ml. All of them were diluted readily with water.

The injection of Nemagon at 3.2 grams per cubic foot into the soil of potted *Ligustrum ovalifolium* at 60° F. did not kill all the third-instar grubs within 3 weeks. The 6.4-gram dosage killed all of them within 1 week. These treatments were not injurious to the plants. The tests were not continued because of the high dosage of Nemagon required to kill the grubs. (Fleming et al. unpublished)

Balled Nursery Stock.—The injection of the miscible formulation at 12.8 grams of Nemagon per cubic foot into the soil of balled and burlapped *Ligustrum ovalifolium* was not always ef-

fective in killing all third-instar grubs within 5 weeks at 60° F. (Fleming et al. unpublished).

Nicotine

Burgess et al. (unpublished) injected nicotine in an acetone solution at 85 ml. per cubic foot into the soil of potted *Gardenia jasminoides* and *Piper* sp. at 57° F. The nicotine killed 44 percent of the third-instar grubs within 3 weeks, and it did not injure the plants.

Vapam

Vapam (sodium *N*-methyldithiocarbamate) injected into the soil of potted *Ligustrum ovalifolium* at 0.4 gram per cubic foot at 60° F. killed all third-instar grubs within 3 weeks. The mortality was 90 percent with the 0.2-gram dosage in that period. However, when Tween 20 was added to the Vapam, 0.2 gram killed all grubs within 1 week. These treatments killed all the plants. (Fleming et al. unpublished)

The injection of 0.4 gram into the soil of balled and burlapped *Ligustrum ovalifolium* killed the grubs and the plants (Fleming et al. unpublished).

Insecticides of Plant Origin Mixed With Soil

Derris is the ground root of *Derris elliptica*, hellebore the ground root of *Veratrum album* or *V. viride*, pyrethrum the ground dried flowers of *Chrysanthemum cinerariaefolium*, and mowrah meal the ground press cake left after removal of oil from the seeds of *Madhuca indica*. Rotenone and deguelin are the toxic constituents in derris, helleborine in hellebore, pyrethrin I, pyrethrin II, cinerin I, and cinerin II in pyrethrum, and mowrine in mowrah meal. All these insecticides have a very low mammalian toxicity.

Derris was a good contact insecticide and repellent for the adult beetle (Fleming and Baker *1936b*; Fleming and Metzger *1936a*, *1936b*; Osburn *1934b*). Sprays containing pyrethrum were very effective in killing beetles, but the residue afforded no protection to plants (Fleming *1933*; Van Leeuwen and Vander Meulen *1926*, *1927*, *1928*). Mowrah meal was effective in controlling earthworms in soil (Carlos *1926*).

When derris, hellebore, pyrethrum, and mowrah meal were mixed with soil, they had little effect on third-instar grubs. The

mortality with 4,000 pounds per acre of these insecticides ranged from 22 to 50 percent. (Fleming *1942*)

Fertilizers and Soil Conditioners Mixed With Soil

The density of the grub population was modified by the pH of the soil. In State-wide surveys in Ohio the most dense populations of grubs were found in soils with a pH below 4.9 and the lowest populations in soils with a pH above 6.5. The grubs increased in numbers more rapidly in the more acid soils, and high grub populations persisted year after year in these soils as long as favorable weather prevailed during the egg and young grub stages. (Polivka *1960a*)

Wessel and Polivka (*1952*) adjusted the pH of the soil in plots from 3.9 to 6.8 by applying sulfur and pulverized limestone. The eggs and first-instar grubs per square foot ranged in 1949 from 39 with a pH of 3.9 to 18 with a pH of 6.8 and in 1950 from 11 to 3, respectively, indicating that the female beetle favored the more acid soils for oviposition.

When lime was applied to an acid soil, the grub population was invariably reduced. During a 7-year period the grub population in part of a cemetery that had been limed to raise the pH of the soil from 4.3 to 7.0 was one-tenth to one-half that in the unlimed part (Polivka *1960b*). The application of lime to an acid soil reduced the emergence of beetles about 75 percent (Metzger *1938*).

Ammonium sulfate, acid phosphate, ferrous sulfate, limonite, and greensand marl at up to 8,000 pounds per acre had no effect on the grubs (Fleming *1942*; Fleming and Burgess unpublished).

Calcium cyanamid at more than 1,000 pounds per acre was toxic to third-instar grubs. The population was reduced 4, 20, 52, and 80 percent, respectively, by 1,000, 2,000, 4,000, and 8,000 pounds (Fleming *1942*).

Naphthalene Mixed With Soil

Naphthalene mixed with soil was more toxic to grubs than 1-naphthol, 2-naphthol, 1-naphthylamine, 1-nitronaphthalene, dibromonaphthalene, or nitronaphthylamine (Fleming *1928b*).

Toxicity to Immature Stages

When third-instar grubs were removed from soil and exposed for 24 hours at 80° F. to the vapors of several organic chemicals, the vapor of naphthalene was much more toxic to the grubs

than the vapor of carbon disulfide and *p*-dichlorobenzene (Fleming *1925a*).

Potting Soil

Naphthalene diffused to a limited extent in soil because of its low vapor pressure. The crystals had to be mixed with soil to kill all eggs, grubs, and pupae. When soils were screened to remove lumps, stones, and debris, and crystals were intimately mixed with the soils, 0.6 pound of naphthalene per cubic yard killed all third-instar grubs in a sandy loam and in a clay loam within 10 days at 50° F. The dosage had to be increased to 1.7 pounds per cubic yard to kill them in a 1:1 mixture of clay loam and peat. However, when the soils were not screened and lumps large enough to conceal a grub were present, the dosage had to be increased to 5 pounds per cubic yard to kill all the grubs in the different soils within 1 week at 50°. A 10-pound treatment was not completely effective when the soils were wet. (Fleming and Baker *1930*, *1934*)

Fleming and Baker (*1934*) found that naphthalene disappeared rapidly from potting soil. Only a few of the grubs introduced into soil 10 days after the 5-pound treatment were killed. Analyses of the soils showed that the average loss in clay loam was 0.8 pound in 2 days, 1.4 pounds in 4 days, 4.2 pounds in 6 days, and 4.99 pounds in 8 days, and in a 1:1 mixture of clay loam and peat the average loss was 1.4, 4.8, 4.99, and 5.0 pounds, respectively. When the soils were re-treated, practically all the naphthalene disappeared within 3 days. The rapid development of filamentous fungi in the re-treated soils was a clue that the loss of naphthalene was due largely to decomposition by micro-organisms. Tattersfield (*1928*) and Thornton (*1928*) attributed the rapid loss of naphthalene in soil under favorable conditions to decomposition by bacteria. Gray and Thornton (*1928*) isolated many types of bacteria that decomposed naphthalene. This rapid loss of the chemical is of practical importance in that it eliminates the residue problem.

When 5 pounds of the crystals were mixed with a cubic yard of potting soil and the soil was used immediately for potting *Hydrangea macrophylla*, 43 percent of the plants were killed and the remainder were retarded. The plants were not injured when the soil was held until no odor of the chemical could be detected. The plant species grown successfully in the treated soil included—

Antirrhinum majus
Araucaria excelsa
Aspidestra elatior
Calendula officinalis
Cyclamen sp.
Dahlia spp.
Dracaena fragrans
Euphorbia pulcherrima
Ficus elastica
Hedera helix
Howea fosteriana
Hydrangea macrophylla
Iris spp.
Pandanus australis
Phlox spp.
Rhododendron indicum
Rhododendron obtusum
Rosa spp.
Sansevieria zeylanica

The treatment of potting soil with naphthalene at 5 pounds per cubic yard was authorized in 1929 to kill grubs in the soil. The soil could be certified after holding for 7 days at not lower than 50° F. It could remain in a certified status until exposed to reinfestation by the adult beetle. The treatment was used by only a few nurserymen. Middleton and Cronin (*1952*) reported that during 1929–50 naphthalene had been used to treat 557 cubic yards of potting soil.

Plots

Fleming and Baker (*1934*) applied 435 pounds of naphthalene per acre at weekly intervals during July and August and mixed the crystals with the upper 2 inches of soil between the rows of plants in a flower garden. The odor of naphthalene was very pronounced during the summer. The treatment did not protect the plants from attack by the beetles nor did it prevent oviposition. In the fall the average population in the treated garden was three grubs in 10 square feet.

Third-instar grubs were eliminated in unplanted plots within 1 week in the early fall by applying 2,000 pounds of naphthalene per acre and mixing the crystals by cultivation with the upper 3 inches of soil. The mortality of the grubs with 1,000 pounds approached 100 percent.

The application of 2,000 pounds of naphthalene per acre mixed with the upper 3 inches of soil was authorized in 1929 to destroy grubs in unplanted plots at not lower than 50° F. The treatment could not be used when adult beetles were present. A plot could be in a certified status until exposed to reinfestation by the adult beetle. Middleton and Cronin (*1952*) reported that during 1929–50 the naphthalene treatment had been used on 500,576 square feet of unplanted plots in commercial nurseries.

p-Dichlorobenzene Mixed With Soil

p-Dichlorobenzene ($C_6H_4Cl_2$) is a white crystalline chemical at room temperature. It melts at 53° C. and boils at 173.4°. The vapor pressure ranges from 0.08 mm. at 0° to 1.5 mm. at 30°. (Chisholm *1952*; Roark and Nelson *1929*)

Toxicity to Grubs

Third-instar grubs were removed from soil and exposed in a saturated atmosphere of *p*-dichlorobenzene. All grubs were killed by an exposure for 4 days at 45° F., 2 days at 70°, and 1 day at 80°. (Fleming *1925a*; Lipp unpublished)

Potting Soil

Third-instar grubs in a sandy loam, mixtures of sandy loam and peat, and peat were killed by mixing crystals of *p*-dichlorobenzene with the soils. The grubs were killed within 7 days with 0.5 pound and within 3 days with 2 or more pounds per cubic yard at 46° to 60° F. The 0.5-pound dosage was not completely effective in killing grubs introduced into the soils 1 week after the chemical was applied. The 2-pound dosage was effective for 2 weeks in peat and in a 3:1 mixture of peat and sandy loam, for 3 weeks in 1:1 and 1:3 mixtures of peat and sandy loam, and for 6 weeks in the sandy loam. A 5-pound dosage was effective for 7 weeks in peat and the sandy loam-peat mixtures and for 11 weeks in the sandy loam. (Lipp unpublished)

Azaleas and *Hydrangea macrophylla* potted in soil to which 2 pounds of *p*-dichlorobenzene had been applied were seriously injured or killed. Subjecting the roots of the plants to a saturated atmosphere of *p*-dichlorobenzene for 2 or more weeks was more than the roots could endure. (Lipp unpublished)

Plunging Soil

Lipp (unpublished) found that third-instar grubs in balled and potted plants were killed by plunging the soil masses into soil treated with *p*-dichlorobenzene so that there was about 1 inch of treated soil under, around, and over each mass of soil. With an exposure of 5 days at 50° to 65° F. all grubs in soil masses up to 6 inches in diameter were killed by plunging them in soil containing 10 pounds of the chemical per cubic yard. The dosage was increased to 20 pounds for masses of soil 6 to 8 inches in

diameter. All grubs were not dead at the end of the 5-day exposure, but those surviving were moribund and died within the following 2 weeks. These results were confirmed by Burgess et al. (unpublished) several years later.

Analyses of the soil treated with the 10-pound dosage showed that on an average 2.5 pounds of *p*-dichlorobenzene were lost during the 5-day treatment. Some of the chemical was in the plant balls and some was lost by evaporation. It would be possible for a large nursery to have the plunging soil analyzed and then add sufficient *p*-dichlorobenzene to restore the original concentration rather than discarding the soil after using it once. (Burgess et al. unpublished)

The reaction of plants to having their soil balls plunged into *p*-dichlorobenzene-treated soil for 5 days was modified by the species and the condition of the plants. Plants growing vigorously were more likely to be injured than dormant or semidormant plants. Species with an asterisk (*) in the following list were severely injured or killed by the treatment.

Anemone japonica
Aquilegia chrysantha
Artemisia dracunculus
Aster alpinus
Astilbe sp.
Campanula medium
Cerastium biebersteinii
Ceratostigma plumbaginoides
Chrysanthemum sp.
Corchorus capsularis
Delphinium grandiflorum
Dianthus caryophyllus
Digitalis purpurea
Eupatorium coelestinum
**Fragaria* sp.
Franklinia alatamaha
Helianthemum glaucum
**Hydrangea macrophylla*
Iberis amara
**Ilex opaca*
**Limonium latifolium*
Magnolia soulangeana
Myosotis alpestris
Pachysandra terminalis
**Papaver nudicaule*
**Papaver orientale*
Phlox sp.
Rhododendron indicum
Rhododendron obtusum
Santolina chamaecyparissus
Sedum acre
Stokesia laevis
Thymus serpyllum
Vaccinium sp.
Viola sp.

The treatment was authorized in 1935 as a basis for certification. It was popular with many of the growers. Middleton and Cronin (*1952*) reported that during 1935–50 a total of 692,676 plants, mostly azaleas, had been treated and certified for shipment.

Inorganic Residual Insecticides Mixed With Soil

According to Browne (*1928*), Chinese farmers for many years applied crude arsenic to soil to destroy earthworms. Hawe (*1899*) used arsenious oxide to control wireworms in soil. Jarvis (*1916*) used arsenious oxide to control grubs attacking the roots of sugarcane. There is no record of inorganic residual insecticides being used in the United States to control soil insects until Leach (*1926*) demonstrated that certain inorganic arsenates mixed with soil killed *Popillia* grubs.

The grubs were killed when they ingested a residual insecticide while burrowing through soil or feeding on the roots of plants growing in this soil. When a grub consumed a lethal dosage of a poison, it ceased burrowing and feeding, discharged the contents of the alimentary tract, became flabby, and eventually died. The action of a residual stomach poison in the soil is complex. It is affected by the development, activity, and susceptibility of the grubs, the nature and the concentration of the poison, the physical and chemical characteristics of the soil, and the temperature. (Fleming *1942*)

In contrast to fumigants that persist for only a short time in the soil, a residual insecticide may be effective for several years in killing successive generations of grubs that hatch in the soil. This type of treatment made it possible for large wholesale nurseries to treat plots of nursery stock en masse, instead of individual plants, and to dig and prepare the plants for shipment in the usual manner. As developed later, however, a persistent insecticide in soil was not always advantageous, particularly if it affected the soil bacteria, fungi, and other micro-organisms and the fertility of the soil or if other crops than ornamentals were to be grown in the soil.

Lead Arsenate

Lead arsenate ($PbHAsO_4$) was the most common insecticide in the 1920's for controlling chewing insects on plants, but so far as can be determined it had not been used prior to 1922 in the United States to control grubs in the soil.

Toxicity to Grubs.—In a preliminary experiment in 1922 Leach (*1926*) determined that the velocity of poisoning third-instar grubs at 60° F. increased with the increment in the quantity of lead arsenate in the soil as follows:

Lead arsenate per acre (pounds)	*Period for 100-percent mortality (days)*
500	40
1,000	18
1,500	10
2,000	7
3,000	5

This quick insecticide action, however, was not confirmed by subsequent experiments.

Fleming and Maines (*1944a*, unpublished) found a joint functional relationship between the mortality of the grubs and their development, the quantity of lead arsenate in the soil, the temperature, and the exposure. In these experiments the arsenical was thoroughly mixed with moist sifted Sassafras sandy loam at 10.4 to 41.6 grams per cubic foot, which was equivalent to incorporating, respectively, 250 to 1,000 pounds of the chemical with the upper 3 inches of an acre of soil. The relationship between 100-percent mortality of the grubs and these factors is presented in table 14.

The velocity of insecticide action was not accelerated significantly by increasing the quantity of lead arsenate from 1,000 pounds to 1,500 or 2,000 pounds per acre, showing that the maximum rate of poisoning was reached with 1,000 pounds. The insecticide action progressed about five times as fast with first-instar as with third-instar grubs. The rate of poisoning of both instars at 50° F. was about one-half that at 60° and about one-fourth that at 80°. The long period required for 100-percent mortality of third-instar grubs below 70° would indicate that lead arsenate would be only partially effective against third

TABLE 14.—*Effect of temperature, stage of development of Japanese beetle grubs, and dosage on insecticide action of lead arsenate in Sassafras sandy loam*

Temperature (° F.)	Period for 100-percent mortality of grubs with indicated pounds per acre					
	First instars			Third instars		
	250	500	1,000	500	750	1,000
	Days	*Days*	*Days*	*Days*	*Days*	*Days*
50		60	33			160
60	45	30	17	160	136	80
70	30	20	11	106	91	53
80	23	15	8	80	68	40

instars in the field. The 1,000 pounds applied in the field on October 1 killed 98 percent of the third-instar grubs before pupation in June; the treatment on March 1 killed 87 percent before pupation. With the warmer soils during the summer 500 pounds applied before the eggs hatched killed all the grubs by September; 250 pounds killed 95 percent of them.

When 500 pounds of lead arsenate were in the soil before the eggs hatched, first-instar grubs died before molting. The average duration of the first-instar stage is longer than the period required to kill the grubs. It was 160, 101, 30, and 17 days, respectively, at 59°, 64°, 68°, and 77° F. (Ludwig *1928b, 1932*). A temperature of 50° is below the threshold for the development of eggs and grubs (Ludwig *1928a*; Payne *1928*). Grubs were practically inactive below this temperature (Fox *1935*; Hawley *1944*; Ludwig *1928b*).

Some third-instar grubs ceased feeding and transformed to the prepupal stage before ingesting a lethal dosage when less than 1,000 pounds of lead arsenate per acre was in the soil (Fleming and Maines *1944a*, unpublished). The transformation to the prepupal stage began in 50 days at 80° F., in 67 days at 70°, and in 100 days at 60°. The evidence demonstrated that elimination of a grub population in the soil can be expected only when the chemical is in the soil at the time the eggs hatch.

Insecticide Action in Different Soils.—Arable soil is a complex mixture of rock debris and plant and animal residues. It is formed in situ from underlying rock or is transported by water, ice, or wind. A classification of soils similar to that of plants and animals has been developed by pedologists. Soils essentially alike in all characteristics, except the texture of the surface horizon, make up a soil series, which corresponds to the genus of closely related groups of plants or animals. The texture corresponds to the species. Each type of soil where soils have been classified is identified by its series and the texture of the surface layer, e.g., Sassafras sand, Sassafras sandy loam, and Sassafras loam.

The toxicity of lead arsenate to third-instar grubs differed greatly in 39 types of soil in Illinois, Missouri, New Jersey, North Carolina, Pennsylvania, South Carolina, and Virginia. Fleming et al. (*1936*, unpublished) determined the quantity of lead arsenate per acre required in these soils to produce a toxicity equivalent to that of 1,000 pounds per acre of the freshly applied chemical in Sassafras sandy loam and the weathering required for 50-percent loss of toxicity in each soil. These results are summarized in table 15.

TABLE 15.—*Toxicity of lead arsenate to third-instar Japanese beetle grubs in different types of soil*

Soil type	Lead arsenate per acre equivalent in toxicity to standard [1]	Period for 50-percent toxicity loss	Soil type	Lead arsenate per acre equivalent in toxicity to standard [1]	Period for 50-percent toxicity loss
	Pounds	*Months*		*Pounds*	*Months*
Durham sandy loam	450	12	Croton silt loam	1,100	34
Appling sandy loam	550	12	Ocklockonee sandy loam	1,100	27
Marlboro sandy loam	550	14	Washington loam	1,100	22
Lakewood sand	600	35	Manor loam	1,200	48
Sassafras sand	600	14	Hagerstown silt loam	1,250	27
Cecil sandy loam	750	8	Bermudian silt loam	1,300	20
Norfolk sandy loam	750	22	Shrewsbury sandy loam	1,300	30
Dunellen sandy loam	800	20	Keyport loam	1,350	30
Helena sandy loam	800	20	Conestoga silt loam	1,400	22
Lakewood sandy loam	800	24	Wethersfield gravelly loam	1,400	30
Elkton clay loam	900	20	Berks shale loam	1,450	35
Muscatine silt loam	950	12	Merrimac sandy loam	1,500	24
Collington sandy loam	1,000	20	Penn shale loam	1,500	40
Dover loam	1,000	20	Chester gravelly loam	1,550	48
Elkton silt loam	1,000	20	Iredell loam	1,650	1
Lansdale silt loam	1,000	24	Memphis silt loam	1,650	27
Sassafras sandy loam	1,000	20	Georgeville silt loam	1,700	20
Woodstown sandy loam	1,000	48	Colts Neck loam	2,000+	?
Dutchess shale loam	1,050	24	Davidson clay loam	2,000+	?
Clyde loam	1,100	23			

[1] Standard = 1,000 pounds per acre of freshly applied lead arsenate in Sassafras sandy loam.

Lead arsenate became insecticidally inactive more readily in the heavy soils with a high capacity for converting the chemical to an unavailable form than in the light soils with low fixing power. Only 450 pounds per acre were required in Durham sandy loam, but more than 2,000 pounds per acre in Colts Neck loam and Davidson clay loam were needed to obtain an initial toxicity equivalent to that of 1,000 pounds per acre in Sassafras sandy loam. The fixation of lead arsenate progressed rapidly because the chemical was applied to the soils shortly before introducing the grubs to determine the initial toxicity. The insecticide action with the 1,000-pound dosage was faster in the light than in the heavy soils.

The exposure to weathering required for these soils to lose 50 percent of their initial toxicity varied considerably. The average time was 18, 24, and 29 months, respectively, with 450–800, 900–1,100, and 1,200–1,700 pounds per acre.

It is not practical, however, to conduct bioassays and adjust the dosage of lead arsenate to obtain the same initial toxicity in all soils. It is best to apply a dosage that will be effective in most of the soils, recognizing that in some heavy soils the treatment might not be completely effective. One thousand pounds applied before the eggs hatched killed all first-instar grubs in all of these soils, except Colts Neck loam and Davidson clay loam. This dosage was effective for 1 to 4 years in eliminating first instars in most of the other soils.

Potting Soil.—It was the practice in commercial nurseries to pot rooted cuttings of plants in the spring and place them in coldframes and beds where the soil was exposed to oviposition by the adult beetle. Lead arsenate was mixed with friable potting soil to kill grubs hatching in the soil during the summer. At 60° F. first-instar grubs were killed within 22 days by mixing 2 pounds of lead arsenate with a cubic yard of soil. A dosage of 1.5 pounds was effective within 27 days and 1 pound within 36 days. A 0.5-pound dosage was not completely effective in 45 days.

Mixing 2 pounds of lead arsenate with a cubic yard of soil was authorized in 1929 as a basis for certifying these potted plants. Since eggs could be deposited in the treated soil during July and August when the soil was above 60° F., the plants could be certified for shipment 4 weeks after the adult beetles had disappeared in the area.

In cooperative studies with commercial nurseries and greenhouses, many plant species were potted with soil containing 2 pounds of lead arsenate. The species with an asterisk (*) were retarded or killed by the arsenical. (Fleming *1937*)

HERBACEOUS PERENNIALS

Achillea filipendulina
Achillea millefolium
Adenophora potaninii
Aethionema cordifolium
Alyssum argenteum
Alyssum saxatile
Amsonia tabernaemontana
Anthemis tinctoria
Artemisia vulgaris
Aster alpinus
**Aster amellus*
Aster subcoeruleus
**Bellis perennis*
**Boltonia asteroides*
Boltonia latisquama
**Campanula lactiflora*
**Campanula medium*
Centaurea dealbata
Centaurea montana
Centranthus ruber
**Cerastium tomentosum*
Chrysanthemum leucanthemum
**Chrysanthemum uliginosum*
Coreopsis lanceolata
Dianthus barbatus
Dianthus caryophyllus
Dianthus deltoides
Digitalis ambigua
Digitalis purpurea
**Dracocephalum ruyschianum*
**Erigeron speciosus*
Eryngium amethystinum
Erysimum asperum
Eupatorium rugosum
**Euphorbia pulcherrima*
Gaillardia aristata
**Geum chiloense*
Gypsophila paniculata
Gypsophila repens
**Helenium hoopesi*
**Helianthemum nummularium*
Iberis amara
**Lavandula officinalis*
Linum perenne
**Lobelia cardinalis*
**Lobelia siphilitica*
**Lychnis alpina*
Lychnis chalcedonica
Lychnis coronaria
Lychnis flosjovis
Lychnis haageana
Lythrum salicaria
**Myosotis scorpioides*
Oenothera fruticosa
Oenothera speciosa
**Papaver nudicaule*
**Penstemon digitalis*
Penstemon hirsutus
Penstemon torreyi
Physalis alkekengi
**Polemonium pulcherrimum*
**Potentilla macnabiana*
Prunella grandiflora
**Rosmarinus officinalis*
Rudbeckia speciosa
Saponaria ocymoides
Scabiosa japonica
Sidalcea malvaeflora
Stachys grandiflora
Stachys lanata
**Stokesia laevis*
Tunica saxifraga
**Valeriana officinalis*
Verbascum olympicum
Veronica repens
Veronica spicata
**Viola cornuta*

DECIDUOUS PLANTS

Acer palmatum
Actinidia arguta
Aronia arbutifolia
Baccharis halimifolia
Berberis thunbergii
Berberis verruculosa
Callicarpa japonica
Caryopteris incana
Clematis heracleaefolia
Clematis paniculata
Clethra alnifolia
Cornus alternifolia
Cornus florida
Cornus kousa
Cotinus coggygria
Elaeagnus multiflora
Elsholtzia stauntoni
Fagus sylvatica
Forsythia intermedia
Forsythia suspensa
Hypericum frondosum
Hypericum moserianum
Jasminum nudiflorum
Lespedeza thunbergii
Ligustrum lucidum
Ligustrum ovalifolium
Lonicera japonica
Lonicera maackii
Magnolia sieboldii
Magnolia soulangeana
Magnolia tripetala
Magnolia virginiana
Mahoberberis neuberti
Nandina domestica
Parthenocissus tricuspidata
Philadelphus coronarius
Philadelphus lemoinei
Rhus aromatica
Rosa wichuraiana
Sorbaria aitchisoni
Spiraea thunbergii
Staphylea colchica
Stephanandra incisa
Symplocos paniculata
Syringa chinensis
Syringa persica
Syringa vulgaris
Viburnum acerifolium
Viburnum cassinoides
Viburnum dentatum
Viburnum lantana
Viburnum prunifolium
Viburnum rhytidophyllum
Viburnum tomentosum
Virburnum trilobum
Weigela florida
Weigela hortensis

EVERGREENS

Abies concolor
Buxus sempervirens
Chamaecyparis obtusa
Chamaecyparis pisifera
Cotoneaster horizontalis
Cotoneaster microphylla
Daphne cneorum
Euonymus alatus
Euonymus bungeanus
Euonymus fortunei
Euonymus kiautschovicus
Hedera helix
Ilex crenata
Ilex glabra
Juniperus chinensis
Juniperus communis
Juniperus excelsa
Juniperus sabina
Juniperus sphaerica
Leucothoe catesbaei
Pachysandra terminalis
Picea abies
Picea glauca
Pieris japonica
Pyracantha coccinea
Rhododendron mucronatum
Rhododendron obtusum
Rhododendron yedoense
Taxus baccata
Taxus brevifolia
Taxus canadensis
Taxus cuspidata
Taxus media
Thuja occidentalis
Thuja orientalis
Thuja plicata
Tsuga canadensis

Unplanted Plots.—Plants potted in soil treated with lead arsenate and plunged in coldframes and beds during the summer and uninfested perennial and deciduous stock heeled in plots were not certified unless the coldframes, beds, and plots also contained no grubs.

In a preliminary experiment in 1922 Leach (*1926*) mixed lead arsenate at 1,000 pounds per acre with the upper 4 inches of soil, covered the plot with a cage, and during July introduced beetles periodically into the cage. On September 1 no grubs were found in the treated plot, but 475 were found in the untreated plot.

Reference was made by Howard (*1923*), Leach (*1926*), and Leach et al. (*1924*) to lead arsenate being applied at 1,500 pounds per acre to the soil of coldframes and heeling-in plots in commercial nurseries during 1923, but information on these experiments is not now available. Howard (*1924*) reported that these experiments had been completed. There was no explanation for the increase to 1,500 pounds per acre.

Lead arsenate at 1,500 pounds per acre mixed with the upper 3 inches of soil was authorized in 1924 as a basis for certifying the soil of coldframes and heeling-in plots, but there was no provision for analyzing the soil in subsequent years to determine whether there was sufficient residue to destroy grubs hatching in the soil during the summer.

It was the practice to apply 1,500 pounds of lead arsenate per acre annually to the soil of coldframes and heeling-in plots until 1928 when, apparently at the discretion of the quarantine official, the quantity could be reduced to 1,000 pounds per acre annually for soil to which the 1,500 pounds had been applied for 2 successive years. As a result of this practice, some coldframes and heeling-in plots received 5,000 or more pounds of the chemical per acre during a 4-year period. The situation was not only chaotic but wasteful of the chemical and a definite hazard to the nursery stock. (Hadley unpublished)

In 1930 the soil in the treated coldframes and heeling-in plots was analyzed, using a procedure developed by Koblitsky (*1939*). From then until 1941 the soil was continued indefinitely in a certified status when sufficient lead arsenate was applied annually before the flight of the beetle so that 1,500 pounds per acre were in the upper 3 inches of soil during the summer. An appraisal of the situation that year showed that the 1,500-pound dosage was much higher than needed and not justified by the experimental data. At that time it was authorized that the soil of coldframes and heeling-in plots could be continued in a certified

status without re-treatment when it was determined by analyses that the upper 3 inches of soil contained at least 1,000 pounds of lead arsenate per acre.

Planted Plots.—In 1929 the 1,500-pound treatment was authorized as a basis for certifying plots of established nursery stock before digging. It was required that the chemical be applied over the entire area of a plot and be mixed by cultivation with the upper 3 inches of soil before August 1 and that a survey in September demonstrate that no living grubs were in the soil. The plants then could be certified for shipment during the fall and the following spring. A plot could be in a certified status indefinitely during the fall and spring shipping seasons when the soil was analyzed each year and sufficient lead arsenate was applied before August 1 to restore the initial dosage in the upper 3 inches of soil.

The decision to extend the 1,500-pound treatment to include plots of established nursery stock was apparently based on the surveys of treated unplanted plots, which showed no surviving grubs in September, and on the tolerance of many deciduous and evergreen plants to lead arsenate in potting soil.

Several modifications were made in this authorized treatment. In 1931 the treatment had to be completed by July 1 instead of August 1 to have the chemical in the soil while the adult beetle was on the wing, the annual surveys of treated plots were eliminated, and the nursery stock could be certified for shipment on October 1. Later these dates were adjusted according to the development of the insect in different parts of the regulated area. In 1941 the initial application was reduced to 1,100 pounds per acre, and 1,000 pounds per acre were an acceptable residue in previously treated plots. In 1942 these dosages were reduced to 1,000 and 900 pounds, respectively. These changes did not modify the effectiveness of the treatment and they reduced the hazard to the plants.

In cooperative studies with commercial nurseries observations were made on the reaction of many plant species in plots treated with 1,500 pounds of lead arsenate per acre. The species with an asterisk (*) were retarded or were killed by the arsenical. (Fleming *1937*)

HERBACEOUS PERENNIALS

Acanthus mollis	*Alyssum argenteum*
**Achillea ptarmica*	**Alyssum saxatile*
Ajuga reptans	*Amsonia tabernaemontana*

**Aquilegia chrysantha*
Arabis alpina
**Arenaria montana*
Armeria pseudoarmeria
Aster alpinus
Aster novae-angliae
Aster subcoeruleus
**Astilbe davidii*
**Baptisia australis*
**Bellis perennis*
Boltonia latisquama
**Calimeris incisa*
Callirhoe involucrata
**Campanula carpatica*
Campanula latifolia
**Campanula medium*
**Campanula persicifolia*
**Campanula pyramidalis*
Centaurea dealbata
Centaurea macrocephala
Centaurea montana
Centranthus ruber
Cephalaria tatarica
**Ceratostigma plumbaginoides*
Cheiranthus cheiri
Crambe cordifolia
Chrysanthemum carinatum
Chrysanthemum leucanthemum
Chrysanthemum maximum
Chrysanthemum uliginosum
Coreopsis lanceolata
Delphinium formosum
Dianthus deltoides
**Dicentra formosa*
**Digitalis lanata*
Echinacea purpurea
**Echinops ritro*
**Erigeron pulchellus*
**Erigeron speciosus*
**Eryngium amethystinum*
**Eryngium planum*
Eupatorium rugosum
Gaillardia aristata
**Geum chiloense*
**Gypsophila repens*
**Halimium halimifolium*
**Hebe speciosa*
**Helenium hoopesi*
**Heliopsis helianthoides*
**Heliopsis scabra*
Hemerocallis dumortieri
**Heuchera sanguinea*
Hosta japonica
Hypericum frondosum
Hypericum moserianum
Iberis sempervirens
Iris germanica
Iris kaempferi
Iris xiphium
Jasminum nudiflorum
Kniphofia uvaria
**Lavandula officinalis*
Lespedeza thunbergii
**Limonium latifolium*
Linum perenne
Lychnis chalcedonica
Lychnis coronaria
Lychnis haageana
Lythrum salicaria
Monarda didyma
**Myosotis scorpioides*
Nierembergia rivularis
Oenothera fruticosa
Oenothera speciosa
**Opuntia microdasys*
**Opuntia vulgaris*
Pachysandra terminalis
**Papaver nudicaule*
**Papaver orientale*
Penstemon barbatus
**Penstemon heterophyllus*
**Penstemon laevigatus*
Penstemon ovatus
**Penstemon puniceus*
**Penstemon smalli*
Phlox divaricata
Phlox maculata
Phlox paniculata
Physalis alkekengi
**Physostegia virginiana*
**Polemonium pulcherrimum*
Polemonium reptans
**Potentilla macnabiana*
**Potentilla nepalensis*
Pueraria thunbergiana
**Rudbeckia maxima*
Rudbeckia nitida
Salvia azurea
Saponaria ocymoides
Scabiosa caucasica

*Scabiosa japonica
*Sedum spectabile
*Senecio pulcher
Sidalcea candida
Silene schafta
*Silphium perfoliatum
Solidago canadensis
Stachys grandiflora
Stachys lanata
*Stokesia laevis
Tanacetum vulgare
Thalictrum glaucum
*Thymus vulgaris
*Tradescantia virginiana
*Tritonia crocosmaeflora
Tuncia saxifraga
Valeriana officinalis
Veronica maritima
*Veronica repens
*Veronica spicata
Vinca minor
*Viola cornuta

DECIDUOUS PLANTS

Abelia grandiflora
Acer japonicum
Acer palmatum
Actinidia arguta
Aronia arbutifolia
Baccharis halimifolia
Berberis gagnepainii
Berberis ilicifolia
Berberis julianae
Berberis sargentiana
Berberis thunbergii
Berberis triacanthophora
Berberis verruculosa
Berberis vulgaris
Buddleia davidii
Callicarpa dichotoma
Callicarpa japonica
Caryopteris incana
Cercis chinensis
*Clematis heracleaefolia
*Clematis integrifolia
Clematis paniculata
Clethra alnifolia
Cornus alba
Cornus alternifolia
Cornus florida
Cornus kousa
Cotinus coggygria
Deutzia gracilis
Deutzia lemoinei
Elaeagnus multiflora
Elsholtzia stauntoni
Enkianthus campanulatus
Exochorda racemosa
Fagus sylvatica
Forythia intermedia
Forsythia suspensa
Hibiscus syriacus
Hydrangea arborescens
*Hydrangea macrophylla
Hydrangea paniculata
Larix decidua
Ligustrum lucidum
Ligustrum ovalifolium
Lonicera fragrantissima
Lonicera japonica
Lonicera maackii
Lonicera tatarica
Magnolia kobus
Magnolia sieboldi
Magnolia soulangeana
Magnolia stellata
Mahonia aquifolium
Malus floribunda
Malus halliana
Malus ioensis
Malus scheideckerii
Malus sieboldii
Nandina domestica
Parthenocissus tricuspidata
Philadelphus coronarius
Philadelphus lemoinei
Prunus serrulata
Quercus robur
Rhus aromatica
Rosa spp.
Rosa wichuraiana
Sorbaria aitchisoni
Spiraea thunbergii
Spiraea vanhouttei
Staphylea colchica
Stephanandra incisa
Symphoricarpos albus
Symphoricarpos orbiculatus

Symplocos paniculata
Syringa chinensis
Syringa persica
Syringa vulgaris
Tamarix odessana
Viburnum acerifolium
Viburnum cassinoides
Viburnum dentatum
Viburnum lantana
Viburnum prunifolium
Viburnum rhytidophyllum
Viburnum tomentosum
Weigela florida
Weigela hortensis

EVERGREENS

Abies concolor
Abies veitchi
Andromeda glaucophylla
Buxus sempervirens
Cedrus atlantica
Cedrus deodara
Chamaecyparis lawsoniana
Chamaecyparis nootkatensis
Chamaecyparis obtusa
Chamaecyparis pisifera
Cotoneaster acuminata
Cotoneaster acutifolia
Cotoneaster apiculata
Cotoneaster dammeri
Cotoneaster divaricata
Cotoneaster francheti
Cotoneaster horizontalis
Cotoneaster hupehensis
Cotoneaster microphylla
Cotoneaster nitens
Cotoneaster racemiflora
Cotoneaster rotundifolia
Cotoneaster salicifolia
Cotoneaster simonsii
Cryptomeria japonica
Daphne cneorum
Euonymus alatus
Euonymus bungeanus
Euonymus europaeus
Euonymus fortunei
Euonymus japonicus
Euonymus kiautschovicus
Hedera helix
Ilex aquifolium
Ilex crenata
Ilex glabra
Juniperus chinensis
Juniperus communis
Juniperus excelsa
Juniperus horizontalis
Juniperus sabina
Juniperus sphaerica
Juniperus squamata
Juniperus virginiana
**Kalmia latifolia*
Leucothoe catesbaei
Mahonia aquifolium
Pachysandra terminalis
Phillyrea decora
Picea abies
Picea glauca
Picea pungens
Pieris floribunda
Pieris japonica
Pinus densiflora
Pinus mugo
Pinus nigra
Pinus ponderosa
Pinus resinosa
Pinus strobus
Pinus sylvestris
Pinus thunbergii
Pseudotsuga taxifolia
Pyracantha coccinea
**Rhododendron arborescens*
**Rhododendron calendulaceum*
**Rhododendron carolinianum*
**Rhododendron catawbiense*
**Rhododendron caucasicum*
**Rhododendron maximum*
**Rhododendron molle*
**Rhododendron mucronatum*
**Rhododendron obtusum*
**Rhododendron occidentale*
**Rhododendron schlippenbachii*
**Rhododendron simsii*
**Rhododendron vaseyii*
**Rhododendron viscosum*
**Rhododendron yedoense*
Rosmarinus officinalis
Taxus baccata

Taxus brevifolia
Taxus canadensis
Taxus cuspidata
Taxus media
Thuja occidentalis
Thuja orientalis
Thuja plicata
Thuja standishi
Tsuga canadensis
Tsuga caroliniana

During 1929–50 the lead arsenate field treatment was used extensively in commercial nurseries. Middleton and Cronin (*1952*) reported that lead arsenate had been applied to 38,442,721 square feet and that 4,335,425 plants had been certified for shipment.

Nursery Seedbeds.—In cooperative studies with the U.S. Forest Service, Fleming et al. (*1937*) sowed seeds of coniferous trees in the soil of nursery seedbeds to which lead arsenate had been applied at 500 to 1,500 pounds per acre and mixed with the upper 3 inches of soil. The seed of *Thuja plicata* did not germinate in either the treated or untreated soil. Only a few seeds of *Larix occidentalis* and *Picea glauca* germinated. The germination of the seeds of the other species was adequate to determine their reaction to the chemical. The arsenical in the soil did not reduce significantly the number of seedlings of *Pinus caribaea, P. echinata, P. jeffreyi, P. monticola, P. palustris, P. resinosa,* and *P. strobus,* but it reduced progressively with the increment in the dosage the number of seedlings of *Picea engelmanni, P. pungens, P. rubens, Pinus banksiana, P. ponderosa, P. taeda,* and *Pseudotsuga taxifolia.* At the end of the growing season only the seedlings of *Picea rubens* and *Pseudotsuga taxifolia* were normal in color and in growth. The other species were retarded or there was browning of the foliage, which varied from a browning of the needle tips to complete discoloration.

Cover Crops.—It was the general practice in the more progressive nurseries to sow one or more cover crops in the field plots after the nursery stock had been harvested, and sometimes cover crops were sown between the rows of ornamental plants to increase the fertility of the soil and to reduce erosion. Rye and the clovers were the only cover crops that grew well in soil containing 1,500 pounds of lead arsenate per acre. Oats, barley, wheat, corn, soybeans, and cowpeas sometimes grew satisfactorily, but they were usually retarded by the arsenical. The retardation was more pronounced in light sandy soils than in the heavy clay and silt loams.

Garden Vegetables.—Some people grew nursery stock under contract with the larger nurseries in soil treated with lead arsenate, and when the nursery stock was harvested, they grew vegetables in the soil, a practice that was not encouraged. There

were also many inquiries from the public about growing vegetables on land to which lead arsenate had been applied several years previously.

McLean et al. (*1944*) planted the common vegetables in plots containing from 250 to 1,000 pounds of lead arsenate per acre in the upper 4 inches of soil. String beans and pea beans did not germinate or were killed in the early stages of growth. Other vegetables grew satisfactorily. When the plants were analyzed, none to a trace of arsenious oxide was found in string beans, a trace in broccoli, carrots, eggplant, peppers, and tomatoes, 0.10 to 0.16 p.p.m. in beet roots and lettuce, 0.11 to 0.17 p.p.m. in beet tops, 0.035 to 0.29 p.p.m. in radish roots, and 0.23 to 0.80 p.p.m. in radish tops.

Fleming et al. (*1948*) planted the common vegetables in plots containing from 1,000 to 2,000 pounds of lead arsenate per acre in the upper 3 inches of soil and from 2,000 to 4,000 pounds of the chemical in the upper 6 inches. When the chemical was mixed to a depth of 3 inches, lima beans germinated and died. The first planting of string beans failed, but a satisfactory stand was obtained with the second planting. The initial growth of the vegetables was retarded, but after the roots penetrated below the poisoned layer of soil, the plants grew normally. The yields of the vegetables were not reduced except onions. With the 2,000-pound treatment less than 0.7 p.p.m. of arsenious oxide was found in the edible part of beets, cabbage, corn, cucumbers, eggplant, muskmelon, parsnips, peppers, string beans, sweetpotatoes, tomatoes, and white potatoes; 0.7 to 1.4 p.p.m. in carrots and onions; and 1.4 to 2.8 p.p.m. in lettuce, radishes, and turnips.

When the arsenical was mixed with the upper 6 inches of soil, the plants had to draw a large part of their nourishment from the poisoned layer. Lima beans and string beans did not grow and turnips did not reach maturity. The yields of cabbage, carrots, corn, cucumbers, lettuce, muskmelon, onions, parsnips, pumpkin, and radishes were reduced, but normal yields were produced by beets, eggplant, peppers, sweetpotatoes, tomatoes, and white potatoes. With the 4,000-pound treatment less than 0.7 p.p.m. of arsenious oxide was found in beets, cabbage, carrots, corn, cucumbers, eggplant, muskmelon, parsnips, peppers, sweetpotatoes tomatoes, and white potatoes; 2.1 p.p.m. in lettuce; 4.0 p.p.m. in radishes; and 8.4 p.p.m. in onions. Except when 1,500 pounds of lead arsenate were applied annually, the quantity of the arsenical in the soil rarely exceeded 2,000 pounds per acre.

McLean et al. (*1944*) concluded that there was no danger in growing vegetables on soils that had received applications of

lead arsenate for one purpose or another, because if the arsenic in a soil was high, the plants did not grow. No tolerance had been established for arsenic assimulated by crops from the soil. Since some arsenic was assimulated and the hazard to human health was unknown, nurserymen and the public were advised that no vegetables should be grown on land treated with lead arsenate until it was established that such a practice was safe.

Leaching and Fixation in Soil.—Since arsenicals poison soil and impair its fertility, lead arsenate was not popular as a soil insecticide with the nurserymen, although it was very useful to them in satisfying the requirements of the quarantine (Fleming *1957*). The problem of arsenic residues in soil, however, was greater than the use of lead arsenate to eliminate the grubs. Boswell (*1952*) pointed out that the greatest accumulation of arsenic in orchard soils was in the Pacific Northwest where lead arsenate sprays had been applied annually to the trees for several years. A similar situation occurred in orchards of other States. Although the accumulation of arsenic in the upper layers of soil did not seem to be harmful to trees in old orchards, legume cover crops tended to become progressively poorer, and toxic symptoms appeared among young trees planted on land from which old sprayed orchards had been removed.

The toxicity of lead arsenate was modified by the nature of the soil. Smaller quantities of the arsenical were required to kill grubs in light sandy soil than in the heavier clay and silt loams (Fleming et al. *1936*, unpublished). Legumes on acid sandy soils were injured by small quantities of the arsenates, but were not injured by much larger applications on the heavier clay soils (Boswell *1952*; McMurtrey and Robinson *1938*).

The injurious action of lead arsenate to plants may be attributed largely to the water-soluble arsenic (arsenic acid) in the soil. Lead arsenate ($PbHAsO_4$) is relatively stable at pH 2 to pH 5, but under neutral and alkaline conditions water-soluble arsenic is liberated and the arsenate is changed to tri-lead arsenate ($Pb_3(AsO_4)_2$) or to lead hydroxy arsenate ($Pb_4(PbOH)(AsO_4)_3$) or ($Pb_5(PbOH)_2(AsO_4)_4$), compounds that are not toxic. There was, however, no relationship between the total and the water-soluble arsenic in soils. More water-soluble arsenic was produced in a sand by 500 pounds of lead arsenate per acre than in a clay loam by 2,000 pounds per acre, especially when the clay contained iron. The water-soluble arsenic reacts with the iron to form insoluble ferric arsenate ($FeAsO_4$), the mineral scorodite.

The active lead arsenate in soil may be lost by the leaching of the water-soluble arsenic and by conversion to inactive ar-

senates. Fleming (*1942*) determined the total lead arsenate by chemical analysis and the active lead arsenate by bioassy in the upper 6 inches of Sassafras sandy loam. Metzger (*1933*) had applied the arsenical to the soil 3 years previously to prevent adult beetles emerging during the winter in a greenhouse where roses were growing. The loss of lead arsenate by leaching and fixation in an inactive form is summarized in table 16.

During the 3-year period the average loss of the initial applications was 27 percent by leaching and 26 percent by fixation in the soil, indicating that several years would be required to remove the active lead arsenate by these natural processes. More loss by leaching and less by fixation in sands would be expected and less loss by leaching and more by fixation in heavy soils.

Coated Lead Arsenate.—It seemed that the phytotoxic action of lead arsenate in soil might be overcome by coating the particles of the arsenical with a nontoxic substance. Moore (*1922*) demonstrated that lead arsenate coated with lead oleate or lead stearate was more palatable to adult beetles and more effective in killing them than the uncoated arsenical.

Vander Meulen (unpublished) coated lead arsenate with various metallic soaps and with paraffin by dissolving the coating material in benzene or ethyl alcohol, mixing the solution with lead arsenate, and evaporating the solvent. Paraffin was the most promising coating because it is practically inert chemically. The final formulation contained 62.5 percent of lead arsenate and 37.5 percent of paraffin.

When mixed with soil the paraffin-coated lead arsenate was equally as toxic to grubs and to plants as the uncoated arsenical (Leach *1926;* Leach et al. *1924*). Fleming (*1927*) found that 40

TABLE 16.—*Loss of lead arsenate by leaching and fixation from upper 6 inches of Sassafras sandy loam in greenhouse during 3 years*

Quantity applied per acre (pounds)	Quantity recovered per acre		Quantity lost per acre	
	Total	Active	Leaching	Fixation
	Pounds	*Pounds*	*Pounds*	*Pounds*
1,000	800	600	200	200
1,500	1,200	700	300	500
2,000	1,500	[1] 950	500	550
2,500	1,700	1,100	800	600
3,000	2,100	1,350	900	750

[1] Estimated.

days after mixing the coated lead arsenate with soil the water-soluble arsenic in the soil was much higher than had been anticipated. The water-soluble arsenic in the soil with the coated lead arsenate was almost as much as with the uncoated arsenical, showing that the paraffin coating was being removed in the soil.

Fleming (*1927*) found that adding uncoated lead arsenate to Sassafras sandy loam greatly increased the number of bacteria but had no effect on the fungi in the soil. The coated arsenical had no effect on the bacteria but greatly increased the number of fungi. Paraffin had the same effect as the coated arsenical. The response of the fungi to the introduction of paraffin and paraffin-coated lead arsenate into the soil showed that these micro-organisms were using the paraffin in their metabolism. Earlier Greaves (*1916*) had found that arsenic stimulated the nitrogen-fixing bacteria, and Gainey (*1917*) had observed that the disappearance of paraffin in soil was accompanied by an enormous increase in the fungi.

Accelerating Fixation in Soil.—In preliminary experiments Mell (*1941*, unpublished) found that the water-soluble arsenic in the upper 6 inches of soil, containing 2,000, 3,000, and 4,000 pounds of lead arsenate per acre, was reduced to a low level within 30 days by applying ferrous sulfate ($FeSO_4$), limonite ($2\ Fe_2O_3 \cdot 3\ H_2O$), or greensand marl (a silicate of iron and potassium). The limonite and greensand marl overcame to a large extent the retarded growth of beans by lead arsenate. Ferrous sulfate was injurious to the plants, but a mixture of ferrous sulfate and lime was not harmful to them. These experiments, however, were not sufficiently extensive to establish how much of these iron compounds was required to fix a given quantity of lead arsenate in different soils. More extensive experiments were not conducted.

The experimental evidence in the 1930's was not sufficient to recommend these iron compounds to overcome arsenic toxicity in soil. Deep plowing was the only practical method available to reduce injury to cover crops by lead arsenate. This operation buried much of the arsenical and reduced the quantity in the upper layers of soil. Several years later Bear (*1957*) recommended the application of 1 to 2 tons of iron sulfate per acre to overcome the phytotoxicity of arsenical residues in soil.

Other Inorganic Arsenicals

Arsenates.—In preliminary tests with the inorganic arsenates in soil, Leach (*1926*) found that basic lead, ferric, and

magnesium arsenates were not toxic to third-instar grubs. Tricalcium arsenate seemed to have about the same toxicity as lead arsenate. Copper and zinc arsenates were slower in killing than lead arsenate. The tests were not conclusive because only a small number of grubs was used in determining their reaction to the different arsenates.

Fleming (*1942*) and Fleming and Baker (*1936a*) determined more definitely the reaction of third-instar grubs to different arsenates in Sassafras sandy loam. The quantity of each arsenate needed to obtain a toxicity equivalent to that of 1,000 pounds of lead arsenate per acre is as follows:

Arsenate	*Pounds per acre required*
Dicalcium	300
Tricalcium	550
Magnesium	600
Manganese	600
Barium	750
Zinc	750
Ferric	975
Aluminum	1,000
Basic lead	2,000+

The length of the toxic action in soil varied with the different arsenates. Fifty percent of the initial toxicity was lost in 1 year by dicalcium and tricalcium arsenates, in 2 years by barium, ferric, magnesium, manganese, and zinc arsenates, and in 4 years by aluminum and lead arsenates.

Hydrangea macrophylla was severely injured or killed within 2 weeks after applying the arsenates of aluminum, barium, dicalcium, magnesium, manganese, tricalcium, and zinc to the soil, and it was retarded by ferric and lead arsenates. Rye was slightly retarded by ferric and lead arsenates and was markedly retarded or killed by the other arsenates. Basic lead arsenate did not injure *Hydrangea macrophylla* or rye. Although ferric arsenate and lead arsenate cannot be used promiscuously to kill grubs, they were the safest of the arsenates to apply to soil about the roots of growing plants.

Arsenious Oxide.—Pierson and Nash (*1931*) recommended arsenious oxide (As_2O_3) to control white grubs in the soil of forest nurseries. Fleming (*1942*) found that 400 pounds of arsenious oxide per acre were equally as toxic to *Popillia* grubs as 1,000 pounds of lead arsenate per acre. It lost 50 percent of its toxicity in 4 years. However, rye, grasses, and nursery plants

were intolerant of insecticide concentrations of arsenious oxide in soil.

Arsenious Sulfide.—Arsenious sulfide (As_2S_3) had the same toxicity to grubs as arsenious oxide, but its toxicity was reduced more rapidly in Sassafras sandy loam. It lost 50 percent of its toxicity in 1 year. Arsenious sulfide was very detrimental to the growth of plants. Rye made only a feeble growth in soil containing as little as 50 pounds of the sulfide per acre. (Fleming *1942*)

Paris Green and Its Homologs.—The homologs of paris green (copper acetoarsenite) were prepared by Dearborn (*1935, 1936*). Toxicity to the grubs equivalent to that of 1,000 pounds of lead arsenate per acre was produced by the following pounds of paris green homologs: Copper oleoarsenite 250, paris green 400, copper stearoarsenite 1,000, copper lauroarsenite 1,300, and copper palmitoarsenite 1,600. All these arsenites were very detrimental to the growth of rye. (Fleming *1942*)

Inorganic Borates

The inorganic borates were only slightly toxic to the grubs. To obtain toxicity equivalent to that of 1,000 pounds of lead arsenate per acre in Sassafras sandy loam required the following pounds of borates: Zinc 2,200, nickel, sodium, and strontium 2,400, magnesium 2,500, calcium 2,800, and lead 3,200. All these borates were very detrimental to the growth of rye. (Fleming *1942*)

Inorganic Fluorides

The inorganic fluorides were only slightly toxic to the grubs. Toxicity equivalent to that of 1,000 pounds of lead arsenate per acre was produced by 2,000 pounds of zinc fluoride. Four thousand pounds of the fluorides of aluminum, barium, calcium, copper, lead, magnesium, or strontium did not raise the toxicity to this level. Rye grew well in soil containing these fluorides. (Fleming *1942*)

Inorganic Hexafluorosilicates

Preliminary tests by Lipp (*1929*) and Metzger (*1933*) indicated that some of the inorganic hexafluorosilicates might be of value in controlling the grubs in the soil.

A toxicity equivalent to that of 1,000 pounds of lead arsenate per acre was obtained with the following pounds of hexafluorosilicates: Sodium 750, potassium 900, barium 975, and magnesium 1,250. Two thousand pounds of calcium hexafluorosilicate did not

raise the toxicity in the soil to this level. After weathering for 6 months in the soil, the following hexafluorosilicates lost a certain percent of their toxicity: Sodium 81, potassium 78, barium 65, and magnesium 55, indicating rapid decomposition in the soil. At these rates the hexafluorosilicates were not toxic to rye. It would be necessary to apply them annually in the spring to kill grubs hatching in the soil during the summer. (Fleming *1942*)

Residual Chlorinated Hydrocarbon Insecticides Mixed With Soil

Since 1943 many chlorinated hydrocarbon insecticides have become available, including DDT, chlordane, toxaphene, aldrin, dieldrin, endrin, heptachlor, benzene hexachloride, lindane, endosulfan, and methoxychlor. In contrast to the halogenated hydrocarbons previously available, such as ethylene dibromide, ethylene dichloride, *p*-dichlorobenzene, chloropicrin, and methyl bromide, these newer chlorinated hydrocarbons were less volatile and more persistent in soil.

DDT

Commercial DDT is dichlorodiphenyl-trichloroethane. The principal constituent is 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane. It was the first of the residual chlorinated hydrocarbon insecticides to be tested as a substitute for lead arsenate to control Japanese beetle grubs.

Toxicity to Grubs.—DDT did not inhibit the female beetle in depositing eggs in soil or prevent the hatching of the eggs, but, as compared with lead arsenate, only a small quantity of DDT was needed to kill the grubs. The grubs developed tremors by contact with DDT or by ingesting soil containing the chemical, and they lost their ability for coordinated movement. With their uncoordinated convulsive movement the grubs near the soil surface often worked their way out of their natural environment and died on the surface. These dead and dying grubs were not usually attractive to birds. (Fleming and Maines unpublished)

Grubs have to come in contact with particles of DDT to be affected. Third-instar grubs in moist soil were sealed in a fumigation box containing 22 pounds of DDT per 1,000 cubic feet so that they were exposed only to the vapor at 80° F. Four weeks later when the box was opened, there was a pronounced odor of the chemical, but none of the grubs had any symptoms of poisoning. (Fleming and Maines unpublished)

DDT was much more toxic to the grubs than lead arsenate. The speed of insecticide action of 5 pounds of DDT per acre with first-instar grubs was equivalent to that of 500 pounds of lead arsenate per acre. Ten pounds of DDT per acre had about the same toxicity to third-instar grubs as 1,000 pounds of lead arsenate per acre. (Fleming *1948b*; Fleming and Maines *1944a, 1944b*)

Fleming and Maines (*1944b*, unpublished) found that the mortality of the grubs was modified by their age, the quantity of DDT in the soil, the temperature, and the prolongation of exposure to the chemical. The days required to kill all first- and third-instar grubs with different quantities of DDT per acre in Sassafras sandy loam at 50° to 80° F. are summarized in table 17. The velocity of insecticide action increased progressively with the increment in the temperature. The velocity at 60° was twice that at 50°; at 70° it was tripled and at 80° quadrupled.

Fleming and Maines (*1947*) collected soils from six physiographic areas in New Jersey and determined the effect of the soil origin on the insecticide action of DDT on third-instar grubs. The Appalachian Mountain soils have been derived by weathering of gneiss rock, the piedmont plateau soils from underlying crystalline and sedimentary rocks, and the limestone valley soils by weathering of limestone. The glacial till soils have been derived from unstratified till or drift, and the glacial lake and river terrace soils from stratified glacial deposits. The coastal plain soils have been developed from unconsolidated sand, gravel, and clay, which have been transported by water from older land areas and deposited in part under marine conditions. The effect of the origin of the soils on the insecticide action is summarized in table 18.

TABLE 17.—*Effect of temperature and DDT dosage on length of exposure required to kill Japanese beetle grubs in Sassafras sandy loam*

Temperature (° F.)	Exposure for 100-percent mortality of grubs with indicated pounds per acre				
	First instars		Third instars		
	2.5	5	10	25	50
	Days	*Days*	*Days*	*Days*	*Days*
50	-	-	160	120	76
60	--	- -	80	60	38
70		- - -	53	40	25
80	28	14	40	30	19

TABLE 18.—*Effect of origin, texture, and natural drainage of New Jersey soils on insecticide action of DDT on third-instar Japanese beetle grubs*

Soil origin	Soils	Exposure for 98-percent mortality at 80° F. with indicated pounds per acre		
		10	25	50
	Number	*Days*	*Days*	*Days*
Appalachian Mountains	2		26	18
Piedmont plateau	5		27	19
Limestone valley	1		25	19
Glacial till	3		27	20
Glacial lake and river terrace	2		28	20
Coastal plain	15		29	19
Texture				
Sand	2	32	20	16
Gravelly and shale loam	4	51	27	19
Sandy loam	9	51	31	19
Loam	8	53	28	19
Silt loam	5	53	28	20
Natural drainage				
Good	18		25	18
Inadequate	10		33	21

The soil origin was not an important factor. The velocity of insecticide action in soils within a physiographic area varied more than that from different areas.

The texture of the New Jersey soils did modify the speed of insecticide action, as shown in table 18. The mortality progressed faster in the sands than in the various loams, but the rates in the different loams were not significantly different. (Fleming *1950a;* Fleming and Maines *1947,* unpublished)

Some of the New Jersey soils have been developed under conditions of good drainage. In these well-aerated soils the oxidation process is not interrupted and leaching and alluviation are not inhibited. The minerals are in various stages of oxidation, denitrification is inhibited, and the nitrates tend to accumulate. Usually these soils contain little organic matter. Other soils have been developed under conditions of poor or inadequate drainage. In these soils the oxidation process, leaching, and alluviation are depressed and the restricted supply of air favors the accumulation of organic matter and the loss of nitrogen. As shown in table 18, the insecticide action of DDT was retarded in

poorly drained and poorly aerated soils. The retardation is probably associated with the higher content of organic matter in these soils. The waterlogged condition of these soils during some parts of the year also inhibits grub activity. (Fleming and Maines *1947*)

New Jersey soils of different series differ in their chemical composition and absorptive capacity. Fleming and Maines (*1947*) and Fleming (*1950a*) determined the average velocity of insecticide action of DDT in 21 of these soil series, as shown in table 19. The speed of the 25-pound dosage in killing third-instar grubs was modified more by the change in the chemical composition of the soils than was the 50-pound dosage. The insecticide action was the most rapid in soils of the Lakewood and Sassafras series and was the most retarded in the heavier soils of the Croton, Portsmouth, Woodstown, and Keyport series.

TABLE 19.—*Effect of variation in chemical composition of different series of New Jersey soils on insecticide action of DDT on third-instar Japanese beetle grubs*

Soil series	Exposure for 98-percent mortality at 80° F. with indicated pounds per acre	
	25	50
	Days	*Days*
Lakewood	18	15
Sassafras	18	15
St. Johns	21	16
Washington	21	18
Lansdale	23	17
Colts Neck	24	17
Berks	25	17
Dunellen	25	19
Hagerstown	25	19
Penn	27	18
Chester	28	19
Gloucester	28	20
Collington	30	18
Merrimac	31	20
Elkton	31	22
Shrewsbury	32	19
Wethersfield	32	22
Croton	35	24
Portsmouth	35	21
Woodstown	36	22
Keyport	37	22

The quantity of DDT applied to a cultivated plot should be sufficient to reduce substantially the third-instar grub population before pupation and to eliminate several successive generations of grubs hatching in the soil. The 10-pound dosage was too slow to be effective in killing the grubs when the temperature was as low as 60° F. The velocity of the insecticide action with 25 pounds at this temperature indicated that a large proportion of the third-instar grubs could be killed before pupation. Eighty percent of this dosage could be lost or inactivated without reducing its effectiveness in killing grubs hatching in the soil. The 50-pound dosage was excessive. From these considerations it was decided to use DDT at 25 pounds per acre mixed with the upper 3 inches of soil for experimentation in field plots of commercial nurseries.

Persistence.—When 25 pounds of DDT per acre were applied in the spring as a dust or granular formulation to the surface of fallow land and left there, the chemical dissipated rapidly. Chemical analyses and bioassays showed that 16, 28, 40, and 50 percent of the chemical was lost in 1, 2, 3, and 4 weeks, respectively.

Fleming and Maines (*1958a*) determined the insecticidally active DDT in 84 mineral soils from New England, New York, Ohio, New Jersey, and North Carolina during a 4-year period of weathering after mixing 25 pounds of the chemical with the soils. Third-instar grubs were used in the bioassays of the soils. The average active DDT found in soils from these areas is summarized in table 20. At the end of this period the average active DDT was 16.5 pounds per acre. This persistence of the chemical in soil is in contrast to that on the soil surface. Less DDT was lost and inactivated during 4 years in soil than during 4 weeks on the surface. There seemed to be a trend for active DDT to be

TABLE 20.—*Persistence of insecticidally active DDT in soils of different areas when applied at 25 pounds per acre*

Area	Soils	Average active DDT per acre after indicated years			
		1	2	3	4
	Number	*Pounds*	*Pounds*	*Pounds*	*Pounds*
New England	30	24.3	22.0	18.7	14.4
New York	2	24.8	21.8	18.5	14.0
Ohio	15	24.1	21.4	17.9	14.6
New Jersey	28	24.8	23.9	21.6	18.9
North Carolina	9	24.5	23.6	22.5	21.3
Total or average	84	24.5	22.5	19.8	16.5

more persistent in the soils of New Jersey and North Carolina than in the soils of New England, New York, and Ohio.

Other investigators, including Cullinan (*1949*), Foster (*1950, 1951*), Foster et al. (*1956*), and Smith (*1948*), found that DDT persisted for a long time in soil.

Fleming and Maines (*1953a*) found that the persistence of insecticidally active DDT in the soils of these areas was modified by their texture. The effect of texture on the persistence of the 25-pound dosage is summarized in table 21. The half-life of the treatment, a 50-percent reduction in the insecticidally active DDT, was more than 8 years in the sands, about 8 years in the sandy loams, about 6 years in the shale and stony loams, loams, silt loams, and probably clay loams, and a little over 1 year in muck

The soils of the different areas belonged to 67 soil series. As noted previously, the soils in a series are alike in all characteristics except the texture of the surface layer. Fleming and Maines (*1953a*) classified the series according to the amount of the 25-pound dosage that was insecticidally active after weathering for 3 years as follows:

Group 1, 90–100 percent.—Caneadea, Cecil, Chester, Chicopee, Davidson, Dunellen, Durham, Gloucester, Hadley, Hartford, Helena, Iredell, Lakewood, Marlboro, Merrimac, Newfield, Painesville, Penwood, St. Johns, Sassafras, Whippany, and Wooster.

Group 2, 75–90 percent.—Agawam, Berks, Brookston, Charlton, Collington, Colts Neck, Croton, Dover, Elkton, Georgeville, Hagerstown, Keyport, Lansdale, Mahoning, Ontario, Penn, Portsmouth, Shrewsbury, Washington, Wethersfield, and Woodstown.

Group 3, 50–75 percent.—Appling, Ashe, Bernardston, Berrien, Bridgehampton, Brookfield, Cheshire, Mentor, Narragansett, Ondawa, Paxton, Pittsfield, Reynolds, Suffield, Toledo, Wauseon, Wingdale, and Woodbridge.

Group 4, 25–50 percent.—Chenango, Coloma, Essex, Lorain, Menlo, and Plymouth.

The average active DDT in each group was determined at intervals and is also summarized in table 21. The half-life of the 25-pound dosage was more than 8, 6, and 4 years in the soils of group 1, 2, and 3, respectively, and between 2 and 3 years in the soils of group 4.

Potting Soil.—To facilitate the mixing of small quantities of DDT with potting soil, the chemical was applied as a dilute dust or as a dilute emulsion. At 80° F. complete mortality of newly

TABLE 21.—*Persistence of insecticidally active DDT in soils of different texture and different series groups when applied at 25 pounds per acre*

Texture	Soils	Average active DDT per acre after indicated years					
		1	2	3	4	6	8
	Number	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Sand	3	24.8	23.3	22.0	21.0	20.0	18.5
Sandy loam	37	24.6	22.9	20.4	16.8	14.9	12.0
Shale and gravelly loam	6	24.8	22.3	18.6	14.6	12.0	10.0
Loam	21	24.0	21.9	18.7	14.3	13.5	9.4
Silt loam	12	24.9	23.9	21.1	16.7	11.7	8.5
Clay loam	5	23.4	21.4	18.7	...	...	...
Muck	1	14.0	9.5	6.0	3.5	...	...
Series group							
1	27	25.0	24.9	24.5	22.4	18.0	13.2
2	30	24.7	23.4	20.7	17.7	13.1	10.4
3	20	24.1	20.7	16.5	12.6	8.8	6.2
4	7	22.4	16.4	8.6	6.3	...	...

hatched grubs in a sandy loam was not obtained with 2.7 grams per cubic yard in 2 weeks, but all first instars were killed by 5.4, 10.8, 27, and 54 grams in 5, 4, 3, and 2 days, respectively. Third-instar grubs were more resistant. To obtain complete mortality required 50 days with 10.8 grams, 28 days with 27 grams, and 19 days with 54 grams. (Fleming *1947a*)

There was a close relationship between the temperature and the rate of poisoning. To kill third-instar grubs in this soil with the 27-gram dosage required 28, 42, 56, and 112 days at 80°, 70°, 60°, and 50° F., respectively. (Fleming *1947a*)

The type of soil affected the velocity of poisoning with the 27-gram dosage. To kill all grubs at 80 F. required 20 days with sands, 31 days with sandy loams, and 28 days with loams. The addition of organic matter to the soils retarded the insecticide action. (Fleming *1947a*)

Third-instar grubs obtained a toxic dosage of DDT long before they died in soil containing 27 grams of the chemical per cubic yard. The velocity of poisoning in a sandy loam at 60° F. progressed at the same rate with grubs left in the soil for 28 days as with those left in the soil until all were dead. (Fleming and Maines unpublished)

DDT mixed with potting soil at 27 grams per cubic yard was authorized in 1946 as a basis for certifying potting soil. The soil could be certified in 30 days at temperatures at least 80° F., in 45 days at 70°–79°, in 60 days at 60°–69°, and in 120 days at 50°–59°. The treatment was rarely used below 60°. Later the soil could be certified after holding for 28 days at not lower than 60°. Treated potting soil could be in a certified status for 5 years, but the longevity of the certified period was determined by soil analysis.

During 1946–50 DDT was applied to 3,765 cubic yards of potting soil in commercial nurseries (Middleton and Cronin *1952*).

Planted and Unplanted Plots.—As an inducement to nurserymen to cooperate in experiments to determine the effectiveness of DDT in eliminating grubs in nursery beds and plots, the Japanese Beetle Laboratory arranged with the then Division of Japanese Beetle Control (U.S. Dept. Agr.) that consideration would be given to certifying the plants if surveys in September demonstrated that no living grubs were in the treated beds and plots.

In the spring of 1944 DDT was applied at 20, 30, and 50 pounds per acre and mixed by cultivation with the upper 3 inches of soil before planting the plots. A survey of the plots

was made in September. Two grubs were found in the plot with 20 pounds of DDT, none in the plots with the 30 and 50 pounds, and 257 in the untreated plot. The plots were prepared for planting the following spring by disking; no additional DDT was applied. In the fall of 1945 no grubs were found in the treated plots and 30 in the untreated plot. (Fleming and Maines *1947*; Hadley and Fleming *1945*)

In the spring of 1945 and 1946 DDT was applied at 25 pounds per acre and mixed with the upper 3 inches of soil at several commercial nurseries. It was applied before planting and to plots of established nursery stock. In the surveys in the fall of 1945 only one moribund grub was found in the treated plots and 1,490 in the untreated plots. No grubs were found in the treated plots in the fall of 1946. (Fleming *1947a*; Fleming and Maines *1947*)

When DDT was applied as a dust or as a spray to the surface of nursery beds and plots at 25 pounds per acre and *not* mixed with the upper 3 inches of soil, the grub population was markedly reduced, but complete elimination was not always obtained. A survey of the plots treated in this manner at several nurseries showed a total of 38 grubs in the treated and 491 in the untreated plots in September. This survey emphasized the importance of mixing the DDT with the soil soon after application. (Fleming and Maines *1947a*)

In more extensive field tests 25 pounds of DDT per acre, applied and mixed with the upper 3 inches of soil before the eggs hatched, eliminated by early fall six to eight annual broods of grubs that hatched subsequently in the nursery plots.

DDT applied at 25 pounds per acre and mixed by cultivation with the upper 3 inches of soil was authorized in 1946 as a basis for certifying beds and plots of nursery stock before digging. The treatment had to be completed within 14 days after emergence of the adult beetle at a locality. The plants could be certified 60 days later. This period provided adequate time for grubs and pupae of the previous generation to be killed or emerge and to eliminate grubs hatching in the soil. A treated plot or bed could be in a certified status for 5 years, but its actual longevity was determined by analysis of the soil.

During 1946–50 DDT was applied to 63,459,413 square feet of soil in nursery beds and plots, and the plants in them were certified for shipment (Middleton and Cronin *1952*).

Nursery and Greenhouse Plants.—The preliminary tests with annual flowers, vegetables, and a few nursery plants showed that many of the species grew normally in potting soil con-

taining 27 grams of DDT per cubic yard and in beds containing 25 pounds of the chemical per acre in the upper 3 inches of soil. Increasing the dosage to 54 grams per cubic yard and to 50 pounds per acre retarded the growth of some species. During 1945–47 in cooperative studies with 73 nurseries and greenhouses in Connecticut, Maine, Massachusetts, New Jersey, New York, North Carolina, Ohio, Pennsylvania, Rhode Island, and Vermont, Fleming (*1947b*, *1948b*) conducted tests to determine the effect of these treatments on nursery and greenhouse plants under various environmental conditions. Over 500 species of plants were grown satisfactorily in the DDT-treated soil. The species grown without apparent injury were as follows:

Abutilon vitifolium
Acalypha hispida
Acanthus montanus
Acer coriaceum
Acer palmatum
Achillea filipendulina
Achillea millefolium
Achillea ptarmica
Aconitum carmichaelii
Aconitum napellus
Acorus gramineus
Adiantum pendatum
Adonis amurensis
Aechmea calyculata
Aechmea coelestis
Aechmea fasciata
Aechmea fulgens
Aechmea weilbachia
Aeschynanthus pulcher
Ageratum houstonianum
Aglaonema commutatum
Aglaonema costatum
Aglaonema simplex
Ajuga genevensis
Ajuga reptans
Allamanda cathartica
Allium schoenoprasum
Alocasia cuprea
Alocasia macrorhiza
Alocasia sanderiana
Alpinia sanderae
Alsophila australis
Althaea rosea
Alyssum saxatile
Amomum cardamon
Amsonia tabernaemontana
Ananas comosus
Ananas porteanus
Anchusa azurea
Anemone japonica
Anemone pulsatilla
Anthemis tinctoria
Anthurium album
Anthurium andraeanum
Anthurium carneum
Anthurium crystallinum
Anthurium ferrierense
Anthurium ornatum
Anthurium scherzerianum
Anthurium selloum
Anthurium warocqueanum
Aquilegia alpina
Aquilegia chrysantha
Aquilegia coerulea
Aquilegia longissima
Aquilegia vulgaris
Arabis alpina
Araucaria excelsa
Ardisia crenulata
Aregelia marmorata
Aregelia spectabilis
Aregilia tristis
Arenaria verna
Aristolochia durior
Armeria maritima
Armeria pseudoarmeria
Aronia arbutifolia
Artemisia dracunculus
Artemisia lactiflora
Artemisia vulgaris
Aruncus sylvester
Asclepias tuberosa

Asperula odorata
Aspidistra elatior
Asplenium bulbiferum
Asplenium nidus
Aster alpinus
Aster dumosus
Aster novae-angliae
Aster novi-belgii
Aster subcoeruleus
Aster thomsonii
Astilbe astilboides
Astilbe thunbergii
Aubrieta deltoidea
Baptisia australis
Begonia aconitifolia
Begonia coccinea
Begonia comata
Begonia foliosa
Begonia fuchsioides
Begonia glaucophylla
Begonia haageana
Begonia heracleifolia
Begonia imperialis
Begonia luxurians
Begonia maculata
Begonia manicata
Begonia metallica
Begonia nitida
Begonia rex cultorum
Begonia semperflorens
Begonia ulmifolia
Bellis perennis
Beloperone guttata
Berberis cretica
Berberis mentorensis
Berberis thunbergii
Berberis verruculosa
Bertolonia pubescens
Billbergia amoena
Billbergia nutans
Billbergia pyramidalis
Billbergia sanderiana
Billbergia zebrina
Boltonia latisquama
Bougainvillea spectabilis
Bougainvillea trolli
Bouvardia humboldti
Bouvardia hybrida
Brunfelsia latifolia
Brunnera macrophylla
Buddleia davidi
Buxus balearica
Buxus sempervirens
Calathea lietzei
Calathea lindeniana
Calathea makoyana
Calathea ornata
Calathea vandenheckei
Calathea zebrina
Calceolaria crenatiflora
Calendula officinalis
Calluna vulgaris
Camellia japonica
Campanula carpatica
Campanula garganica
Campanula medium
Campanula persicifolia
Campanula poscharskyana
Campanula rotundifolia
Canna generalis
Capsicum frutescens
Caryopteris incana
Cassia marilandica
Catananche caerulea
Centaurea dealbata
Centaurea macrocephala
Centaurea montana
Centranthus ruber
Cerastium tomentosum
Ceratostigma plumbaginoides
Cereus candelaris
Chamaecyparis lawsoniana
Chamaecyparis nootkatensis
Chamaecyparis obtusa
Chamaecyparis pisifera
Chelone glabra
Chelone lyoni
Chlorophytum elatum
Chorizema ilicifolium
Chrysanthemum amaliae
Chrysanthemum arcticum
Chrysanthemum cinerariae-folium
Chrysanthemum coccineum
Chrysanthemum maximum
Chrysanthemum morifolium
Chrysanthemum uliginosum
Cibotium schiedei
Cimicifuga foetida
Cimicifuga racemosa
Cissus discolor
Cissus rhombifolia

Cissus sicyoides
Cissus striata
Citrus limon
Citrus taitensis
Clematis paniculata
Clerodendron thomsonae
Codiaeum variegatum
Coleus autrani
Colocasia antiquorum
Convallaria majalis
Cordyline terminalis
Coreopsis grandiflora
Coreopsis lanceolata
Coreopsis tinctoria
Cornus florida
Cotoneaster acutifolia
Cotoneaster horizontalis
Crassula arborescens
Croton punctatus
Cryptantha acaulis
Cryptantha beuckeri
Cryptantha zonatus
Cryptomeria japonica
Ctenanthe oppenheimiana
Cyclamen neapolitanum
Cyperus alternifolius
Cypripedium acaule
Cypripedium calceolus
Cypripedium reginae
Cyrtomium falcatum
Daphne cneorum
Davallia fijiensis
Davallia pentaphylla
Delphinium elatum
Delphinium grandiflorum
Deutzia gracilis
Deutzia scabra
Dianthus barbatus
Dianthus caryophyllus
Dianthus deltoides
Dianthus latifolius
Dianthus plumarius
Dicentra cucullaria
Dicentra eximia
Dicentra spectabilis
Dictamnus albus
Dieffenbachia bausei
Dieffenbachia imperialis
Dieffenbachia picta
Dieffenbachia splendens
Digitalis ambigua
Digitalis purpurea
Doronicum caucasicum
Doronicum clusi
Doronicum pardalianches
Dracaena deremensis
Dracaena fragrans
Dracaena godseffiana
Dracaena goldicana
Dracaena gracilis
Dyckia sulphurea
Echinacea purpurea
Echinops humilis
Echinops ritro
Epimedium alpinum
Epimedium macranthum
Epimedium pinnatum
Episcia cupreata
Eremurus robustus
Erica carnea
Erica mediterranea
Erica stricta
Erica tetralix
Erigeron speciosus
Erinus alpinus
Erodium chamaedryoides
Eryngium alpinum
Eryngium amethystinum
Erysimum asperum
Eucharis grandiflora
Euonymus alatus
Euonymus fortunei
Eupatorium coelestinum
Euphorbia epithymoides
Euphorbia pulcherrima
Fatsia japonica
Ficus elastica
Ficus pumila
Ficus radicans
Ficus rubiginosa
Filipendula hexapetala
Filipendula purpurea
Filipendula rubra
Fittonia verschaffelti
Forsythia intermedia
Forsythia suspensa
Fuchsia magellanica
Gaillardia aristata
Gardenia jasminoides
Gentiana acaulis
Gentiana andrewsii
Gentiana linearis

Geranium platypetalum
Geranium sanguineum
Geum borisi
Geum chiloense
Gillenia trifoliata
Ginkgo biloba
Globularia cordifolia
Grevillea robusta
Guzmania musaica
Gypsophila paniculata
Gypsophila repens
Gypsophila viscosa
Hebe lyalli
Hedera canariensis
Hedera helix
Helenium autumnale
Helenium tenuifolium
Helianthemum nummularium
Helianthus annuus
Heliconia bihai
Heliconia illustris
Heliopsis scabra
Heliotropium arborescens
Helleborus niger
Helxine soleiroli
Hemerocallis fulva
Hemerocallis thunbergii
Hemigraphis colorata
Hepatica americana
Herniaria glabra
Heuchera lithophila
Heuchera sanguinea
Hibiscus rosa-sinensis
Hoffmannia ghiesbreghti
Hoffmannia refulgens
Homalocladium platycladum
Homalomena wallisii
Hosta caerulea
Hosta fortunei
Hosta plantaginea
Hosta undulata
Hoya carnosa
Hydrangea arborescens
Hydrangea macrophylla
Hydrangea paniculata
Hypericum calycinum
Iberis sempervirens
Ilex cornuta
Ilex glabra
Ilex opaca
Impatiens sultanii
Iris cristata
Iris pumila
Ixora coccinea
Ixora congesta
Jacobinia carnea
Jasminum mesnyi
Jasminum officinale
Jasminum sambac
Juniperus chinensis
Juniperus communis
Juniperus excelsa
Juniperus horizontalis
Juniperus sabina
Juniperus scopulorum
Juniperus squamata
Juniperus virginiana
Juniperus wallichiana
Kalanchoe blossfeldiana
Kalanchoe globulifera
Kniphofia uvaria
Laburnum anagyroides
Lathyrus latifolius
Lavandula officinalis
Leontopodium alpinum
Liatris pycnostachya
Liatris scariosa
Ligularia kaempferi
Lilium longiflorum
Linum flavum
Linum perenne
Lobelia cardinalis
Lobelia siphilitica
Lonicera canadensis
Lonicera henryi
Lonicera ruprechtiana
Lonicera tatarica
Lychnis viscaria
Lysimachia nummularia
Lysimachia punctata
Lythrum salicaria
Magnolia liliflora
Magnolia soulangeana
Magnolia stellata
Mahonia aquifolium
Maranta arundinaceae
Maranta bicolor
Maranta leuconeura
Marcgravia elegans
Matricaria tchihatchewi
Mazus japonicus
Medinilla magnifica

Mentha piperita
Mentha rotundifolia
Mertensia virginica
Mimosa pudica
Monarda didyma
Monarda fistulosa
Mondo japonicum
Muehlenbeckia axillaris
Musa nana
Myosotis alpestris
Myosotis scorpioides
Neomarica gracilis
Neomarica northiana
Nepenthes atrosanguinea
Nepenthes domini
Nepenthes intermedia
Nepenthes williamsi
Nepeta mussini
Nephrolepis exaltata
Nephthytis afzelii
Nidularium fulgens
Nidularium innocenti
Nierembergia rivularis
Oenothera fruticosa
Oenothera glauca
Oenothera missouriensis
Opuntia tuna
Origanum vulgare
Osmanthus fortunei
Osmanthus fragrans
Osmanthus ilicifolius
Pachysandra procumbens
Pachysandra terminalis
Pandanus baptistii
Pandanus sanderii
Pandanus veitchii
Panicum capillare
Papaver orientale
Parthenocissus tricuspidata
Passiflora alatocaerulea
Passiflora quadrangularis
Passiflora racemosa
Pelargonium domesticum
Pelargonium grandiflorum
Pelargonium odoratissimum
Pellaea viridis
Pellionia daveauana
Pellionia pulchra
Penstemon barbatus
Penstemon digitalis
Penstemon garretti
Penstemon laevigatus
Penstemon torreyi
Peperomia crassifolia
Peperomia obtusifolia
Peperomia rotundifolia
Peperomia sandersi
Petrea volubilis
Petunia hybrida
Philodendron acuminatissimum
Philodendron andreanum
Philodendron cordatum
Philodendron corsinianum
Philodendron erubescens
Philodendron lucerum
Philodendron mamei
Philodendron selloum
Philodendron verrucosum
Phlox decussata
Phlox divaricata
Phlox frondosa
Phlox glaberrima
Phlox nivalis
Phlox ovata
Phlox paniculata
Phlox subulata
Phyllitis scolopendrium
Physalis alkekengi
Physostegia virginiana
Picea abies
Picea glauca
Picea pungens
Pieris japonica
Pilea nummulariaefolia
Pilea pumila
Pinus mugo
Piper magnificum
Piper nigrum
Piper ornatum
Platycerium angolense
Platycerium bifurcatum
Platycerium grande
Platycerium hilli
Platycerium veitchi
Platycerium willinckii
Platycodon grandiflorum
Plectranthus discolor
Plumbago rosea
Polemonium pulcherrimum
Polemonium reptans
Polygonatum multiflorum
Polypodium aureum

Polypodium subauriculatum
Polyscias balfouriana
Polystichum acrostichoides
Polystichum tsussimense
Potentilla andicola
Potentilla fruticosa
Potentilla tridentata
Potentilla verna
Primula auricula
Primula denticulata
Primula florindae
Primula japonica
Primula juliae
Primula polyantha
Primula sieboldi
Primula veris
Primula vulgaris
Prunus nipponica
Pseudotsuga taxifolia
Pteris cretica
Pteris ensiformis
Pteris multifida
Pteris quadriaurita
Pteris tremula
Pulmonaria angustifolia
Pulmonaria saccharata
Puya alpestris
Pyracantha coccinea
Rhapidophyllum hystrix
Rhektophyllum mirabile
Rhododendron calendulaceum
Rhododendron catawbiense
Rhododendron gandavense
Rhododendron indicum
Rhododendron maximum
Rhododendron molle
Rhododendron obtusum
Rhododendron ponticum
Rhododendron rutherfordiana
Rhododendron sanderi
Rhoeo discolor
Rorippa nasturtium-aquaticum
Rosa multiflora
Rosa odorata
Rosa rugosa
Rosmarinus officinalis
Rubus reflexus
Rudbeckia laciniata
Sagina glabra
Saintpaulia ionantha
Saintpaulia kewensis
Salvia nemorosa
Salvia officinalis
Salvia pitcheri
Salvia pratensis
Salvia virgata
Sansevieria zeylanica
Santolina chamaecyparissus
Santolina virens
Saxifraga sarmentosa
Scabiosa caucasica
Scabiosa fischeri
Schismatoglottis neo-guinecnsis
Scindapsus aureus
Scindapsus pictus
Scirpus cernuus
Sedum acre
Sedum hybridum
Sedum kamtschaticum
Sedum reflexum
Sedum selskianum
Sedum sieboldi
Sedum spectabile
Sedum spurium
Sedum stoloniferum
Selaginella caulescens
Selaginella denticulata
Selaginella emmeliana
Selaginella uncinata
Selaginella willdenovi
Sempervivum arachnoideum
Sempervivum arenarium
Sempervivum ruthenicum
Sempervivum tectorum
Senecio cruentus
Senecio pulcher
Serissa foetida
Silene schafta
Sonerila margaritacea
Sparmannia africana
Spathyphyllum floribundum
Stenanthium robustum
Stenotaphrum secundatum
Stephanotis floribunda
Stokesia laevis
Strelitzia reginae
Syringa vulgaris
Taxus baccata
Taxus brevifolia
Taxus canadensis

Taxus cuspidata
Taxus hunnewelliana
Taxus media
Tetrastigma voinierianum
Teucrium chamaedrys
Thalictrum aquilegifolium
Thalictrum dipterocarpum
Thalictrum glaucum
Thermopsis caroliniana
Thuja occidentalis
Thuja orientalis
Thuja plicata
Thymus serpyllum
Thymus vulgaris
Tiarella cordifolia
Tibouchina semidecandra
Tillandsia lindeniana
Tolmiea menziesi
Trachelospermum jasminoides
Tradescantia fluminensis
Tradescantia fuscata
Tradescantia reginae
Tradescantia virginiana
Trichostigma peruvianum
Trillium erectum
Trillium grandiflorum
Trillium sessile
Trillium undulatum
Trollius europaeus
Trollius ledebouri
Tropaeolum majus
Tsuga canadensis
Vaccinium spp.
Valeriana officinalis
Veronica incana
Veronica latifolia
Veronica maritima
Veronica spicata
Veronica spuria
Viburnum burkwoodii
Viburnum carlesii
Viburnum dilatatum
Viburnum opulus
Viburnum setigerum
Viburnum tomentosum
Vinca minor
Viola calcarata
Viola canadensis
Viola cornuta
Viola kitaibeliana
Viola odorata
Viola pedata
Viola tricolor
Vitis spp.
Vriesia cardinalis
Vriesia erecta
Vriesia hieroglyphica
Vriesia poelmani
Vriesia speciosa
Weigela florida
Xanthosoma lindeni
Yucca filamentosa
Zebrina pendula
Zinnia elegans

The plants grown in DDT-treated soil and certified for shipment totaled 40,975,031 during 1946–50 (Middleton and Cronin *1952*).

Soil Improvement Crops.—Nurseries using rye, soybeans, and corn in rotation with nursery stock to improve the fertility of their soil had no difficulty in growing these crops on land treated with DDT. Fleming and Maines (*1952*) used rotations of rye, soybeans, and corn, soybeans, corn, and rye, and corn, rye, and soybeans in studying the effect of DDT in the upper 3 inches of soil on the yields of these crops. DDT was applied at 25 and 60 pounds per acre and mixed by cultivation with the upper 3 inches of soil by disking before planting. After each crop was harvested, it was disked into the soil. Each spring the land was prepared for planting by disking. The analyses of the soil during the fourth

year showed that practically all the residual DDT was within the upper 3-inch layer of soil.

The DDT retarded the early seedling growth of rye, but it had no effect on the germination and growth of corn and soybeans. When the yields on the plots with the 25- and 60-pound dosages were compared with those on untreated plots, the relative average yields over the 4-year period were 86 and 81 percent with rye, 99 and 93 percent with soybeans, and 96 and 101 percent with corn, respectively. However, the variation in the yields on plots receiving the same treatment was such that the differences in yield between the treated and untreated plots were not significant, except those with rye the first year.

TDE

TDE, a byproduct in the production of DDT, is closely related to DDT in chemical structure and in properties. The commercial product is largely 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethane.

Fleming and Maines (*1950a*) found that TDE was slightly less toxic to grubs than DDT. In laboratory tests with third-instar grubs, 13 pounds of TDE per acre were required to produce a toxicity in the soil equivalent to that of 10 pounds of DDT. In field tests both chemicals were applied at 25 pounds per acre in May. TDE reduced the third-instar grub population by 42 to 76 percent before pupation, and by September it had eliminated 84 to 89 percent of the grubs hatching in the soil during the summer. DDT reduced the third-instar grub population by 75 to 78 percent and eliminated 99.4 to 99.8 percent of the first instars. Probably if TDE had been applied at 33 pounds per acre, the control of the grubs would have been equivalent to that with the 25 pounds of DDT. Although a potential substitute for DDT, the possibilities of TDE were not explored further because of the higher dosage required to control the grubs.

Methoxychlor

Methoxychlor is closely related to DDT in that the two *p*-chlorophenyl groups of DDT are replaced by *p*-methoxyphenyl groups. Technical methoxychlor is largely 1,1,1-trichloro-2,2-bis (*p*-methoxyphenyl) ethane. Other designations are methoxy DDT, DMDT, and Marlate.

In laboratory tests with third-instar grubs, 100 pounds of methoxychlor per acre were required to produce a toxicity in the

soil equivalent to that of 10 pounds of DDT. In view of its relatively low toxicity the possibilities of methoxychlor were not explored further. (Fleming and Maines *1950b*)

Toxaphene

Toxaphene is chlorinated camphene containing 67–69 percent of chlorine.

Toxicity to Grubs.—Toxaphene did not inhibit the hatching of eggs. A few first-instar grubs transformed into second instars in soil containing 1.25 pounds of toxaphene per acre, but all were killed within 2 weeks at 80° F. by a 2.5-pound dosage. A 2.5-pound dosage of DDT killed 99.6 percent of the newly hatched grubs. (Fleming et al. unpublished)

The relative toxicity of toxaphene and DDT to third-instar grubs was determined in 71 types of soils occurring in Connecticut, Massachusetts, New Jersey, New York, North Carolina, Rhode Island, and Virginia. The days required to kill 98 percent of the grubs at 80° F. with 25 pounds per acre of these chemicals in these soils grouped according to texture are summarized in table 22. Toxaphene tended to be slightly slower than DDT in killing the grubs, but an analysis of the variance showed that the differences between the chemicals were not significant. (Fleming and Maines *1951*)

The addition of finely divided peat to Sassafras sandy loam retarded the insecticide action of the 25-pound dosage of toxa-

TABLE 22.—*Effect of texture of different soils on insecticide action of toxaphene and DDT on third-instar Japanese beetle grubs*

Texture	Soils	Exposure for 98-percent mortality at 80° F. with 25 pounds per acre of—	
		Toxaphene	DDT
	Number	*Days*	*Days*
Sand	3	16	15
Gravelly and shale loam	4	17	18
Sandy loam	29	18	16
Loam	20	19	18
Silt loam	11	14	16
Silty clay loam	2	20	12
Clay loam	1	11	11
Muck	1	41	30
Total or average	71	19	17

phene. When third-instar grubs were introduced into the soil-peat mixtures immediately after applying the chemical, 98-percent mortality of the grubs was obtained in 7 days at 80° F. with the soil and 3:1 mixture of soil and peat, in 11 days with 1:1 and 1:3 mixtures, and in 12 days with peat. When grubs were introduced 9 weeks later, this level of mortality was reached in 8 days with the soil, 23 days with 3:1 mixture, 24 days with 1:1 mixture, 29 days with 1:3 mixture, and 41 days with peat. (Fleming and Maines *1951*)

The temperature of the soil also modified the velocity of the insecticide action with the 25-pound dosage of toxaphene in Sassafras sandy loam. To kill all third-instar grubs with this dosage required an exposure of 30, 40, 60, and 120 days at 80°, 70°, 60°, and 50° F., respectively. (Fleming et al. unpublished)

In comparative field tests toxaphene and DDT were applied in May at 25 pounds per acre. Toxaphene reduced the third-instar grub population by 68–69 percent before pupation and by September had eliminated 97–98 percent of the grubs hatching in the soil during the summer. DDT reduced the third-instar population by 75–78 percent and by September had eliminated 99 percent of the newly hatched grubs. (Fleming and Maines *1951*)

Pound for pound toxaphene was equivalent to DDT in toxicity to the grubs.

Persistence.—Toxaphene disappeared rapidly when applied as a dust at 25 pounds per acre and left on the surface of fallow ground. Chemical analyses showed that 12, 24, 34, 42, 64, and 92 percent of the chemical were lost in 1, 2, 3, 4, 8, and 16 weeks, respectively. (Fleming et al. unpublished)

When toxaphene was mixed with Sassafras sandy loam at 25 pounds per 3-inch acre and exposed to weathering in the field, the persistence of this insecticidally active chemical in the soil was about the same as that of DDT. The bioassays of this soil treated with toxaphene and with DDT are summarized in table 23. After weathering for 7 years 58 percent of the toxaphene and 54 percent of the DDT were insecticidally active. The residues of both chemicals at that time were more than needed to eliminate grubs hatching in the soil. (Fleming et al. unpublished)

Potting Soil.—All the newly hatched Japanese beetle grubs were not killed by mixing 1.4 grams of toxaphene with a cubic yard of potting soil, but none of them survived when the dosage was increased to 2.7 grams per cubic yard. (Fleming et al. unpublished)

TABLE 23.—*Persistence of insecticidally active toxaphene and DDT in Sassafras sandy loam when applied at 25 pounds per acre*

Weathering (years)	Active insecticide per acre	
	Toxaphene	DDT
	Pounds	*Pounds*
1	23.5	23.5
2	22.0	22.0
3	20.5	20.0
4	19.0	18.5
5	17.5	17.0
6	16.0	15.5
7	14.5	13.5

Third-instar grubs were killed within 28 days at 80° F. by mixing 27 grams of toxaphene with a cubic yard of soil. The exposure had to be prolonged for 42 days at 70°, 56 days at 60°, and 112 days at 50° before all the grubs were dead at these temperatures. However, the grubs obtained a toxic dosage long before they were all dead. The velocity of poisoning at 60° progressed at the same rate with grubs left in the soil for 28 days as with those left in the soil until all of them were dead. (Fleming and Maines unpublished)

Toxaphene mixed with potting soil at 27 grams per cubic yard was authorized in 1953 as a basis for certification. The soil could be certified in 30 days at temperatures at least 80° F., in 45 days at 70°–79°, in 60 days at 60°–69°, and in 120 days at 50°–59°. Later the soil could be certified after holding for 28 days at not lower than 60°.

There is no record of toxaphene being used for the treatment of potting soil in commercial nurseries. When toxaphene was authorized, DDT had been used for several years for treating the soil and there was no advantage in substituting toxaphene for DDT.

Planted and Unplanted Plots.—In experimental plots toxaphene had been shown to be equivalent to DDT in eliminating grubs hatching in the soil during the summer. The application of 25 pounds of toxaphene per acre, mixed by cultivation with the upper 3 inches of soil, was authorized tentatively in 1953 with the understanding that consideration would be given to certifying the plants if surveys in September demonstrated that no living grubs were in the soil. It was not possible to arrange with commercial nurseries to apply toxaphene to planted or unplanted plots because they did not want to assume the potential risk that the

plots would not be certified in the fall. This attitude was understandable because the nurseries had been using DDT with satisfactory results for several years.

Nursery and Greenhouse Plants.—In preliminary tests of the tolerance of plants to toxaphene in soil, Fleming and Maines (*1951*, unpublished) found that broccoli, cabbage, celery, corn, eggplant, kale, lettuce, peppers, radishes, squash, string beans, tomatoes, and turnips were not affected by toxaphene applied at 27 grams per cubic yard of soil or 25 pounds per acre, but beets and cucumbers were retarded in growth. Asters, poppies, and snapdragons also tolerated the chemical in the soil.

The reaction of nursery and greenhouse plants to toxaphene in the soil was not determined because of the lack of interest by the growers. However, a small-scale test with several varieties of azaleas indicated that these plants were not affected by toxaphene at 50 pounds per acre.

Chlordane

Pure chlordane is 1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene. There are at least eight possible stereoisomers. Technical chlordane is a complex mixture containing 60 to 75 percent of *alpha* and *beta* chlordane and several associated compounds resulting from the manufacturing process. All the ingredients of technical chlordane are considered to be insecticidally active.

One of the principal producers reported that technical chlordanes with the same chlorine content may differ widely in insecticide properties, and technical chlordanes differing greatly in chlorine content may have similar insecticide properties. This producer bioassayed each batch of technical chlordane, using crystalline *alpha* chlordane as a standard and the house fly (*Musca domestica* L.) as the test insect. The marketed product had a toxicity index of not less than 150.

Fleming and Maines (unpublished), using third-instar grubs as test insects and crystalline *alpha* chlordane as the standard, determined the relative toxicity of technical chlordanes with the manufacturer's indices between 130–145 and 175–190. With the toxicity of *alpha* chlordane rated as 100, the relative toxicity to the grubs of the first group of technical chlordanes ranged from 34 to 132 and that of the second group from 54 to 112. There was no correlation between the manufacturer's toxicity indices and the toxicity of the technical chlordanes to the grubs. There was, however, a high negative correlation between the chlorine

content of the technical chlordanes and their toxicity. The average relative grub toxicity ranged from 122 with chlordanes containing 61–62 percent of chlorine to 11 with those containing 64–65 percent of chlorine.

This situation was disturbing because it indicated that chlordane formulations available on the market could differ greatly in their toxicity to the grubs. Tests were made with formulations obtained from several processors who used technical chlordane made by the two principal producers. These tests showed that the chlordane formulations on the market did not differ significantly in their toxicity to the grubs. (Fleming *1948a*)

Toxicity to Grubs.—Chlordane did not inhibit the female beetle from depositing eggs in the soil or prevent their hatching. It was more toxic to the grubs than DDT. Newly hatched grubs in Sassafras sandy loam were killed in 14 days by 0.4 pound of chlordane and 5 pounds of DDT per acre at 80° F. Under these conditions 3 pounds of chlordane and 50 pounds of DDT killed third-instar grubs in 19 days. Chlordane was 13 to 14 times as toxic as DDT to the grubs. (Fleming and Maines unpublished)

The mortality of the grubs was modified by their age, the quantity of chlordane in the soil, the temperature, and the duration of the exposure, as shown in table 24. The velocity of insecticide action at 60° F. was twice that at 50°; at 70° it was tripled and at 80° quadrupled. The poisoning of first-instar grubs was not accelerated by increasing the dosage from 0.4 to 1 pound per acre; the poisoning of third-instar grubs was not accelerated significantly by increasing the dosage from 5 to 10 pounds per acre. The maximum rate of poisoning of first- and third-instar grubs was obtained with 0.4 and 5 pounds per acre, respectively. (Fleming and Maines unpublished)

TABLE 24.—*Effect of temperature and chlordane dosage on length of exposure required to kill Japanese beetle grubs in Sassafras sandy loam*

Temperature (° F.)	Exposure for 100-percent mortality with indicated pounds per acre					
	First instars		Third instars			
	0.4	1	1	2	5	10
	Days	*Days*	*Days*	*Days*	*Days*	*Days*
50					56	52
60			56	42	28	26
70	19	18			19	18
80	14	14	28	21	14	13

Fleming (*1948a*) and Fleming and Maines (unpublished) collected soils from six physiographic areas in Connecticut, Massachusetts, New Jersey, and New York and determined the effect of the origin of the soils on the insecticide action of chlordane on third-instar grubs. The effect of the origin and the natural drainage of these soils on the insecticide action is summarized in table 25. The origin of the soil was not important in modifying the effectiveness of chlordane. The variations in insecticide action in soils within the physiographic areas were as great as the differences between the areas. The insecticide action tended to be slower in the inadequately drained soils than in the well-drained, adequately aerated soils.

The effect of soil texture on the rate of insecticide action of 10 pounds of chlordane was determined with 84 soils occurring in New England, New Jersey, New York, North Carolina, Ohio, and Virginia. The days required to kill 98 percent of the third-instar grubs at 80° F. in soils of different texture are summarized in table 26. The insecticide action progressed faster in the sands than in the various loams, but the rates in gravelly and shale loams, sandy loams, loams, silt loams, and clay loams were not significantly different. The insecticide action was slower in muck than in the mineral soils. However, a difference of only 3 days for

TABLE 25.—*Effect of soil origin and natural drainage on insecticidal action of chlordane on third-instar Japanese beetle grubs*

Soil origin	Natural drainage	Soils	Exposure for 98-percent mortality at 80° F. with indicated pounds per acre	
			5	10
		Number	*Days*	*Days*
Appalachian Mountains	Good	2	10	9
Piedmont plateau	do	4	9	9
	Imperfect	1	20	16
Limestone valley	Good	1	14	8
Glacial till	do	8	11	12
	Imperfect	2	15	14
Glacial lake and river terrace	Good	9	11	10
	Imperfect	1	9	11
Coastal plain	Good	7	12	9
	Imperfect	9	9	9

TABLE 26.—*Effect of soils of different texture on insecticide action of 10 pounds of chlordane per acre on third-instar Japanese beetle grubs*

Texture	Soils	Exposure for 98-percent mortality at 80° F.
	Number	*Days*
Sand	4	8
Gravelly and shale loam	5	9
Sandy loam	37	10
Loam	20	10
Silt loam	12	9
Clay loam	5	10
Muck	1	11

the mortality to reach 98 percent in the sands and the muck showed that the texture of the soil was only of minor importance. (Fleming *1948a;* Fleming and Maines unpublished)

Persistence.—When 10 pounds of chlordane per acre were applied in the spring as a dust or granular formulation to the surface of fallow land and left there, the chemical dissipated rapidly. Chemical analyses and bioassays showed that 46, 60, 73, and 84 percent of the chemical were lost in 1, 2, 3, and 4 weeks, respectively. (Fleming et al. unpublished)

Fleming and Maines (unpublished) mixed chlordane with Sassafras sandy loam, exposed the soil to weathering, and periodically determined the active chlordane in the soil by bioassay. The duration of the effectiveness of 1 to 10 pounds of the chemical per acre was considered to be the number of months elapsing before the active residue decreased to 0.4 pound per acre, the dosage needed to kill newly hatched grubs. The longevity of effectiveness of 1, 2, 5, and 10 pounds was 12, 24, 42, and 60 months, respectively. This persistence of the 10 pounds in soil is in contrast to the rapid disappearance of the chemical from the surface of soil. The dosage of chlordane applied is dependent on the period of effectiveness required. The 10-pound dosage of chlordane was selected for nursery treatments because its longevity of effectiveness approached that with lead arsenate and DDT.

The effect of the area where 83 mineral soils occur on the persistence of 10 pounds of chlordane is summarized in table 27. At the end of the first year of weathering the active residual chlordane varied from 4.2 pounds in Ohio soils to 7 pounds per acre in North Carolina soils. As the weathering was prolonged

TABLE 27.—*Persistence of insecticidally active chlordane in soils of different areas and different textures when applied at 10 pounds per acre*

Area	Soils	Average active chlordane per acre after indicated years			
		1	2	3	4
	Number	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Ohio	15	4.2	2.0	1.1	0.6
New England	30	5.1	2.5	1.1	.4
New York	2	5.4	2.4	1.1	.5
New Jersey	28	6.5	3.4	1.4	.5
North Carolina	8	7.0	3.8	1.6	.7
Total or average	83	5.6	2.7	1.3	.5
Texture					
Sand, shale, and gravelly loam	9	6.1	3.1	1.5	0.7
Sandy loam	38	5.9	3.1	1.3	.5
Loam	19	5.1	2.5	1.1	.5
Silt loam	12	5.5	2.5	1.0	.4
Clay loam	5	4.5	2.4	1.3	.6
Muck	1	1.5	.3		

the differences became progressively less until after 4 years about the same amount of active chlordane was present in all of them. The active chlordane at that time was approaching the minimum needed to kill grubs hatching in the soils. (Fleming and Maines *1954*)

Fleming and Maines (*1954*) found that the persistence of insecticidally active chlordane in the soils of these areas was modified by the soil texture. The effect of texture on the persistence of the 10-pound dosage is also summarized in table 27. The half-life of the treatment, a reduction of 50 percent in the insecticidally active chlordane, occurred in less than 1 year in the clay loams and in muck, and in a little over 1 year in the sands, gravelly and shale loams, sandy loams, loams, and silt loams. The minimum dosage to kill newly hatched grubs was reached in the muck in less than 2 years. This minimum was approached in the other soils in about 4 years.

Potting Soil.—To facilitate the mixing of small quantities of chlordane with potting soil the chemical was applied as a dilute dust or as a dilute emulsion. A dosage of 11.2 grams of chlordane per cubic yard of soil killed all third-instar grubs within 13, 18, 26, and 52 days at 80°, 70°, 60°, and 50° F., respectively. Later the velocity of poisoning at 60° progressed as rapidly with grubs

removed from the treated soil in 14 days as with grubs left in the soil for 26 days. (Fleming and Maines unpublished)

The treatment of potting soil with chlordane at 11.2 grams per cubic yard was authorized in 1948. The soil could be certified in 14 days at temperatures at least 80° F., 21 days at 70°–79°, 28 days at 60°–69°, and 56 days at 50°–59°. The treatment was rarely used below 60°. Later the soil could be certified after holding for 14 days at not lower than 60°. The treated soil could be in a certified status for 4 years, but the longevity of the certified period was determined by analysis of the soil.

Planted and Unplanted Plots.—By 1948 most of the commercial nurseries were applying DDT to beds and plots to obtain certification. To acquire information on the effectiveness of chlordane in eliminating newly hatched grubs, the Japanese Beetle Laboratory arranged with the then Division of Japanese Beetle Control (U.S. Dept. Agr.) that if chlordane was substituted for DDT in the treatment of some plots, consideration would be given to the certifying of the chlordane-treated plots provided in September no living grubs were found in the soil. The nurseries agreed to this arrangement and substituted chlordane in the treatment of 12 plots with an area of about 5 acres.

Chlordane was applied at 10 pounds per acre and mixed by cultivation with the upper 3 inches of soil. The chemical was applied during April, May, and July. The third-instar grubs in the plots treated in April were practically eliminated by the time of pupation. No living grubs were found in any of the treated plots in September. This treatment eliminated three or four annual broods that hatched subsequently in the treated plots. (Fleming *1948a*; Fleming and Maines unpublished)

Chlordane at 10 pounds per acre was authorized in 1948 for the certification of nursery beds and plots both planted and unplanted. The initial application had to be completed at the time the eggs began to hatch, approximately 14 days after emergence of the beetle. Mixing the chemical with the upper 3 inches of soil had to be completed within 24 hours after applying it. A plot could be certified 60 days after the eggs began to hatch. It could be in a certified status for 3 or 4 years, but the longevity of certification was determined by analysis of the soil.

Some nursery crops would be harvested within 1 or 2 years after the application of chlordane. The 10-pound treatment usually provided a longer period of certification than was needed for these crops. It was authorized in 1960 that a plot could be in a certified status for at least 1 year by applying 5 pounds and for

at least 2 years by applying 7 pounds of chlordane per acre, except 10 pounds were required when the soil was high in organic matter.

During 1949 and 1950 chlordane largely replaced DDT for the treatment of nursery plots. Middleton and Cronin (*1952*) reported that during 1948–50 chlordane had beeen applied to 6,619,966 square feet of cultivated plots in commercial nurseries.

Nursery and Greenhouse Plants.—In a preliminary test with annual and perennial flowers, 22.4 grams of chlordane per cubic yard of potting soil, double the quantity needed to kill third-instar grubs, did not affect the germination and growth of *Althaea rosea, Aster novae-angliae, Calendula officinalis, Centaurea cyanus, Chrysanthemum carinatum, C. coccineum, Cosmos bipinnatus, Gaillardia aristata, Iberis odorata, Lathyrus odoratus, Portulaca grandiflora, Scabiosa* sp., *Tropaeolum majus, Verbena* sp., *Viola tricolor,* and *Zinnia elegans. Dianthus deltoides* was retarded by 22.4 grams but was not affected by 11.2 grams of chlordane. *Antirrhinum majus* and *Papaver orientale* were retarded by 5.6 grams. (Fleming *1948a*)

In preliminary tests in commercial greenhouses *Cyclamen* sp., *Pteris tremula, Rhododendron indicum,* and *R. obtusum* grew normally in soil containing 11.2 grams of chlordane per cubic yard. After the treatment was authorized, many other plants were grown satisfactorily in the treated soil, but the records on the species treated are not now available. (Fleming *1948a*)

In the large-scale field test in 1948 in commercial nurseries over 28,000 evergreens, including *Buxus sempervirens, Chamaecyparis lawsoniana, C. nootkatensis, C. obtusa, C. pisifera, Juniperus communis, Rhododendron indicum, R. obtusum, Taxus cuspidata, T. hunnewelliana, Thuja occidentalis,* and *T. orientalis,* grew normally in plots treated with 10 pounds of chlordane per acre (Fleming *1948a*). After the treatment was authorized, many species were grown in treated plots in commercial nurseries. Information on the species treated is not now available.

During 1948–50, 720,833 plants grown in chlordane-treated soil were certified for shipment (Middleton and Cronin *1952*).

Soil Improvement Crops.—Nurseries using rye, soybeans, and corn in rotation with nursery stock to improve the fertility of their soil had no difficulty in growing these crops on land treated with chlordane. Fleming and Maines (*1952*) used rotations of rye, soybeans, and corn, soybeans, corn, and rye, and corn, rye, and soybeans in a study on the effect of chlordane in the upper 3 inches of soil on the yields of these crops. Chlordane

was applied at 11 and 19 pounds per acre and mixed by cultivation with the upper 3 inches of soil before planting. After each crop was harvested, it was disked into the soil. Each spring the land was prepared for planting by disking. The analyses of the soil during the fourth year showed that practically all the residual chlordane was within the upper 3-inch layer of soil.

When the yields on the plots with the 11- and 19-pound dosages were compared with those on untreated plots, the relative average yields over the 4-year period were 98 and 96 percent with rye, 100 and 107 percent with soybeans, and 96 and 98 percent with corn, respectively. The differences in the yields were not significant.

Aldrin

Technical aldrin contains not less than 95 percent of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8,-dimethanonaphthalene. Other designations that have been used are compound 118, Octalene, and HHDN.

Toxicity to Grubs.—Aldrin was more toxic to the grubs than chlordane. Newly hatched grubs did not survive for 2 weeks at 80° F. in Sassafras sandy loam containing 0.07 pound of aldrin per acre. A few grubs completed their development to the second instar in loam with the 0.04-pound dosage. A 0.4-pound dosage of chlordane was required to eliminate first-instar grubs. The toxicity of 3 pounds of aldrin per acre was equivalent to that of 10 pounds of chlordane to third-instar grubs. (Fleming et al. unpublished)

The insecticide action of aldrin in Sassafras sandy loam was modified more by the temperature than by the dosage, as shown in table 28. The velocity of poisoning at 60° F. was twice that at 50°; at 70° it was tripled and at 80° quadrupled. The speed of action on the first-instar grubs was not enhanced by increasing the dosage from 0.07 to 0.15 pound per acre. The 6-pound dosage was only slightly faster than the 3 pounds in killing third-instar grubs; the 9-pound dosage was no faster than 6 pounds. (Fleming et al. unpublished)

Fleming and Maines (unpublished) determined the effect of soil texture on the velocity of poisoning third-instar grubs by the 3-pound dosage in 29 soils occurring in New England, New York, North Carolina, and Ohio. The results are summarized in table 29. The grubs were killed faster in the gravelly loams than in the other loams. The insecticide action was slowest in the clay loams.

TABLE 28.—*Effect of temperature and aldrin dosage on length of exposure required to kill Japanese beetle grubs in Sassafras sandy loam*

Temperature (° F.)	Exposure for 100-percent mortality of grubs with indicated pounds per acre				
	First instars		Third instars		
	0.07	0.15	3	6	9
	Days	*Days*	*Days*	*Days*	*Days*
50			56	52	
60			28	26	26
70			21	18	
80	14	14	14	13	

Persistence.—When 3 pounds of aldrin per acre were applied in the spring as a dust or granular formulation to the surface of fallow land and left there, the chemical dissipated very rapidly. Chemical analysis and bioassays showed that 76 percent of the chemical was lost in 1 week, 93 percent in 2 weeks, 96 percent in 3 weeks, and practically all of it was gone in 4 weeks. (Fleming et al. unpublished)

Fleming and Maines (unpublished) mixed aldrin with 29 mineral soils and a muck at 3 pounds per acre, exposed the soils to weathering, and periodically determined the active residual aldrin by bioassay. The persistence of aldrin in these soils is summarized in table 30. The residue approached the minimum needed to kill newly hatched grubs in 4 years in the gravelly loams, sandy loams, loams, silt loams, and clay loams and in 2 years in muck.

Potting Soil.—To facilitate the mixing of small quantities of aldrin with potting soil, the chemical was applied as a dilute dust

TABLE 29.—*Effect of soils of different texture on insecticide action of 3 pounds of aldrin per acre on third-instar Japanese beetle grubs*

Texture	Soils	Exposure for 98-percent mortality at 80° F.
	Number	*Days*
Gravelly loam	2	11
Sandy loam	14	12
Loam	8	13
Silt loam	2	13
Clay loam	3	15

TABLE 30.—*Persistence of insecticidally active aldrin in soils of different texture when applied at 3 pounds per acre*

Texture	Soils	Average active aldrin per acre after indicated years			
		1	2	3	4
	Number	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Gravelly loam	2	1.9	0.5	0.2	0.1
Sandy loam	14	1.7	.7	.4	.2
Loam	8	1.2	.6	.2	.1
Silt loam	2	.8	.6	.4	.2
Clay loam	3	1.1	.5	.2	.1
Muck	1	.2	.1		

or as a dilute emulsion. A dosage of 3.4 grams of aldrin per cubic yard of soil killed all third-instar grubs within 14 days at 80° F., 21 days at 70°, 28 days at 60°, and 56 days at 50° (Fleming and Maines unpublished). Later it was found that the velocity of poisoning at 60° progressed as rapidly with grubs removed from the treated soil in 7 days as with grubs left in the soil for 28 days.

The treatment of potting soil with aldrin at 3.4 grams per cubic yard was authorized in 1953. The soil could be certified in 14 days at temperatures at least 80° F., in 21 days at 70°–79°, 28 days at 60°–69°, and 56 days at 50°–59°. Later the soil could be certified after holding for 14 days at not lower than 60°. The treated soil could be in a certified status for 4 years, but the longevity of the certified period was determined by analysis of the soil.

Planted and Unplanted Plots.—Commercial nurseries were not interested in large-scale tests to demonstrate the effectiveness of aldrin in eliminating grubs hatching in plots during the summer because both DDT and chlordane were authorized and were being used as a basis for certification. They did not want to assume the risk with another chlorinated hydrocarbon of not having their plots certified in the fall. It was necessary therefore to conduct the tests in ¼-acre unplanted experimental plots.

When aldrin was applied in September at 3 pounds per acre and mixed by cultivation with the upper 3 inches of soil, the third-instar grubs were reduced by 67 to 86 percent within 4 weeks and were eliminated before pupation in the spring. The application of the chemical in April practically eliminated the

third-instar grubs before pupation. Both the fall and the spring applications eliminated by September three annual broods that hatched subsequently in the treated soil. The effectiveness of the treatments on the fourth annual brood was not determined. (Fleming and Maines unpublished)

Aldrin at 3 pounds per acre was authorized in 1953 for certifying planted and unplanted nursery beds and plots. The initial application had to be completed when the eggs began to hatch, about 14 days after emergence of the beetle. Mixing the chemical with the upper 3 inches of soil had to be completed within 24 hours after applying it. A plot could be certified 60 days after the eggs began to hatch. It could be in a certified status for 3 years, but the longevity of certification was determined by analysis of the soil.

Some nursery crops would be harvested within 1 or 2 years after applying aldrin. It was authorized in 1960 that a plot could be certified for 1 year by applying 1.5 pounds of aldrin per acre and for 2 years by applying 2 pounds, except 3 pounds were required when the soil was high in organic matter.

Nursery and Greenhouse Plants.—In a preliminary test aldrin at 6.7 grams per cubic yard of potting soil, double the quantity needed to kill third-instar grubs, did not affect the germination and growth of *Calendula officinalis, Centaurea cyanus, Chrysanthemum carinatum, Dianthus* sp., *Ipomoea purpurea, Lathyrus odoratus, Papaver orientale,* and *Zinnia elegans,* but it retarded the growth of *Althaea rosea* and *Verbena* sp. The 3.4-gram dosage was tolerated by *Verbena* but not by *Althaea rosea.* (Fleming and Maines *1953b*)

Only limited tests were made in commercial nurseries because of lack of interest by the growers in using the chemical. *Pelargonium* sp. was not injured when potted in soil containing 10.2 grams of aldrin per cubic yard. Over 4,500 azaleas, including 17 varieties of *Rhododendron indicum* and *R. obtusum,* grew normally in beds treated with aldrin at 3 pounds per acre. (Fleming and Maines unpublished)

Soil Improvement Crops.—Some nurseries used rye, soybeans, and corn in rotation with nursery stock to improve the fertility of their soil. Fleming and Maines (unpublished) used rotations of rye, soybeans, and corn, soybeans, corn, and rye, and corn, rye, and soybeans in a study to determine the effect of 3 pounds of aldrin per acre in the upper 3 inches of soil on the yields of these crops. Over a 3-year period the yields of these crops were slightly less on the treated plots than on the untreated. The relative

average yields on the treated plots over this period were 91 percent with rye, 93 percent with soybeans, and 96 percent with corn.

Dieldrin

Technical dieldrin contains not less than 85 percent of 1,2,3,4,-10, 10-hexachloro-6, 7-epoxy-1,4,4a,5,6,7,8, 8a-octahydro-1, 4-*endo-exo*-5,8-dimethanonaphthalene. Other designations are Octalox, compound 497, and HEOD.

Toxicity to Grubs.—Dieldrin was less toxic than aldrin and more toxic than chlordane to newly hatched grubs. A dosage of 0.07 pound of aldrin per acre killed all the first-instar grubs, but 0.3 pound of dieldrin and 0.4 pound of chlordane were required to kill them. However, 3 pounds of dieldrin per acre were needed to kill third-instar grubs. (Fleming et al. unpublished)

The velocity of the insecticide action of 3 pounds of dieldrin on third-instar grubs in Sassafras sandy loam was modified by the temperature in the same manner as were the rates of poisoning by lead arsenate, DDT, toxaphene, chlordane, and aldrin. To kill all third-instar grubs with this dosage required 14 days at 80° F., 21 days at 70°, 28 days at 60°, and 56 days at 50°. (Fleming and Maines unpublished)

Fleming and Maines (unpublished) determined the effect of soil texture on the velocity of poisoning third-instar grubs by the 3-pound dosage in 29 soils occurring in New England, New York, North Carolina, and Ohio. As shown in table 31, the insecticide action progressed at about the same rate in the gravelly loams, sandy loams, loams, and silt loams, but it was slower in the clay loams.

TABLE 31.—*Effect of soils of different texture on insecticide action of 3 pounds of dieldrin per acre on third-instar Japanese beetle grubs*

Texture	Soils	Exposure for 98-percent mortality at 80° F.
	Number	*Days*
Gravelly loam	2	12
Sandy loam	14	12
Loam	8	12
Silt loam	2	11
Clay loam	3	15

Persistence.—When 3 pounds of dieldrin per acre were applied in the spring as a dust or granular formulation to the surface of fallow land and left there, it was more persistent than chlordane or aldrin and had about the same persistency as DDT and toxaphene. Chemical analysis and bioassays showed that 17 percent of the chemical was lost in 1 week, 30 percent in 2 weeks, 37 percent in 3 weeks, and 40 percent in 4 weeks. (Fleming et al. unpublished)

Fleming and Maines (unpublished) mixed dieldrin with Sassafras sandy loam at 3 pounds per acre, exposed the soil to weathering in the field, and periodically determined the active residue in the soil. It was found that 2.6 pounds of active dieldrin remained after 1 year, 2.2 pounds after 2 years, 1.9 pounds after 3 years, 1.6 pounds after 4 years, 1.4 pounds after 5 years, and 1.2 pounds after 6 years. The half-life of the chemical in this soil was about 4½ years. After weathering for 6 years four times as much dieldrin was in the soil as was needed to kill newly hatched grubs.

The persistence of the 3-pound dosage was also determined in 29 other soils during a 4-year period of weathering. These results are summarized in table 32. The persistence of active dieldrin in these soils was much like that in Sassafras sandy loam. The texture of the soil was not important in modifying the longevity of the chemical.

Potting Soil.—A dosage of 3.4 grams of dieldrin per cubic yard of potting soil killed all third-instar grubs within 14 days at 80° F., 21 days at 70°, 28 days at 60°, and 56 days at 50°. Later it was found that the velocity of poisoning at 60° progressed as rapidly with grubs removed from the treated soil in 7 days as with grubs left in the soil for 28 days. (Fleming and Maines unpublished)

TABLE 32.—*Persistence of insecticidally active dieldrin in soils of different texture when applied at 3 pounds per acre*

Texture	Soils	Average active dieldrin per acre after indicated years			
		1	2	3	4
	Number	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Gravelly loam	2	2.5	2.0	1.4	0.9
Sandy loam	14	2.5	2.5	2.0	1.5
Loam	8	2.3	2.2	2.0	1.5
Silt loam	2	2.6	2.6	2.1	1.6
Clay loam	3	1.9	1.9	1.6	1.1

The treatment of potting soil with dieldrin at 3.4 grams per cubic yard was authorized in 1953. The soil could be certified in 14 days at temperatures at least 80° F., in 21 days at 70°–79°, 28 days at 60°–69°, and 56 days at 50°–59°. Later the soil could be certified after holding for 14 days at not lower than 60°. The treated soil could be in a certified status for 5 years, but the longevity of the certified period was determined by analysis of the soil.

Planted and Unplanted Plots.—Commercial nurseries were not interested in large-scale tests to demonstrate the effectiveness of dieldrin in eliminating grubs hatching in plots during the summer because both DDT and chlordane were authorized and were being used as a basis for certification. The nurseries did not want to assume the risk with another chlorinated hydrocarbon of not having their plots certified in the fall. It was necessary therefore to conduct the tests in ¼-acre unplanted experimental plots.

When dieldrin was applied in September at 3 pounds per acre and mixed by cultivation with the upper 3 inches of soil, the third-instar grubs were reduced by 72 to 89 percent within 4 weeks and were eliminated before pupation in the spring. When applied before the eggs hatched, dieldrin eliminated by September five annual broods of grubs that hatched subsequently in the soil. (Fleming and Maines unpublished)

Dieldrin at 3 pounds per acre was authorized in 1953 for certifying planted and unplanted nursery beds and plots. The initial application had to be completed when the eggs began to hatch, about 14 days after emergence of the beetle. Mixing the chemical with the upper 3 inches of soil had to be completed within 24 hours after applying it. A plot could be certified 60 days after the eggs began to hatch. It could be in a certified status for 5 years, but the longevity of certification was determined by analysis of the soil.

Most nursery crops would be harvested in less than 5 years after applying dieldrin to the soil. In 1960 a plot could be certified for 3 years by applying 1.5 pounds of dieldrin and for 4 years by applying 2 pounds per acre, except 3 pounds were required when the soil was high in organic matter.

Nursery and Greenhouse Plants.—In a preliminary test dieldrin at 6.7 grams per cubic yard of potting soil, double the quantity needed to kill third-instar grubs, did not affect the germination and growth of *Aster novae-angliae, Calendula officinalis, Chrysanthemum carinatum, Ipomoea purpurea, Lathyrus odora-*

tus, Papaver orientale, and *Zinnia elegans,* but it retarded the growth of *Althaea rosea, Dianthus* sp., and *Verbena* sp. The 3.4-gram dosage was tolerated by all these species. (Fleming and Maines *1953b*)

Only limited tests were made in commercial nurseries because of lack of interest by the growers in using the chemical. *Pelargonium* sp. grew normally in potting soil containing 10.2 grams of dieldrin per cubic yard. Over 4,500 azaleas, including 17 varieties of *Rhododendron indicum* and *R. obtusum,* grew normally in beds treated with dieldrin at 3 pounds per acre. (Fleming and Maines unpublished)

Soil Improvement Crops.—Some nurseries used rye, soybeans, and corn in rotation with nursery stock to improve the fertility of their soil. Fleming and Maines (unpublished), using rotations of rye, soybeans, and corn, soybeans, corn, and rye, and corn, rye, and soybeans, found that 3 pounds of dieldrin per acre in the upper 3 inches of soil did not modify significantly the yields of these crops. When the yields on the treated plots were compared with those on untreated plots, the relative average yields on the treated plots over a 3-year period were 99 percent with rye, 100 percent with soybeans, and 96 percent with corn.

Heptachlor

Heptachlor is largely 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene.

Toxicity to Grubs.—Heptachlor was of the same toxicity to newly hatched grubs as aldrin. A dosage of 0.07 pound per acre of either chemical killed all first-instar grubs. Heptachlor was more toxic than aldrin to third-instar grubs; 2 pounds of heptachlor per acre were equivalent in toxicity to 3 pounds of aldrin. (Fleming et al. unpublished)

The temperature modified the velocity of the insecticide action of 2 pounds of heptachlor on third-instar grubs. All third instars were killed by this dosage in 14 days at 80° F., in 21 days at 70°, 28 days at 60°, and 56 days at 50°. (Fleming and Maines unpublished)

The velocity of poisoning third-instar grubs by 2 pounds of heptachlor was not modified significantly by the texture of 19 soils occurring in New Jersey. The insecticide action progressed at about the same rate in sands, gravelly and shale loams, sandy loams, loams, and silt loams. (Fleming and Maines unpublished)

Persistence.—When 3 pounds of heptachlor per acre were applied in the spring as a dust or granular formulation to the sur-

face of fallow land and left there, the chemical dissipated almost as fast as aldrin. Chemical analysis and bioassays showed that 63 percent of the heptachlor was lost in 1 week, 73 percent in 2 weeks, 86 percent in 3 weeks, and 93 percent in 4 weeks. (Fleming et al. unpublished)

Fleming and Maines (unpublished) mixed heptachlor with 19 mineral soils from New Jersey and with a muck, exposed the soils to weathering, and periodically determined the active residual heptachlor by bioassay. The persistence of heptachlor in the mineral soils—sands, gravelly and shale loams, sandy loams, loams, and silt loams—and in muck is summarized in table 33. Information is not given for each type of mineral soil because the texture was not important in the longevity of the chemical in the soil. The 2 pounds of heptachlor per acre approached the minimum needed to kill newly hatched grubs in 2 years in the mineral soils and in about 1 year in the muck. The longevity of the effectiveness of 3 pounds was about 4 years in the mineral soils and about 2 years in the muck.

Potting Soil.—Third-instar grubs in potting soil were killed within 14 days at 80° F. by mixing 3.4 grams of heptachlor with each cubic yard of soil. It required 21 days at 70°, 28 days at 60°, and 56 days at 50° to kill all the grubs. Later it was found that the velocity of poisoning at 60° progressed as rapidly with grubs removed from the treated soil in 7 days as with grubs left in the soil for 28 days. (Fleming and Maines unpublished)

The treatment of potting soil with heptachlor at 3.4 grams per cubic yard was authorized in 1953. The soil could be certified in 14 days at temperatures at least 80° F., in 21 days at 70°–79°, 28 days at 60°–69°, and in 56 days at 50°–59°. Later the soil could be certified after holding for 14 days at not lower than 60°. The treated soil could be in a certified status for 4 years, but the

TABLE 33.—*Persistence of insecticidally active heptachlor in mineral soils and in muck*

Soils	Chemical per acre	Average active heptachlor per acre after indicated years			
		1	2	3	4
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Mineral	2	0.3	0.1		
	3	.5	.3	0.2	0.1
Muck	2	.06			
	3	.12	.08		

longevity of the certified period was determined by analysis of the soil.

Planted and Unplanted Plots.—When heptachlor was applied late in May at 2 and 3 pounds per acre and mixed by cultivation with the upper 3 inches of soil, the chemical had little effect on the third-instar grubs before pupation. The 2-pound dosage was effective in eliminating two annual broods that hatched subsequently in the treated soil, and 3 pounds were effective in eliminating three annual broods. Commercial nurseries were not interested in large-scale tests to demonstrate the effectiveness of heptachlor in eliminating grubs hatching in plots during the summer because both DDT and chlordane were authorized and were being used as a basis for certification. They did not want to assume the risk with another chlorinated hydrocarbon of not having their plots certified in the fall. (Fleming and Maines unpublished)

Heptachlor at 3 pounds per acre was authorized in 1953 for certifying planted and unplanted nursery beds and plots. The initial application had to be completed when the eggs began to hatch, approximately 14 days after emergence of the beetle. Mixing the chemical with the upper 3 inches of soil had to be completed within 24 hours after applying it. A plot could be certified 60 days after the eggs began to hatch. It could be in a certified status for 3 years, but the longevity of certification was determined by analysis of the soil.

Some nursery crops would be harvested within 1 or 2 years after applying heptachlor. In 1960 a plot could be certified for 1 year by applying 1.5 pounds of heptachlor and for 2 years by applying 2 pounds per acre, except 3 pounds were required when the soil was high in organic matter.

Nursery and Greenhouse Plants.—In a preliminary test Fleming and Maines (*1953b*) used the common garden vegetables to determine their reaction to heptachlor in soil. Heptachlor at 4 pounds per acre did not modify significantly the germination and growth of beets, broccoli, cabbage, carrots, corn, cucumbers, eggplant, lettuce, lima beans, peppers, radishes, soybeans, spinach, squash, tomatoes, turnips, and watermelon. The 8-pound dosage retarded the growth of corn, soybeans, and watermelon, but it did not affect the growth of the other vegetables.

Only limited tests were made in commercial nurseries because of lack of interest by the growers in using the chemical. Thirty-one varieties of azaleas, including those of *Rhododendron indicum*, *R. obtusum*, and *R. rutherfordiana*, grew normally in

potting soil containing 3.4 grams of heptachlor per cubic yard and in beds treated with the chemical at 3 pounds per acre. (Fleming and Maines unpublished)

Endrin

Technical endrin is largely 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,-4,4a,5,6,7,8,8a-octahydro-1,4-*endo-endo*-5,8-dimethanonaphthalene.

Toxicity to Grubs.—Endrin was equivalent to chlordane in toxicity to newly hatched grubs. The mortality was 31 percent with 0.1 pound of the chemical per acre, 83 percent with 0.2 pound, and 100 percent with 0.4 pound. It was equivalent to heptachlor in toxicity to third-instar grubs; 2 pounds per acre of both chemicals killed all the third instars. (Fleming et al. unpublished)

The velocity of the insecticide action of 2 pounds of endrin on third-instar grubs was modified by the temperature of the soil. Fifty percent of the grubs were killed in 4 days at 80° F., 9 days at 60 , and 22 days at 50°. (Fleming and Maines unpublished)

Persistence.—Fleming and Maines (unpublished) mixed endrin with Sassafras sandy loam at 2 pounds per acre, exposed the soil to weathering in the field, and periodically determined the active residue in the soil. After an exposure of 15 months 1.9 pounds of active endrin remained in the soil, indicating that endrin had about the same persistence in soil as DDT, toxaphene, and dieldrin.

Nursery and Greenhouse Plants.—In a preliminary test endrin at 4 pounds per acre, double the quantity needed to kill third-instar grubs, did not affect the germination and growth of *Aster novae-angliae, Chrysanthemum carinatum, Ipomoea purpurea, Lathyrus odoratus, Papaver, orientale, Verbena* sp., and *Zinnia elegans*, but it retarded the growth of *Centaurea cyanus, Dianthus chinensis*, and *Scabiosa* sp. Most of the vegetables grew normally with the 4-pound dosage. (Fleming and Maines *1953b*)

The reaction of nursery and greenhouse plants to endrin in the soil was not determined because endrin seemed to be only a potential substitute for dieldrin as a soil insecticide. The chemicals are very closely related and have many common characteristics. A substitute for dieldrin was not needed in the certification program.

Isodrin

Technical isodrin is largely 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,-8a-hexahydro-1,4-*endo-endo*-5,8-dimethanonaphthalene. It is closely related to aldrin.

Toxicity to Grubs.—Isodrin was very toxic to grubs. A dosage of 0.015 pound of isodrin per acre killed 63 percent of the newly hatched grubs and 0.03 pound killed all of them. A 0.3-pound dosage of isodrin was equivalent in toxicity to 3 pounds of aldrin to third-instar grubs. (Fleming et al. unpublished)

The velocity of insecticide action of isodrin was modified by the temperature. Fifty percent of the third-instar grubs were killed by 0.15 pound in 5 days at 80° F., 10 days at 60°, and 20 days at 50°, and by 0.3 pound in 4 days at 80°, 8 days at 60°, and 16 days at 50°. (Fleming and Maines unpublished)

Nursery and Greenhouse Plants.—Preliminary phytotoxicity tests were made with only some of the garden flowers. The 0.6-pound dosage did not affect the germination or growth of *Althaea rosea, Aster novae-angliae, Calendula officinalis, Centaurea cyanus, Chrysanthemum carinatum, Dianthus chinensis, Ipomoea purpurea, Lathyrus odoratus, Papaver orientale, Verbena* sp., and *Zinnia elegans*, but it retarded the growth of *Scabiosa* sp. The growth of *Scabiosa* was normal with the 0.3-pound dosage. (Fleming and Maines *1953b*)

Experiments with isodrin were discontinued when the chemical was withdrawn from the market by the producer.

Benzene Hexachloride

Benzene hexachloride is a mixture of several isomers of 1,2,3,-4,5,6-hexachlorocyclohexane. Other designations for the insecticide are BHC and Gammexane. An analysis by infrared spectroscopy of the material used in this investigation showed that it contained 25.5 percent of the *alpha* isomer, 3.8 percent of the *beta*, 33.2 percent of the *gamma*, 20.7 percent of the *delta*, 5.5 percent of the *epsilon*, 2.0 percent of other isomers, and 9.3 percent of other compounds.

It was the general practice among entomologists to base the dosage of benzene hexachloride on the percentage of the *gamma* isomer in the insecticide. In this investigation the dosage was based on the insecticide rather than on one of its components.

Toxicity to Grubs.—The toxicity of benzene hexachloride to

newly hatched grubs was not determined. A dosage of 4.5 pounds of benzene hexachloride per acre was equivalent in toxicity to third-instar grubs to 25 pounds of DDT. When freshly applied to Sassafras sandy loam, 1.5 pounds of benzene hexachloride killed all third-instar grubs in 4 weeks at 80° F. A 3-pound dosage was effective in 3 weeks and 7.5 pounds in 2 weeks. Increasing the dosage to 15 pounds did not accelerate significantly the insecticide action. (Fleming and Maines unpublished)

As shown in table 34, the speed of killing third-instar grubs with benzene hexachloride was only retarded slightly by reducing the temperature from 80° F. to 60°, but it was greatly retarded by reducing it to 50°. The velocity of poisoning was adequate only when the temperature was not lower than 60°. (Fleming and Maines unpublished)

Persistence.—Fleming and Maines (unpublished) mixed benzene hexachloride with Sassafras sandy loam, exposed the soil to weathering in the field, and periodically determined the quantity of the active residual chemical by bioassay. A dosage of 1.5 pounds per acre disappeared in about 8 weeks. The 3 pounds were reduced to 1.5 pounds in 8 weeks and only a trace remained after 42 weeks. After weathering for 52 weeks, the 7.5 pounds were reduced to about 3 pounds per acre and the 15 pounds to about 7.5 pounds. To be effective in killing grubs in the soil for more than 1 year, an application of about 7.5 pounds of benzene hexachloride per acre was required.

Nursery and Greenhouse Plants.—In a preliminary test with garden flowers, 7.5 pounds of benzene hexachloride per acre re-

TABLE 34.—*Effect of temperature and benzene hexachloride dosage on length of exposure required to kill third-instar Japanese beetle grubs in Sassafras sandy loam*

Temperature (° F.)	Exposure for 98-percent mortality with indicated pounds per acre		
	3	7.5	15
	Days	*Days*	*Days*
50	93 +	75	49
60	23	16	16
70	20	12	12
80	20	12	11

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tarded the growth of *Althaea rosea, Calendula officinalis, Gallardia aristata, Scabiosa* sp., *Tagetes erecta, Tropaeolum majus,* and *Zinnia elegans.* In commercial nurseries this dosage retarded the growth of *Hydrangea paniculata, Rhododendron obtusum,* and *Vitis* sp. (Fleming and Maines unpublished)

Lindane

Lindane contains more than 99 percent of the *gamma* isomer of 1,2,3,4,5,6-hexachlorocyclohexane.

Toxicity to Grubs.—Newly hatched grubs in Sassafras sandy loam were killed in 14 days by 0.8 pound of lindane per acre at 80° F. Third-instar grubs were killed in 21 days by 4 pounds and in 14 days by 10 pounds. (Fleming and Maines unpublished)

Lindane was about three-fourths as toxic to third-instar grubs in Sassafras sandy loam as benzene hexachloride. Four pounds of lindane and 3 pounds of benzene hexachloride per acre were required to kill third-instar grubs in 21 days at 80° F. and 10 and 7.5 pounds, respectively, to kill them in 14 days at this temperature. These data indicated that other isomers than the *gamma* had a role in the insecticide action of benzene hexacloride. This chemical had about one-half the toxicity of chlordane. As shown in table 24, 2 pounds of chlordane killed third-instar grubs in 21 days and 5 pounds in 14 days.

When the temperature of the soil was reduced from 80° F. to 60°, 28 days were required to obtain 100-percent mortality of third-instar grubs with 10 pounds of lindane. (Fleming and Maines unpublished)

Persistence.—The persistence of lindane in the soil was not determined, but it is probably similar to that of benzene hexachloride. It would be expected that several years would elapse before the 10-pound dosage was reduced to 0.8 pound of active lindane per acre, the dosage to eliminate newly hatched grubs.

Plants.—Fleming and Maines (*1953b*) used common garden vegetables in preliminary tests to determine the reactions of plants to lindane in the soil. Eight pounds of lindane per acre, the highest dosage tested, did not modify significantly the germination and growth of beets, broccoli, cabbage, carrots, cucumbers, lettuce, lima beans, radishes, squash, two varieties of tomatoes, turnips, and watermelon, but it reduced the growth of corn, eggplant, peppers, soybeans, and one variety of tomatoes. The growth

of *Rhododendron obtusum* was not affected by 10 pounds of lindane.

The possibilities of treating nursery stock with lindane were not explored further.

Kepone

Kepone is largely decachlorooctahydro-1,3,4-methano-2*H*-cyclobuta[*cd*]pentalen-2-one.

In comparative tests with third-instar grubs in Sassafras sandy loam at 80° F., the toxicity of 10 pounds of Kepone per acre was equivalent to that of 5 pounds of chlordane (Fleming and Maines unpublished).

Endosulfan

Endosulfan is 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide. It is also known as Thiodan.

The toxicity of 3.5 pounds of endosulfan per acre to third-instar grubs in Sassafras sandy loam at 80° F. was equivalent to that of 5 pounds of chlordane (Fleming and Maines unpublished).

Fleming and Maines (unpublished) mixed endosulfan with Sassafras sandy loam, exposed the soil to weathering in the field, and periodically determined the active chemical in the soil by bioassay. Three months after applying endosulfan to the plots, 3.3 pounds per acre remained of the 3.5 pounds and 5.3 of the 7.4 pounds. Five months later only a trace remained of the 3.5 pounds per acre and 0.08 of the 7.4 pounds. (Fleming and Maines unpublished) These results indicated that endosulfan was of little value as a residual insecticide against the grubs.

Isobenzan

Isobenzan is 1,3,4,5,6,7,8,8-octachloro-1,3,3a,4,7,7a-hexahydro-4,7-methanoisobenzofuran.

Isobenzan was very toxic to third-instar grubs in Sassafras sandy loam at 80° F. A dosage of 0.3 pound per acre killed all the grubs in 14 days. This dosage was equivalent in toxicity to 3 pounds of aldrin per acre. Experimentation was discontinued when the producer stopped development of the chemical. (Fleming and Maines unpublished)

Mixtures of Chlorinated Hydrocarbon Insecticides

A nurseryman could apply any of the authorized chlorinated hydrocarbon insecticides—aldrin, chlordane, DDT, dieldrin, heptachlor, or toxaphene—for the certification of plants in cultivated beds and plots. When additional insecticide was required to continue the certified status of the soil, the insecticide applied initially did not have to be used in the re-treatment. Any of these chlorinated hydrocarbons could be applied. By 1955 many nursery plots contained residues of chlordane and DDT, chlordane and dieldrin, or DDT and dieldrin, and some contained residues of chlordane, DDT, and dieldrin. The chemical analysis of the soil residues became complex. There was also a question whether the insecticide action of the mixtures was at least equivalent to their components.

Preliminary tests showed that a mixture of chlordane and dieldrin in Sassafras sandy loam was slightly more toxic to third-instar grubs than was expected from the toxicity of its components. An exposure of 2 weeks at 80° F. to 1 pound of chlordane or 0.26 pound of dieldrin per acre killed 50 percent of the grubs, but 0.5 pound of chlordane mixed with 0.13 pound of dieldrin killed 55 percent of them. When a pomace fly, *Drosophila melanogaster* Meigen, used as a test insect in bioassay, was exposed for 24 hours at 80° to these amounts of the insecticides in the loam, 1 pound of chlordane or 0.26 pound of dieldrin killed 50 percent of them and the mixture 53 percent. Since about the same evaluation of the toxicity of the mixture was made with the grubs and the flies, the more rapid procedure with the flies was used in more extensive tests with various mixtures of the chlorinated hydrocarbon insecticides. (Fleming et al. *1962*) The deviations from the expected 50-percent mortality with these mixtures are given in table 35.

DDT plus aldrin, chlordane, dieldrin, endrin, heptachlor, or toxaphene was definitely more toxic than DDT or the other components of these binary mixtures. Toxaphene plus aldrin, endrin, or heptachlor was definitely more toxic than toxaphene or the other components of these mixtures. Aldrin plus heptachlor was more toxic than either aldrin or heptachlor. There was no definite change in toxicity with the other binary mixtures. These data demonstrated that any of these chlorinated hydrocarbons could be substituted for the one applied initially in re-treatment of soil without any decrease in the toxicity.

TABLE 35.—*Deviation from expected 50-percent mortality with* Drosophila melanogaster *exposed to Sassafras sandy loam containing binary mixtures of chlorinated hydrocarbon insecticides* [1]

Component A	Component B						
	Aldrin	Chlordane	DDT	Dieldrin	Endrin	Heptachlor	Toxaphene
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Aldrin		+ 0.2	+23.0	− 5.4	+ 4.1	+16.6	+16.7
Chlordane	+ 0.2		+23.2	+ 2.5	+ 1.5	+ 3.8	+ 5.8
DDT	+23.0	+23.2		+15.2	+23.9	+24.4	+17.0
Dieldrin	− 5.4	+ 2.5	+15.2		− 3.0	− 3.3	+ 1.3
Endrin	+ 4.1	+ 1.5	+23.9	− 3.0		+ 8.9	+30.0
Heptachlor	+16.6	+ 3.8	+24.4	− 3.3	+ 8.9		+17.3
Toxaphene	+16.7	+ 5.8	+17.0	+ 1.3	+30.0	+17.3	

[1] A deviation of 8.2 percent is significant and one of 11.2 percent highly significant.

Carbamate Insecticides Mixed With Soil

Fleming (*1963b*) recommended 2 pounds of 50-percent wettable carbaryl in 100 gallons of water as a spray to protect plants from attack by the adult Japanese beetle. Carbaryl and DDT were equally effective in killing beetles. Carbaryl was less poisonous to man and animals than DDT. The deposit on the plants usually protected them from attack by the beetle for about 7 days. Additional applications of carbaryl were used when beetles again began to collect on the plants. In view of the toxicity of carbaryl to the adult beetle, experiments were undertaken to explore the possibility of substituting the carbamates for the chlorinated hydrocarbon insecticides to control grubs in soil.

Survey of Carbamates

Fleming et al. (unpublished) determined the quantities of 14 carbamates per acre required to produce a toxicity to third-instar grubs in Sassafras sandy loam equivalent to that of 3 pounds of dieldrin per acre. The results of these tests are summarized in table 36. Hercules 5727 was slightly more toxic and Landrin slightly less toxic than dieldrin. Propoxur and Shell SD–8786 were three-fifths, carbaryl three-tenths, and Shell SD–8959, carbofuran, Zectran, American Cyanamid E.I. 38906, Bayer 37344, and Bux were about one-fifth as toxic as dieldrin. The other carbamates were less toxic.

TABLE 36.—*Toxicity of carbamates to third-instar grubs in Sassafras sandy loam at 80° F.*

Common name or company designation	Chemical name	Amount per acre equivalent to 3 pounds of dieldrin per acre
		Pounds
Hercules 5727	*m*-Cumenyl methylcarbamate	2
Landrin	75 percent 3,4,5-Trimethylphenyl methylcarbamate; 18 percent 2,3,5-trimethylphenyl methylcarbambate.	4
Propoxur	*o*-Isopropoxyphenyl methylcarbamate	5
Shell SD–8786	2,3,5-Trimethylphenyl methylcarbamate	5
Carbaryl	1-Naphthyl methylcarbamate	10
Shell SD–8959	5-*tert*-Butyl-*m*-tolyl methylcarbamate	13
Carbofuran	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate	14
Zectran	4-(Dimethylamino)-3,5-xylyl methylcarbamate	14
American Cyanamid E.I. 38906	Cyclic ethylene [(methylcarbamoyl)oxy]dithioimidocarbonate	14
Bayer 37344	4-(Methylthio)-3,5-xylyl methylcarbamate	14
Bux	75 percent *m*-(1-Methylbutyl)phenyl methylcarbamate; 25 percent *m*-(1-methylpropyl)phenyl methylcarbamate.	15
Chevron Chemical RE–5655	5-*sec*-Butyl-2-chlorophenyl methylcarbamate	20+
Bayer 42696	3-(Dimethylamino)-*p*-tolyl methylcarbamate	20+
Bayer 50282	4-(Diallylamino)-3,5-xylyl methylcarbamate	20+

Carbaryl

Toxicity to Grubs.—Newly hatched grubs in Sassafras sandy loam at 80° F. were killed within 3 weeks by 4 pounds of carbaryl per acre. A 5-pound dosage eliminated second-instar grubs and 10 pounds eliminated third-instar grubs within 4 weeks. (Fleming et al. unpublished)

Persistence.—Fleming et al. (unpublished) mixed 10 pounds of carbaryl per acre with Sassafras sandy loam, exposed the soil to weathering, and periodically determined the quantity of active residue in the soil by bioassay. The results of these determinations were as follows:

Days after application	*Active carbaryl (pounds per acre)*
10	10.0
20	7.9
30	6.8
40	6.1
60	5.3
80	4.8
100	4.4
120	4.0
160	3.4

The 10-pound dosage of carbaryl was reduced in 120 days to 4 pounds per acre, the minimum to kill newly hatched grubs. This dosage applied when adult beetles appeared in an area would eliminate grubs hatching in the soil during the summer, but it would not be effective against the next generation of grubs. It would be necessary to apply the 10 pounds annually to maintain a plot in a certified status. The annual application of a residual insecticide did not meet the requirements of the nursery industry.

Zectran

Toxicity to Grubs.—Newly hatched grubs in Sassafras sandy loam at 80° F. were killed in 3 weeks by 8 pounds of Zectran per acre. A 14-pound dosage killed third-instar grubs in 3 weeks. (Fleming et al. unpublished)

Persistence.—Fleming et al. (unpublished) mixed 14 pounds of Zectran per acre with Sassafras sandy loam, exposed the soil to weathering, and periodically determined the quantity of active residue in the soil by bioassay. The results of these determinations were as follows:

Days after application	*Active Zectran (pounds per acre)*
10	11.8
20	10.5
30	9.9
40	9.4
50	9.1
60	8.8
70	8.6
80	8.4
90	8.3

Zectran approached the minimum dosage to kill newly hatched grubs in 90 days.

Fourteen pounds of Zectran per acre applied when adult beetles appeared in an area would eliminate grubs hatching in the soil during the summer, but it would not be effective against the next grub generation. The treatment would have to be used annually to maintain a plot in a certified status.

Carbofuran

Toxicity to Grubs.—Third-instar grubs in Sassafras sandy loam at 80° F. were killed in 3 weeks by 14 pounds of carbofuran per acre. The dosage to kill newly hatched grubs was not determined. (Fleming et al. unpublished)

Persistence.—Fleming et al. (unpublished) mixed 14 pounds of carbofuran per acre with Sassafras sandy loam, exposed the soil to weathering, and periodically determined the quantity of active residue in the soil by bioassay. Practically all the carbofuran disappeared from the soil during an exposure of 4 weeks.

It seems reasonable to assume that at least 5 pounds of carbofuran per acre would be required to kill newly hatched grubs. Applying 14 pounds when adult beetles appeared in an area would not be effective in killing newly hatched grubs in the soil 4 weeks later.

Organic Phosphorus Insecticides Mixed With Soil

A spray containing 0.5 pound of malathion in 100 gallons of water was effective in killing adult Japanese beetles. The residue on the plants protected them from attack by beetles for not more than 7 days. Additional applications of malathion were made when beetles again began to collect on the plants. (Fleming *1963*) In view of the toxicity of malathion to the adult beetle,

experiments were undertaken to explore the possibility of substituting the organic phosphorus insecticides for the chlorinated hydrocarbon insecticides to control grubs in the soil.

Survey of Organic Phosphorus Insecticides

Fleming et al. (unpublished) determined the quantities of 23 organic phosphorus insecticides per acre required to produce toxicity to third-instar grubs in Sassafras sandy loam at 80° F. equivalent to that of 3 pounds of dieldrin per acre. The results of these tests are summarized in table 37. Stauffer N–2788 was slightly more toxic to the grubs than dieldrin. Bayer 37289 and Zytron were equivalent in toxicity to dieldrin. Akton ™, Union Carbide 8305, Diazinon, and Shell SD–8803 were slightly less toxic than dieldrin. The quantities of the other phosphorus compounds required ranged from 5 to more than 20 pounds per acre.

A few of the organic phosphorus insecticides were mixed with Sassafras sandy loam, exposed to weathering, and the quantity of active residue was determined periodically by bioassay. The 3 pounds of Zytron and 4 pounds of Diazinon were largely dissipated in 28 days. The 4 pounds of Akton ™ were reduced to 2.7 pounds per acre during that period. The 4 pounds of Union Carbide 8305 were reduced to 3 pounds in 28 days, 1 pound in 70 days, and 0.6 pound in 90 days. The indications were that the organic phosphorus insecticides were not sufficiently persistent to kill grubs hatching in soil during the summer. (Fleming et al. unpublished)

QUARANTINE ON TURF

Nursery stock is grown in some nurseries in plots where a grass cover is maintained. There is no cultivation. The production and distribution of *Zoysia* grass and other turf became an important industry in the quarantined area after World War II. Since turf is a favored place for oviposition by the Japanese beetle, it is an important medium for carrying the immature stages to uninfested areas. In addition to the treatment of turf and nursery stock in grass-covered plots as a basis for certification, another feature of the quarantine was the treatment of turf where isolated colonies of the beetle had become established to reduce the density of the soil population and to retard the normal spread of the insect.

TABLE 37.—*Toxicity of organic phosphorus insecticides to third-instar grubs in Sassafras sandy loam at 80° F.*

Common name or company designation	Chemical name	Amount per acre equivalent to 3 pounds of dieldrin per acre
		Pounds
Stauffer N–2788	*O*-Ethyl *S*-*p*-tolyl ethylphosphonodithioate	2
Bayer 37289	*O*-Ethyl *O*-(2,4,5-trichlorophenyl) ethylphosphonothioate	3
Zytron	*O*-(2,4-Dichlorophenyl) *O*-methyl isopropylphosphoramidothioate	3
Akton [TM]	*O*-[2-Chloro-1-(2,5-dichlorophenyl)vinyl] *O,O*-diethyl phosphorothioate	4
Union Carbide 8305	Phosphorochloridothioic acid cyclic *O,O*-diester with 2-hydroxy-α-methylcyclohexanemethanol.	4
Diazinon	*O,O*-Diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate	4
Shell SD–8803	*O*-[2-Chloro-1-(2,4-dichlorophenyl)vinyl] *O,O*-diethyl phosphorothioate	4
Dasanit	*O,O*-Diethyl *O*-*p*-[(methylsulfinyl)phenyl] phosphorothioate	5
American cyanamid CL 47470	Cyclic propylene *P,P*-diethyl phosphonodithioimidocarbonate	6
Shell SD–8970	1-(5-Bromo-2-chlorophenyl)-2-chlorovinyl diethyl phosphate	8
Shell SD–9052	2-Chloro-1-(4-chloro-2-fluorophenyl)vinyl diethyl phosphate	13
Phorate	*O,O*-Diethyl *S*-[(ethylthio)methyl] phosphorodithioate	14
Shell SD–8753	2-Chloro-1-(*o*-chlorophenyl)vinyl diethyl phosphate	14
Cyolane	Cyclic ethylene *P,P*-diethyl phosphonodithioimidocarbonate	15
American Cyanamid 47772	Cyclic propylene *P,P*-dimethyl phosphonodithioimidocarbonate	20+
Shell SD–8436	2-Chloro-1-(2,4-dibromophenyl)vinyl dimethyl phosphate	20+
Shell SD–7438	*S,S'*-Benzylidene *O,O,O'O'*-tetramethyl phosphorodithioate	20+
Dimethoate	*O,O*-Dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate	20+
Bayer 42524	S-(*p*-Chlorophenyl) *O,O*-dimethyl phosphorodithioate	20+
Gardona	2-Chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate	20+
Malathion	*S*-[1,2-bis(Ethoxycarbonyl)ethyl] *O,O,*-dimethyl phosphorodithioate	20+
Bayer 29492	*O,O,*-Diethyl *O'*[4-methylthio)-*m*-tolyl] phosphorothioate	20+
Abate	*O,O'*-(Thiodi-*p*-phenylene) *O,O,O',O'*-tetramethyl phosphorothioate	20+

During their most extensive feeding in May, July, August, and September, 90 percent or more of the grubs in turf were just below the surface of the soil. In cultivated fields during these periods most of the grubs were within the upper 3 inches of soil (Hawley *1944*). An insecticide applied to the surface therefore is more likely to kill grubs in turf than in cultivated land.

Insecticide Emulsions and Solutions

Since 1918 many emulsions and solutions have been applied to established turf to reduce the density of grub populations and to eliminate the grubs in the soil.

Sodium Cyanide

Sodium cyanide was the first chemical used to control the grubs in turf. It was used in 1918 and 1919 at 165 pounds in 12,000 gallons of water per acre in an attempt to eradicate the grubs in pastures of the heavily infested area. Only 32 acres of pasture were treated because the cost of the treatment, then $65 per acre, restricted the application of the cyanide to those pastures where the grubs were very abundant. The mortality of the grubs ranged from 66 to 100 percent, depending on the environmental conditions. The treatment burned the grass but did not kill it, except in low spots where excessive quantities of the solution collected. (*Davis 1920a, 1920b, 1920c*)

Carbon Disulfide

The application of dilute carbon disulfide emulsion in late August or early September when oviposition was completed and the grubs were feeding close to the surface of the soil was the first practical method for controlling the grubs in pastures, lawns, and golf courses. A 70-percent emulsion diluted 1:200 with water and applied at 1 quart per square foot deposited 4.17 grams of the chemical per square foot or 400 pounds in 10,890 gallons of water per acre. To assure uniform distribution without runoff, one-half of the solution was applied and when that had penetrated into the soil, the remainder was used.

The dilute emulsion was prepared in a tank and applied under low pressure by a special nozzle, which discharged the emulsion in a fanlike stream close to the surface of the turf. When a mixing machine, which injected the concentrated emulsion into water flowing through a hose, was developed, the use of the tank was discontinued.

The carbon disulfide treatment eliminated 95 to 100 percent of the grub population within 1 week when the soil was moist and the grubs were close to the surface. It was less effective when the ground was dry or the grubs were deeper in the soil.

When the turf was not weakened seriously by grubs feeding on the roots, the following grasses were not injured by the carbon disulfide, except in low spots where an excessive quantity of emulsion collected: Redtop (*Agrostis alba*), creeping bentgrass (*Agrostis palustris*), colonial bentgrass (*Agrostis tenuis*), crabgrass (*Digitaria* sp.), red fescue (*Festuca rubra*), perennial ryegrass (*Lolium perenne*), annual bluegrass (*Poa annua*), Kentucky bluegrass (*Poa praetensis*), and white clover (*Trifolium repens*). Some injury to turf occurred when the emulsion was applied above 85° F. The injured grass turned brown within a few days, but when watered daily it became a normal green within 2 weeks. On the other hand, turf damaged severely by grubs had little resistance to the chemical and was usually killed by the treatment.

Further information on the use of dilute carbon disulfide emulsion to control the grubs in turf is given by Fleming and Baker (*1935*), Leach (*1925*), Leach and Johnson (*1923*), Leach and Lipp (*1927a*), Leach and Thomson (*1923*), and Smith (*1925a*).

The application of 4.17 grams of emulsified carbon disulfide in 1 quart of water per square foot of turf was used extensively in the 1920's and 1930's to reduce populations of grubs where isolated colonies had become established. The density of the populations was greatly reduced, and at some sites the grubs were eliminated.

The application of dilute carbon disulfide to turf per se as a basis for certification was not considered in the 1920's and 1930's because no turf was grown then for shipment outside the quarantined area. The certification of nursery stock growing in plots with a grass cover required the application of 5.9 to 10.4 grams of emulsified carbon disulfide in 2.4 gallons of water per square foot of turf depending on the temperature, as did stock growing in cultivated plots.

Chlordane

The application of 10 pounds of emulsified chlordane in 2,400 gallons of water per acre to turf in the spring when the soil was between 48° and 73° F. killed 98 to 100 percent of the third-instar grubs before pupation. No grubs survived when the dosage

was increased to 20 pounds per acre. The 10-pound dosage was effective in eliminating five annual broods of grubs hatching subsequently in the treated turf. (Fleming et al. unpublished; Mason unpublished)

The 10-pound chlordane treatment was not adapted to the needs of the growers who shipped most of their turf in the spring. The 20-pound dosage was excessive. Although the 10 pounds would eliminate grubs hatching in the soil for several years and thus permit certification of the turf in the fall and spring following the application of the chemical, the growers needed a treatment that would make it possible to certify the turf shortly after its use.

Cresol

Mason (unpublished) applied 195 pounds of emulsified cresol in 9,780 gallons of water per acre of turf and killed 54 percent of the third-instar grubs in 1 week and 82 percent of them in 3 weeks.

D-D

The application of 327 pounds of emulsified D-D in 4,356 gallons of water per acre of turf killed all the third-instar grubs in 5 days. The treatment prevented the transformation of 76 percent of the pupae to adult beetles. When the dosage was applied in 8,712 gallons of water per acre, the emergence of adult beetles was reduced by 98 percent. (Mason et al. unpublished)

Dichloroethyl Ether

Mason (unpublished) applied 484 pounds of emulsified dichloroethyl ether in 9,680 gallons of water per acre to turf and killed 96 percent of the third-instar grubs in 7 days and 97 percent of them in 21 days. Later when 60 percent of the grubs had pupated, the treatment killed 80 percent of the population.

Dichloroethyl Formal

The application of 484 pounds of emulsified dichloroethyl formal in 9,680 gallons of water per acre to turf killed 88 percent of the third-instar grubs in 7 days and 95 percent of them in 21 days (Mason unpublished).

Ethylene Dibromide

During the spring and the fall when the soil was between 40° and 75° F. all third-instar grubs in turf were killed by ap-

plying 10 pounds of emulsified ethylene dibromide in 9,680 gallons of water per acre or 20 pounds of the chemical in 4,840 gallons of water per acre (Mason and Chisholm *1945*; Mason et al. unpublished).

The application of 20 pounds of emulsified ethylene dibromide in 4,840 gallons of water per acre was authorized in 1949 as a basis for certifying turf in the spring and in the fall when the soil was between 40° and 75° F. and only grubs were in the soil. When the turf was matted, the volume of water was increased to 9,680 gallons per acre to assure adequate penetration of the chemical into the soil. Later when the treatment was found to be effective against pupae, the application of the emulsified ethylene dibromide was extended through the pupal period. The turf could be certified for shipment 24 hours after application of the chemical. The treated turf was in a certified status until adult beetles appeared in the vicinity. The turf could be continued in a certified status by repeating the treatment after the adult beetles had completed their flight.

Ethylene Dibromide-Aldrin

An emulsifiable mixture containing 2 pounds of ethylene dibromide and 0.5 pound of aldrin per gallon was prepared according to the procedure described by Chisholm et al. (*1946b*).

The application of 20 pounds of emulsified ethylene dibromide and 5 pounds of emulsified aldrin in 4,840 gallons of water per acre killed all eggs in turf within 1 week. Grubs hatching in the treated turf during the remainder of the summer were killed by the residual aldrin. The treatment was effective in eliminating five annual broods of grubs that hatched subsequently in the treated turf. (Fleming et al. unpublished; Mason unpublished)

Although turf treated with ethylene dibromide-aldrin could be certified shortly after the treatment was used and could be continued in a certified status without re-treatment for several fall and spring shipping seasons, the treatment was not authorized, probably because other fumigant-residual treatments had been authorized previously.

Ethylene Dibromide-Chlordane

The emulsifiable mixture of ethylene dibromide and chlordane, described by Chisholm and Koblitsky (*1951*) and Chisholm and Mason (*1948b*), contained 1 pound of ethylene dibromide and 0.5 pound of chlordane per gallon.

Mason (unpublished) killed all eggs in turf by applying 20

pounds of emulsified ethylene dibromide and 10 pounds of emulsified chlordane in 4,840 gallons of water per acre. Grubs hatching from eggs deposited in the treated turf during the remainder of the summer were killed by the residual chlordane. Fleming et al. (unpublished) found that the residual chlordane in the soil eliminated five annual broods of grubs that hatched subsequently in the treated turf.

Third-instar grubs in turf were killed within 2 weeks in the spring and the fall by applying 20 pounds of emulsified ethylene dibromide and 10 pounds of emulsified chlordane in 4,840 gallons of water or 13 pounds of ethylene dibromide and 6.5 pounds of chlordane in 9,680 gallons of water per acre. The treatment was effective when the temperature of the soil was not lower than 40° F. (Fleming et al. unpublished; Mason and Chisholm *1949*)

A few pupae completed their development and emerged as adult beetles from turf to which 30 pounds of emulsified ethylene dibromide and 15 pounds of emulsified chlordane in 4,840 or 9,680 gallons of water per acre had been applied. These adult beetles were in a weakened condition and many of them died within 1 or 2 days. (Mason and Chisholm *1949*)

The turf was not injured by the 20 pounds of ethylene dibromide and 10 pounds of chlordane, but 30 and 15 pounds, respectively, caused some temporary yellowing of the grass in some of the plots (Mason and Chisholm *1949*).

The application of 20 pounds of emulsified ethylene dibromide and 10 pounds of emulsified chlordane in 4,840 gallons of water per acre was authorized in 1956 as a basis for certifying cultivated turf. This volume of water was usually sufficient to adequately wet the soil, but when the turf was matted, additional water was applied for satisfactory penetration of the insecticides into the soil. The soil had to be between 40° and 75° F. The turf could be certified for shipment 24 hours after the treatment. The treated turf could be in a certified status for 4 years, but the longevity of the effectiveness of the treatment was determined by analysis of the soil.

Ethylene Dibromide-DDT

Chisholm et al. (*1946b*) prepared an emulsifiable mixture containing 2 pounds each of ethylene dibromide and DDT per gallon.

Fleming and Maines (unpublished) applied 25 pounds of emulsified ethylene dibromide, 25 pounds of emulsified DDT, and a

mixture of 25 pounds of each chemical in 1,200 gallons of water per acre to infested turf in May. The ethylene dibromide reduced the third-instar grub population by 62 percent in 2 weeks, 79 percent in 4 weeks, and 92 percent in 7 weeks. The DDT reduced the population by 0.4 percent in 2 weeks, 16 percent in 4 weeks, and 36 percent in 7 weeks. The mixture of the chemicals caused a reduction of 57 percent in 2 weeks, 78 percent in 4 weeks, and 96 percent in 7 weeks. If the chemicals had been applied in 4,840 gallons of water per acre, there is no doubt that the mortality in plots to which ethylene dibromide was applied would have been about 100 percent within 4 weeks. These results show that ethylene dibromide was largely responsible for reducing the population by the ethylene dibromide-DDT mixture.

Ethylene dibromide had little effect on the grubs hatching in the treated turf during the summer; the reduction of the first-instar population was only 9 percent. DDT applied alone or in combination with ethylene dibromide reduced the grub population by 99.5 to 100 percent by mid-September. The DDT was effective in eliminating six annual broods that hatched subsequently in the treated turf.

None of these treatments with ethylene dibromide and DDT caused any injury to the turf.

The ethylene dibromide-DDT treatment satisfied the requirements of the growers of certified turf. It was not authorized because the ethylene dibromide-chlordane treatment was being used with satisfactory results and there was no advantage to the growers in substituting DDT for chlordane.

Ethylene Dibromide-Dieldrin

An emulsifiable mixture containing 2 pounds of ethylene dibromide and 0.5 pound of dieldrin per gallon was prepared according to the procedure described by Chisholm et al. (*1946b*).

Applying 20 pounds of emulsified ethylene dibromide and 0.5 pound of emulsified dieldrin in 4,840 gallons of water per acre killed all eggs in turf within 1 week. Grubs hatching in the treated turf during the remainder of the summer were killed by the residual dieldrin. (Mason unpublished) The possibilities of using the ethylene dibromide-dieldrin treatment on turf were not explored further.

Ethylene Dichloride

Applying 240 pounds of emulsified ethylene dichloride in 4,840 gallons of water per acre killed 90 percent of the third-

instar grubs in turf within 3 weeks. All the grubs were killed by applying 480 pounds of the chemical in 9,680 gallons of water per acre. The ethylene dichloride caused slight yellowing of the grass but no permanent injury. (Mason et al. *1943*)

Parathion

Five pounds of parathion in 4,840 gallons of water per acre applied to turf before the eggs hatched killed all the eggs. The treatment killed 96 percent of the eggs deposited 2 weeks later and 77 percent of those deposited 4 weeks later. Increasing the dosage to 10 pounds per acre did not prolong the effectiveness of the treatment. (Mason unpublished)

Topdressing Established Turf

Lead Arsenate

Leach and Lipp (*1926, 1927b*) demonstrated that lawns and golf courses could be protected from damage by the grubs by applying lead arsenate to the soil before seeding. In further experiments Leach (*1928, 1929*) protected established turf from grub damage for 1 or 2 years by topdressing with 150 pounds of lead arsenate per acre and for 3 or 4 years by topdressing with 250 pounds of the chemical per acre. Fleming and Osburn (*1932*) found that the 250-pound treatment reduced the third-instar grub population adequately when there were not more than 150 grubs per square yard of established turf, but when the grubs were more numerous, it was necessary to use not less than 435 pounds per acre to prevent serious damage. The 435-pound treatment prevented the reestablishment and the buildup of a grub population in turf for at least 5 years.

The lead arsenate was applied as a spray or mixed with a filler and applied in a dry state (Fleming *1936*; Fleming and Osburn *1932*). Satisfactory results were obtained by mixing 1 pound of the chemical with each 2 gallons of water in a spray tank with agitation to keep the material in suspension and applying 20 gallons of the spray under 200 to 300 pounds' pressure to each 1,000 square feet of turf. The turf was then washed with water to remove the chemical from the grass before the spray dried. For the application in the dry state with a fertilizer distributor, some of the satisfactory mixtures by weight, each with one part of lead arsenate, were as follows: (1) Four parts of activated sludge, (2) two parts of activated sludge and four

parts of sand, (3) two parts of tankage and four parts of sand, and (4) 10 parts of sand.

Topdressing turf with lead arsenate was not injurious to most of the common grasses, including redtop (*Agrostis alba*), velvet bentgrass (*Agrostis canina*), creeping bentgrass (*Agrostis palustris*) and the varieties of this grass called metropolitan bent and Washington bent, colonial bentgrass (*Agrostis tenuis*), red fescue (*Festuca rubra*), Italian ryegrass (*Lolium multiflorum*), perennial ryegrass (*Lolium perenne*), and Kentucky bluegrass (*Poa pratensis*). Annual bluegrass (*Poa annua*) was sometimes killed by the arsenical. White clover (*Trifolium repens*) was not injured. (Fleming *1936*)

Further information on the use of lead arsenate to protect turf from damage by the grubs is given by Fleming (*1930b, 1936, 1950b*), Fleming et al. (*1934*), Fleming and Metzger (*1936a, 1938*), Fleming and Osburn (*1932*), Leach (*1928, 1929*), and Osburn (*1934a*).

Lead arsenate was not applied as a basis for certification to turf grown for shipment or to nursery stock grown in grass-covered plots. These problems did not arise during the period that lead arsenate was being used.

It was applied extensively in the 1930's as a topdressing to turf where isolated colonies of the beetle had developed. This was part of the program to retard the buildup and the dispersion of the beetle from these colonies. The chemical was usually applied at 500 pounds per acre, but at some sites 1,000 pounds per acre were used. One of the more extensive applications of lead arsenate to turf was in the campaign against the beetle at St. Louis, Mo., during 1934–44, when over 347 tons of the arsenical were applied to over 700 acres of turf. By 1937 the thriving colony of beetles was so reduced that only one beetle was captured in traps. It was caught in a freight yard and was probably a hitchhiker. During the following 7 years only 130 beetles were captured in traps. No doubt the campaign at St. Louis retarded for many years the spread of the insect into the agricultural areas. Further information on the St. Louis campaign is given by Dawson (*1936, 1937, 1938*), Denning and Goff (*1944*), and Stockwell (*1935*).

Chlorinated Hydrocarbon Insecticides

Topdressing established turf with the chlorinated hydrocarbon insecticides was developed originally to protect lawns, golf

courses, cemeteries, and parks from damage by the grubs. DDT and toxaphene were recommended at 25 pounds, chlordane at 10 pounds, and aldrin, dieldrin, and heptachlor at 3 pounds per acre. The application of these chemicals killed a high percentage of the third-instar grubs and eliminated several annual broods of grubs hatching subsequently in the treated turf. The chlorinated hydrocarbon insecticides caused no injury to the various grasses. (Fleming *1947c, 1948a, 1950b, 1950c, 1955, 1958, 1960, 1963*)

The persistence of topdressings of the chlorinated hydrocarbon insecticides in turf was determined by bioassays and chemical analyses of treated turf in Connecticut, Massachusetts, New Jersey, and Pennsylvania (Fleming unpublished; Fleming, Maines, and Coles *1951*). The results of these determinations are summarized in table 38. When these residues are compared with the pounds of the insecticides per acre required to kill first-instar grubs—aldrin 0.07, chlordane 0.4, DDT 5, dieldrin 0.3, heptachlor 0.07, and toxaphene 2.5—it is evident that the effectiveness of 10 pounds of chlordane, 25 pounds of DDT, 3 pounds of dieldrin, and 25 pounds of toxaphene persisted for 7 years, 3 pounds of aldrin for about 5 years, and 3 pounds of heptachlor for about 4 years.

The use of 3 pounds of aldrin, dieldrin, or heptachlor, 10 pounds of chlordane, and 25 pounds of DDT or toxaphene per acre was authorized in 1960 as a basis for certifying nursery stock grown in grass-covered plots. The requirements were the same as for cultivated plots, except the chemicals were not mixed with the upper 3 inches of soil. It was expected that certi-

TABLE 38.—*Persistence of chlorinated hydrocarbon insecticides applied as topdressing to established turf*

Years after application	Average active insecticide per acre					
	Aldrin	Chlordane	DDT	Dieldrin	Heptachlor	Toxaphene
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
0	3.0	10.0	25.0	3.0	3.0	25.0
1	.9	2.8	24.5	2.0	.5	20.0
2	.5	1.5	23.0	1.4	.3	15.5
3	.3	1.0	20.0	1.0	.2	12.0
4	.2	.9	15.5	.7	.1	10.0
5	.1	.7	11.0	.5		8.5
6		.6	9.5	.4		7.0
7		.5	7.5	.3		6.0

fication could be for at least 3 years with aldrin, chlordane, and heptachlor and for at least 5 years with DDT, dieldrin, and toxaphene, but the longevity of the certified period was determined by assays of the soil.

Topdressing turf grown for shipment with the chlorinated hydrocarbon insecticides was not adapted to the requirements of the turf industry because turf treated in the spring could not be certified for shipment until the fall, although it then could be in a certified status for several years.

In addition to applying the chlorinated hydrocarbon insecticides to grass-covered nursery plots as a basis for certifying nursery stock, the chemicals were used to treat turf where isolated colonies of beetles had developed to retard the buildup and dispersion of the insect. Usually chlordane, DDT, and dieldrin were used in these campaigns.

DDT was first applied in 1944 to control an isolated colony of beetles at Blowing Rock, N.C. During 1944–48 the chemical was applied at 25 pounds per acre to 257 acres of turf. The conditions were favorable for the rapid buildup of the beetle population as was shown by the increase in beetles captured by traps in an untreated part of the area where 357 beetles were caught in 1945, 2,612 in 1946, 7,727 in 1947, and 16,798 in 1948. In contrast to this trend, traps in the treated area captured 138,945 beetles in 1944, 41,914 in 1945, 19,623 in 1946, 23,361 in 1947, and 15,008 in 1948. In 1953 very few beetles were found in Blowing Rock, but the insect had spread into the adjacent countryside and was very numerous there. (Fleming and Hawley *1950*)

One of the areas treated with dieldrin was an isolated colony of beetles on farmland along the Illinois-Indiana border. During 1954–58 dieldrin was applied at 2 or 3 pounds per acre to 17,844 acres in Illinois and 3,460 acres in Indiana. The dieldrin killed about 50 percent of the third-instar *Popillia* grubs in the soil when the chemical was applied and practically eliminated the grubs hatching in the soil during the following 4 years. Many other insects of economic importance that came in occasional or frequent contact with the treated soil were controlled for 1 to 5 years. The populations of a few economic insects increased after the application of dieldrin, but the increase was not great enough to warrant additional control measures. Some predators were adversely affected or eliminated by the dieldrin, whereas other predators and parasites appeared to be unharmed. (Luckmann and Decker *1960*)

The campaign against an isolated colony of beetles at Sacra-

mento, Calif., during 1961–64 was conducted by spraying the foliage with carbaryl during the flight of the beetle and applying 10 pounds of chlordane per acre to the turf. The situation at Sacramento is unique in that there is no rainfall during the summer when the beetle is in flight and the city is surrounded by thousands of acres of dry-farmed land where the beetle could not survive. The luxuriant foliage and turf in the residential areas, which are maintained by frequent heavy watering, provided an excellent environment for the development of the beetle and tended to preclude widespread flights of the insect in search of food and oviposition sites. During 1961 and 1962 chlordane was applied to approximately 3,500 acres. Intensive surveys in 1963 and 1964 failed to find any beetles in the Sacramento area. (Gammon *1961*, unpublished)

DETERMINING RESIDUES OF PERSISTENT INSECTICIDES IN SOIL

The quantitative determination of residues of the authorized persistent insecticides in soil was an important feature in the certification of nursery stock grown in plots treated with these insecticides, particularly when the chemical applied was approaching the expected longevity of effectiveness that had been determined experimentally. The certification was based on the knowledge that there was sufficient toxic residue in a plot to eliminate grubs hatching in the soil during the summer. The variation in the types of soil, cultural practices, and environmental conditions throughout the quarantined area made it necessary to determine how much residue remained in the soil of a plot. It has been the practice of the Plant Protection Division, Agricultural Research Service, to analyze the soil of certified plots and to require the application of more insecticide only when needed to continue a plot in a certified status.

Distribution of Residues in Soil

Vertical Distribution

Most of the residues of the persistent insecticides applied to turf and uncultivated land were found close to the soil surface. One year after applying DDT as a topdressing to turf, 92 percent of the residue was in the upper inch of soil and the remainder in the next 2 inches (Chisholm and Koblitsky *1952*). Ninety percent of the DDT recovered in uncultivated orchards was found in the

debris or close to the surface of the ground (Chisholm et al. *1950*). Four months after applying aldrin to uncultivated land nearly all the residue was in the upper 2 inches of soil (Lichtenstein et al. *1962*). One or more years after broadcasting dieldrin over alfalfa fields 87 percent of the residue occurred in the upper inch of soil (Hardee et al. *1964*).

Most of the residues of the chlorinated hydrocarbon insecticides applied to cultivated land were found in the tilled layer. The DDT in the soil of cultivated orchards was found largely in the upper 3 inches (Chisholm et al. *1950*). One year after applying DDT to plots cultivated periodically 88 percent of the residue was in the upper 3 inches of soil and the remainder in the 3- to 6-inch layer (Chisholm and Koblitsky *1952*). One or two years after applying aldrin, DDT, and lindane and rototilling to a depth of 5 inches, 84 to 96 percent of the residues occurred in the upper 3 inches of soil (Lichtenstein *1958a*; Lichtenstein et al. *1962*). Rotary tillage and disking dispersed more aldrin, DDT, dieldrin, and lindane within the upper 2½ inches of soil than in the next 2½ inches (Lange and Carlson *1955*). Four years after applying benzene hexachloride, DDT, and toxaphene 97 percent of the residues were found in the plowed layer (Allen et al. *1954*). Chisholm and Koblitsky (*1959*) reported a similar vertical distribution of the chlorinated hydrocarbon insecticides in plowed land.

Thus the chlorinated hydrocarbon insecticides penetrated little into the soil beyond where they were deposited or were mixed with the soil by tillage. The residual insecticide in the upper 3 inches of soil is the most important in controlling the grubs because most of the feeding occurs within this layer. The residue at lower levels contributed little to the control of the grubs. In sampling plots to determine insecticide residues it has been the practice for many years to take the samples to a depth of 3 inches and to disregard any residue deeper in the soil.

Lateral Distribution

Even when great care was exercised in applying an insecticide to turf or to cultivated land, the lateral distribution was heterogeneous. A year after applying 1,500 pounds of lead arsenate per acre to two cultivated nursery plots Fleming and Koblitsky (unpublished) found the quantity of residue at spots throughout one plot ranged from 280 to 3,875 pounds per acre and throughout the other plot from 180 to 3,515 pounds per acre. Chisholm and Koblitsky (*1952*) found that the variation in a cultivated plot

treated with 1,000 pounds of lead arsenate per acre ranged from 289 to 3,437 pounds per acre. About 1 year after applying 25 pounds of DDT per acre to turf Chisholm and Koblitsky (*1952*) found that the quantity of residue ranged from 1 to 74 pounds per acre.

These heterogeneous distributions of the insecticides were effective in killing grubs in the soil because the grubs while feeding moved laterally in the soil and encountered spots of high and low concentration.

Sampling Soil

Cores of soil, 2 inches in diameter and 3 inches deep, were taken at random in nursery plots and in turf with a soil sampler described by Johnson and Caskey (*1952*) or with a similar device.

With the heterogeneous lateral distribution of an insecticide, a sufficient number of cores must be taken over a treated plot so that the composite sample contains an amount of the chemical equivalent to the average in the plot. Fleming analyzed statistically the lead arsenate content of 228 cores that had been taken over two ½-acre nursery plots. He determined that the average amount of the chemical in each of these plots could be estimated within 18 percent with a composite sample of 10 cores, 11 percent with 25 cores, 8 percent with 50 cores, 7 percent with 75 cores, and 6 percent with 100 cores. The analyses of ten 50-core composite samples were all within 10 percent of the average.

It was authorized in 1931 that in sampling a treated nursery plot to determine the insecticide content of the soil, 50 cores of soil, each 2 inches in diameter and 3 inches deep, must be taken at random over an area of not more than 20,000 square feet. Larger plots were divided into areas of not more than 20,000 square feet and 50 cores were taken from each of them. The soil had to be friable at the time of sampling. The 50-core sample was passed twice through a ¼-inch mesh screen to remove stones and debris and was weighed.

Chemical Analysis

It is customary in reporting chemical analyses of pesticide residues to express the results as parts of pesticide per million parts of product by weight. When the quantity of insecticide in the upper 3 inches of soil is reported as parts per million, there may be little relation between the quantity reported and the actual weight of insecticide in a layer of soil. The weight of an

acre of many field soils to a depth of 3 inches is close to 1 million pounds, but the weight of this layer of soil may range from 800,000 to 1,200,000 pounds. Twenty-five pounds of insecticide in the upper 3 inches of various soils would have no constant value when expressed as parts per million; the parts per million may range from 20 to 30.

Since soils differ in bulk density, insecticide concentrations in various soils can be compared only on a weight-volume basis, such as pounds of insecticide per acre in the upper 3 inches of soil. Twenty-five pounds of insecticide per acre in this layer of soil is 25 pounds per acre in the various soils. Therefore in reporting the quantity of insecticide found in the composites of the 3-inch cores of soil, the results were expressed as pounds of insecticide per acre in the upper 3 inches of soil.

Lead Arsenate

Subsamples of convenient size were taken from the screened composite sample, weighed, exposed in shallow trays until nearly air-dry, and then reweighed. The residues of lead arsenate in the aliquant subsamples were determined according to the procedure described by Koblitsky (*1939*). The method involves the reduction of the lead arsenate with hydrazine-sodium bromide in the presence of hydrochloric acid, removal of the arsenious chloride formed by distillation, and titration of the distillate for arsenic with a standard iodine solution. The equivalent lead arsenate in the aliquant sample was calculated by multiplying the grams of arsenic by the factor 1.6752.

The lead arsenate as pounds per 3-inch acre of soil was calculated by the formula given by Koblitsky and Chisholm (*1949*).

$$\text{Pounds per 3-inch acre} = \frac{U \times W_1 \times W_3 \times 41{,}486}{S \times W_2 \times V}$$

Where U = weight of lead arsenate in aliquant titrated (grams).
S = weight of nearly air-dry subsample represented by aliquant (grams).
W_1 = weight of nearly air-dry subsample (grams).
W_2 = weight of subsample taken from screened composite sample (grams).
W_3 = weight of screened composite sample (grams).
V = volume of composite sample = top area × depth × number of cores (cubic inches).
41,486 = factor to convert grams per cubic inch to pounds per 3-inch acre.

In the analyses of soil containing known quantities of lead arsenate the recoveries ranged from 97.7 to 100.8 percent.

This analytic procedure was authorized in 1930 for determining the quantity of lead arsenate in certified nursery plots. It was used extensively for many years to determine whether there was sufficient residue in the soil to eliminate grubs hatching in the soil.

Chlorinated Hydrocarbon Insecticides

Subsamples of convenient size were taken from the screened composite sample, weighed, exposed in shallow trays until nearly air-dry, and then reweighed. Chisholm and Koblitsky (*1952*) found that when a solvent was used to extract the insecticide from the soil, low recoveries may be obtained with subsamples that have been insufficiently dried or have been thoroughly dried. Subsamples that have not been dried sufficiently tend to form balls during the extraction. Some of the insecticide may be lost by volatilization from thoroughly dried soil, or it may be encased in pellets formed on drying soils high in colloids.

Chisholm and Koblitsky (*1952*) tested many solvents and mixtures of solvents as extractants for the chlorinated hydrocarbon insecticides. Some solvents removed an excessive amount of the natural soil constituents, whereas others did not remove the insecticide completely. The most consistent recoveries were obtained with a 2:1 mixture by volume of benzene and isopropyl alcohol. The slight amount of water that may be extracted from soil by this mixture was easily removed by adding anhydrous sodium sulfate to the extract. Practically complete extraction was obtained by rotating mechanically a mixture of the soil and the solvent for one-half hour. The soil-solvent mixture was filtered to obtain the extract for analysis.

Eventually specific colorimetric and spectrophotometric methods were developed for determining most of the chlorinated hydrocarbon insecticides. Carter (*1955*) pointed out that these methods are tedious and time consuming and that homologous compounds, decomposition products, and plant extractive sometimes interfere with the analysis. Chisholm and Koblitsky (*1952*) found that many soils contain extractable materials that interefere with such determinations. Koblitsky and Chisholm (*1949*) found that the color bodies extracted from soils interfered with the colorimetric method for DDT of Stiff and Castillo (*1945*). The colorimetric determination of DDT by the procedure of Schechter et al. (*1945*) was too time consuming for routine

analyses (Koblitsky and Chisholm *1949*). An adaptation of the method of Clayborn (*1946*) did not give reliable results in the determination of DDT in nursery soils in New Jersey (Chisholm and Koblitsky *1952*). In 1963 G. L. Mack (unpublished) of the New York Agricultural Experiment Station demonstrated that residues of DDT and dieldrin in soil could be determined by gas chromatography.

In the absence of suitable specific methods in 1948, Koblitsky and Chisholm (*1949*) developed a method for determining the total organic chlorine in the extracts. It was based on the procedure described by Umhoefer (*1943*). The method entails evaporating an aliquant part of an extract almost to dryness, adding metallic sodium with isopropyl alcohol to liberate the chlorine in the insecticide, eliminating the undecomposed sodium, acidifying with dilute sulfuric acid, adding standardized silver nitrate solution, removing the precipitated silver chloride, and determining the excess silver nitrate. The difference between the quantity of silver nitrate added and that remaining is the quantity of silver nitrate reacting with the liberated chlorine. The equivalent grams of aldrin, DDT, chlorane, dieldrin, heptachlor, or toxaphene in the aliquant sample were calculated by multiplying the grams of chlorine by the appropriate factor. The pounds of the chlorinated hydrocarbon insecticide per acre to a depth of 3 inches were calculated by the same formula used for lead arsenate.

The recovery of DDT from extracts of sandy, clay, and muck soil containing known quantities of the insecticide ranged from 96 to 108 percent. However, when corrections were made from the results of analyses of untreated soils, which usually were not available in nurseries, the recoveries ranged from 92 to 96 percent. The differences between the analyses of duplicate subsamples from 84 composite samples from nursery plots averaged 1 pound of DDT per 3-inch acre and ranged from 0 to 2.5 pounds. Similar results were obtained in the analyses of soils containing the other chlorinated hydrocarbon insecticides. (Chisholm and Koblitsky *1952*; Koblitsky and Chisholm *1949*)

The organic chlorine method was authorized in 1949 to determine whether one of the chlorinated hydrocarbon insecticides in a nursery plot was sufficient to continue it in a certified status. The method was used extensively. Since a nurseryman did not have to re-treat a plot with the same insecticide applied originally to the soil, in a few years an increasing number of nursery plots contained residues of two or more of the chlorinated hydrocarbon insecticides. The organic chlorine method was

inadequate under these conditions. Although the organic chlorine in soil containing these mixtures could be determined, the residues, which differed in toxicity, could not be identified or the equivalent quantity of each residue calculated. No other chemical method was then available to determine whether a mixture of the chlorinated hydrocarbon insecticides in a soil was sufficient to continue a nursery plot in a certified status.

Bioassay

In the absence of suitable chemical methods, consideration was given to developing a biological procedure for evaluating complex insecticide residues in soil. Davis (*1951*) pointed out that the lack of satisfactory chemical methods had stimulated interest in the application of bioassays to residue problems.

Chemical analyses do not necessarily establish the presence of toxic substances in soil because they do not measure the availability of the residues as insecticides. Toxicity is revealed by its effect on a living organism. Bioassay is a method for determining the quantity of a toxic substance by measuring its effect on a living organism. Bioassays are of interest because of the wide range of chemical compounds to which they can be applied and in some instances to their high sensitivity. Bioassays sometimes indicate the presence of toxic residues that have not been detected by chemical analysis. (Carter *1955*)

Various biological techniques have been used for many years in assays of insecticide formulations and residues. These assays evaluate toxicity but usually give little other information about the toxicant. However, Laug (*1948*) differentiated the *gamma* isomer from the other isomers of benzene hexachloride by their relative toxicities to the house fly (*Musca domestica* L). Fleming, Coles, and Maines (*1951*) distinguished residues of DDT from those of chlordane in the soil by the slower response of *Macrocentrus ancylivorus* Rohwer to DDT and noted differences in the symptoms of intoxication produced by these insecticides. Davidow and Sabatino (*1954*) differentiated chlordane and related cyclodienes from other chlorinated hydrocarbons by differences in the response of goldfish (*Carassius auratus* L.). Harwood and Areekul (*1960*) used four unrelated test animals—a pomace fly, *Drosophila melanogaster* Meigen, the rusty grain beetle (*Cryptolestes ferrugineus* (Stephens)), the mushroom mite (*Tyrophagus putrescentiae* (Schrank)), and the brine shrimp (*Artemia salina* (L.))—and obtained mortality patterns that distinguished

aldrin, allethrin, carbaryl, DDT, dieldrin, heptachlor, and parathion.

The basic principles of quantitative determination used in analytical chemistry apply to bioassay. Gunther and Blinn (*1953*), Healey (*1956*), and Van Middelem (*1958*) reviewed these principles. Hoskins (*1957*) discussed the use of bioassay in entomological research. Dewey (*1958*) and Nagasawa (*1959*) reviewed bioassay techniques for determining pesticide residues.

Bioassays of soil containing the residue of only one insecticide have been made by extracting the residue in the same manner as for chemical analysis and exposing a test organism to the extract, or by exposing a test organism directly to the soil, or by introducing a test organism into the soil. The response of the test organism to the extract or to the soil was compared with its response to a standard with known amounts of the insecticide under standardized conditions, and by interpolation of the response data the amount of toxicant present was calculated.

Bioassays of soil extracts have been made by Banks and Reed (*1956*), Berger and Randolph (*1958*), Fleming, Coles, and Maines (*1951*), Gannon and Bigger (*1958*), Kiigemagi et al. (*1958*), Lange and Carlson (*1955*), Lichtenstein et al. (*1960*), Terriere and Ingalsbe (*1953*), and others. Direct bioassays of an insecticide residue in soil have been made by Edwards et al. (*1957*), Fleming, Coles, and Maines (*1951*), Fleming et al. (*1962*), Fleming and Maines (*1953a, 1954*), Kloke (*1953*), Lichtenstein and Polivka (*1959*), Lichtenstein and Schulz (*1959, 1960*), Lichtenstein et al. (*1960*), Wylie (*1956*), and Young and Rawlins (*1958*).

The bioassay of a soil extract is an evaluation of the total potential toxicity in the soil because the solvent extracts both the free and the adsorbed insecticide. On the other hand, the direct bioassay measures only the active toxicity. The adsorbed part of the residue apparently is not available as an insecticide. The direct exposure methods have certain advantages over the extraction methods in that they are relatively simple and the potential danger of insecticide loss during preparation of the sample or poor extraction is not encountered. Lichtenstein (*1958b*) discussed the advantages and disadvantages of direct bioassay of soil. The active toxicity, not the total potential toxicity, in a soil is the criterion as to whether sufficient insecticide is present to kill the grubs.

The Grub as a Test Insect

The first-instar grub is the logical organism to use in evaluating the active toxicity in soil treated with insecticides to control this stage of the insect, but its usefulness is limited. Grubs at this stage are usually available for only a few weeks during the summer; they have not been propagated successfully in large numbers at other seasons. They must be handled with extreme care because of their susceptibility to mechanical injury and to adverse environmental factors, such as desiccation, excess moisture, or increased carbon dioxide in the soil. The third-instar grub, which is present for 9 months of the year and is more readily handled and less affected by its environment, was a practical substitute for the newly hatched grub. Third-instar grubs were used for many years in evaluating insecticide residues in experimental field plots. (Fleming, Coles, and Maines *1951;* Fleming and Maines *1953a, 1954;* Fleming et al. unpublished)

In sampling the soil of a plot for bioassay, a composite sample of 50 cores was taken at random to a depth of 3 inches in the same manner as for chemical analyses. This composite sample was slightly more than one-fourth cubic foot in volume. It was passed through a ¼-inch mesh sieve to remove stones and debris.

The amounts of lead arsenate and the chlorinated hydrocarbon insecticides in Sassafras sandy loam that killed third- and first-instar grubs are given in table 39. Although the duration of exposure shown in the table is 2 weeks, it was necessary to modify this period because various batches of grubs collected in the field differed in their susceptibility to the insecticides. In a bioassay the exposure was prolonged until 50-percent mortality was obtained with the median dosage of the standard insecticide, which could be lead arsenate or one of the chlorinated hydrocarbon insecticides.

In the bioassay of an insecticide residue in an experimental plot the mortality with the sample was compared with the mortality in the standard, and by interpolation the quantity of active insecticide, expressed as pounds per 3-inch acre, was calculated.

The dosages of endrin, dieldrin, heptachlor, and aldrin to kill 50 percent of the third-instar grubs under these conditions were about the same as those needed to prevent the development of newly hatched grubs. The dosages of DDT, toxaphene, and chlordane for third-instar grubs at this level of mortality were somewhat higher than those required to eliminate first-instar grubs.

TABLE 39.—*Amounts of lead arsenate and chlorinated hydrocarbon insecticides per 3-inch acre of Sassafras sandy loam for 10- to 90-percent mortality of third-instar and 100-percent mortality of first-instar Japanese beetle grubs in 2 weeks at 80° F.*

Mortality (percent)	Lead arsenate	DDT	Toxaphene	Chlordane	Endrin	Dieldrin	Heptachlor	Aldrin
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
			THIRD-INSTAR GRUBS					
10	180	1.8	2.4	0.11	0.08	0.02	0.03	0.02
20	240	3.0	3.4	.24	.13	.04	.04	.03
30	300	4.4	4.3	.41	.20	.08	.06	.04
40	360	6.0	5.3	.66	.28	.15	.08	.06
50	440	8.0	6.5	1.02	.40	.26	.10	.08
60	520	10.7	8.0	1.58	.54	.45	.12	.11
70	630	14.6	10.0	2.52	.76	.81	.16	.15
80	780	21.0	12.7	4.34	1.14	1.64	.23	.22
90	1,050	34.8	18.0	9.23	1.98	4.35	.37	.38
			FIRST-INSTAR GRUBS					
100	500	5.0	2.5	.40	.40	.30	.07	.07

There was better agreement between the dosages of DDT, toxaphene, and chlordane for 50-percent mortality of third-instar grubs and 100-percent mortality of first-instar grubs when the exposure with these insecticides was prolonged for 21 days. However, the exposure could not be modified in this manner when residues of the other insecticides were also in the soil.

In a bioassay of a certified nursery plot containing residues of lead arsenate and one or more of the chlorinated hydrocarbon insecticides, it would be necessary to establish whether the toxicity in the soil was sufficient to eliminate newly hatched grubs. The toxicity would be sufficient to continue the plot in a certified status for at least an additional year when the mortality of the third-instar grubs in the sample of soil was more than 50 percent. Unfortunately the third-instar grub was a suitable test organism only during the fall and winter. The results were confounded by pupation when bioassays were conducted in the spring. Evaluating toxicity in the spring is more important than at other periods of the year, because it shows, just prior to the hatching of the new annual brood, whether there is sufficient insecticide residue to kill the first-instar grubs.

Drosophila melanogaster Meigen as a Test Insect

A search was made for a more suitable organism for bioassays of insecticide residues in soil. A review of the literature showed that many organisms had been used for the detection and quantitative determination of toxic materials. These included bacteria, fungi, yeasts, daphnids, shrimps, several species of fish, collembola, symphilids, the larvae of several species of mosquitoes, several species of insects that attack stored products, the house fly, the pomace fly, termites, wireworms, and several species of white grubs. The most suitable of these organisms appeared to be a pomace fly, *Drosophila melanogaster* Meigen. Fleming et al. (*1962*, unpublished) explored the possibilities of using this fly as a test insect to evaluate insecticide residues in nursery soils.

Although *Drosophila* is probably best known as a well-adapted organism for studies in theoretical biology, it has been used by several investigators as a test insect in bioassay. It was used with other organisms to identify insecticides and acaricides in comparative bioassays (Harwood and Areekul *1960*) and to determine residues of the chlorinated hydrocarbon insecticides in or on fruits and vegetables (Earle et al. *1959*; Glasser et al.

1958; Gyrisco et al. *1955*; Olsen [4]; Pankaskie and Sun *1954*; Sun and Pankaskie *1954*), of parathion on cauliflower (Kasting and Harcourt *1952*), of dieldrin in applesauce (Fisher and Smallman *1954*), of aldrin, dieldrin, and heptachlor on alfalfa (Koehler et al. *1958*; Lichtenstein and Medler *1958*), of isobenzan on tobacco (Guthrie et al. *1959*), and of aldrin, benzene hexachloride, chlordane, DDT, dieldrin, heptachlor, and methoxychlor in soil (Edwards et al. *1957*; Lichtenstein et al. *1960*; Lichtenstein and Polivka *1959*; Lichtenstein and Schulz *1959, 1960*; Wylie *1956*; Young and Rawlins *1958*).

Advantages in using *Drosophila* as a test insect in bioassay were as follows: (1) It was reared in large numbers with reasonable facility throughout the year; (2) continuous propagation did not modify its susceptibility to insecticides; (3) it was handled in large numbers without difficulty; (4) it reacted to small residues of the chlorinated hydrocarbon insecticides in soil; (5) slight changes in the concentration of these insecticides were reflected by modifications in the mortality; (6) an assay with most of the chlorinated hydrocarbon insecticides was completed within 24 hours; and (7) its natural mortality during a bioassay was negligible.

In preparing a nearly air-dry sample of soil for bioassay, Fleming et al. (*1962*) mixed an aliquant part of the sample with a small amount of plaster of paris and added enough of a dilute corn sirup solution containing sodium propionate to make a free-flowing slurry. The plaster of paris was added so that the slurries of all soils presented a moderately hard smooth surface to the flies. The corn sirup provided food for the flies during the bioassay, and the sodium propionate retarded the decomposition of the sugar. The slurry was distributed among several petri dishes. When the plaster of paris had set and there was no free water on the surface, the soil was ready for exposure to the flies. The petri dishes containing the hardened slurries of the samples and those with hardened slurries of Sassafras sandy loam containing known quantities of insecticide were transferred to a well-lighted room maintained at 80° F., and the flies, slightly anesthetized with carbon dioxide, were distributed among the dishes.

Chlorinated Hydrocarbon Insecticides.—*Drosophila* was very sensitive to residues of the chlorinated hydrocarbon insecticides

[4] OLSEN, R. E. THE USE OF THE FRUIT FLY DROSOPHILA MELANOGASTER IN DETECTING MINUTE QUANTITIES OF BENZENE HEXACHLORIDE IN PLANT TISSUES. 1954. [Unpublished Ph. D. thesis. Univ. Microfilms, Ann Arbor, Mich., Pub. 9767.]

in the slurries of soil. The speed of reaction to the residues at 80° F. and constant light varied with the inherent toxicity and the concentration of the insecticides, the nature of the soil, and other factors, but the symptoms of intoxication were much the same for all of the insecticides.

Five stages of intoxication were recognized. In the first stage the insecticide had no visible effect on the flies. They appeared to be normal. They rested quietly, walked leisurely, and flew occasionally. The second stage was a period of excitement. The flies did little resting and appeared to be irritated. They walked and flew rapidly and responded readily to any disturbance, such as a shadow or a tap on the petri dish. In the third stage the flies did more resting and began to walk unsteadily. As the legs became paralyzed, the flies tended to stand in a fixed position with an occasional violent movement of the wings. This movement pushed the flies aimlessly and rapidly over the surface of the soil. The fourth stage was exhaustion. The flies were unable to move from their positions; they lay on their sides or backs with only an occasional slight movement of the legs. The final stage was death. The affected individuals were most easily counted when the intoxication had progressed to the advanced stage of paralysis so that the flies were unable to change their positions or they were dead. Permanent immobilization was selected as the criterion of toxic action.

The amounts of the chlorinated hydrocarbon insecticides in Sassafras sandy loam that killed 10 to 90 percent of the flies and 100 percent of the first-instar grubs at 80° F. are given in table 40. The 24- and 48-hour exposures indicated in the table are only approximations. The exposure of soil samples from the nurseries to the flies was the period of time for the median dosage of one of the insecticides to reach 50 percent. The use of Sassafras sandy loam in the standard was restricted geographically because this type of soil occurs only on the Coastal Plain of the Middle Atlantic States.

Of the several materials tested as a substitute for the soil the most satisfactory was a standard filter-paper tablet, which is readily available at companies dealing in laboratory equipment. When the flies were exposed to this tablet containing 27 μg. of dieldrin, mortality occurred at the same rate during 24 hours as when the soil contained the median dosages of the insecticides. The mortality with the tablet containing 12 μg. of dieldrin progressed at the same rate during 48 hours as when the soil contained the median dosage of DDT or toxaphene. The use of the

TABLE 40.—*Amounts of chlorinated hydrocarbon insecticides per 3-inch acre of Sassafras sandy loam for 10- to 90-percent mortality of* Drosophila *and 100-percent mortality of first-instar Japanese beetle grubs at 80° F.*

Mortality (percent)	24-hour exposure							48-hour exposure	
	DDT	Chlordane	Endosulfan	Endrin	Dieldrin	Heptachlor	Aldrin	DDT	Toxaphene
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
				DROSOPHILA					
10	29.2	0.41	0.08	0.16	0.14	0.014	0.034	2.7	1.9
15	32.0	.49	.19	.19	.16	.028	.038	3.6	2.3
20	35.0	.57	.24	.22	.18	.034	.042	4.5	2.7
25	37.5	.65	.29	.26	.20	.040	.046	5.4	3.0
30	40.0	.73	.35	.29	.22	.045	.050	6.4	3.3
35	42.5	.81	.41	.32	.23	.051	.054	7.5	3.7
40	45.0	.89	.48	.36	.25	.058	.058	8.7	4.1
45	47.5	.98	.55	.40	.26	.065	.062	10.0	4.5
50	50.0	1.07	.64	.44	.28	.073	.066	11.6	4.9
55	52.5	1.18	.74	.49	.30	.082	.070	13.3	5.4
60	55.0	1.30	.86	.54	.32	.092	.075	15.4	5.9
65	58.5	1.42	1.00	.60	.34	.104	.081	17.9	6.5
70	62.0	1.58	1.18	.67	.37	.118	.087	21.0	7.2
75	66.0	1.76	1.40	.76	.40	.136	.094	24.9	8.0
80	71.0	2.00	1.70	.86	.43	.158	.103	30.1	9.0
85	77.0	2.30	2.14	1.01	.48	.189	.114	37.5	10.4
90	85.0	2.76	2.85	1.23	.54	.237	.130		12.4
				JAPANESE BEETLE GRUBS					
100	5.0	.40		.40	.30	.07	.07	5.0	2.5

impregnated tablet to regulate the exposure of the flies to the soil samples removed the geographical limitation on the procedure.

The dosages of endrin, dieldrin, heptachlor, and aldrin for 50-percent mortality of *Drosophila* were about the same as the minimum dosages of these insecticides for 100-percent mortality of first-instar grubs, but the dosages of DDT (48 hours), chlordane, and toxaphene at this level of mortality were about double the quantities needed for the grubs (table 40). The dosage of endosulfan to kill newly hatched grubs was not determined. If only one of these insecticides was in a soil, the exposure could be so adjusted that the dosage for 50-percent mortality of the flies closely agreed with the dosage for the grubs. However, in soil with a conglomerate mixture of DDT or toxaphene with aldrin, chlordane, dieldrin, endosulfan, endrin, or heptachlor, the exposure was governed largely by the exposure required for the more toxic insecticides. This situation tends to make the estimate of the toxicity in a soil a conservative one.

A conglomerate mixture of residues of DDT with those of aldrin, chlordane, dieldrin, endosulfan, or endrin could not be evaluated unless the toxicity of the DDT in the soil was increased so that the bioassay could be completed with a 24-hour exposure. This was accomplished by adding 30 pounds of DDT per 3-inch acre (1.2 grams per cubic foot) to the sample of soil. The dosage of DDT (24 hours) corresponding to the mortality obtained with the fortified soil was determined and 30 pounds were subtracted from it. The mortality corresponding to the latter dosage of DDT with a 48-hour exposure was the measure of the toxicity in the soil.

The sensitivity of an analytical method usually refers to the minimum amount of a substance that can be determined consistently. Accurate evaluations of a residue cannot be made by bioassay when the mortality of the test insect is approaching zero. Since the most accurate biological determinations are made at the 50-percent level of mortality, the sensitivity of a bioassay may be considered to be the amount of an insecticide needed to cause a significant change in the mortality at that level. A deviation of 8.2 percent in the mortality of the flies was usually significant and one of 11.2 percent highly significant. It was estimated that a change of 8.2 percent in the mortality of the flies was caused by the following amounts (pounds) of the chemical in a 3-inch acre of Sassafras sandy loam: Aldrin 0.007, heptachlor 0.01, dieldrin 0.03, endrin 0.07, endosulfan 0.15, chlordane 0.17, and DDT 2.75. Lower dosages of these insecti-

cides would produce a significant change in the mortality in sands, and higher dosages would be required in soils high in organic matter. These values, however, may be considered a good estimate of the general sensitivity of the bioassay procedure.

It was necessary to determine only whether the toxicity in a soil was sufficient to prevent the development of newly hatched Japanese beetle grubs. The toxicity was considered to be inadequate when the mortality of *Drosophila* was less than 50 percent. The toxicity was considered to be sufficient to eliminate the grubs for at least 1 year when the mortality of the flies was between 50 and 90 percent, and sufficient for 2 or more years when the mortality of the flies was more than 90 percent, particularly when 100-percent mortality occurred several hours before completion of the exposure period.

If desired, the toxicity in a soil may be expressed as equivalent pounds per 3-inch acre of any one of the chlorinated hydrocarbon insecticides given in table 40 by comparing the mortality of the flies with the mortality rates given in the table and interpolating the data.

Bioassays were made of the toxicity of insecticide residues in the soil of 89 plots in 27 nurseries in Connecticut, Massachusetts, New Jersey, New York, Pennsylvania, Rhode Island, and Virginia, using both Japanese beetle grubs and *Drosophila* as test insects. Forty of the soil samples were from plots containing residues of lead arsenate, chlordane, DDT, dieldrin, or heptachlor, alone and in combination, 34 of them were from plots where the foliage of the plants had been sprayed with aldrin, bordeaux mixture, carbaryl, chlordane, DDT, endrin, lead arsenate, lindane, malathion, parathion, TDE, or toxaphene, and 15 of them were from plots where insecticides had been applied to the soil and to the foliage. With the exception of one plot where foliar sprays were used and the toxicity was rated as adequate by the grub bioassay and inadequate by the *Drosophila* bioassay, the same evaluation of toxicity was obtained with both test insects. The toxicity was adequate to prevent the development of newly hatched grubs in 75 percent of the plots where the insecticides had been applied to the soil, in 45 percent of the plots where only foliar sprays were used to control other pests, and in 87 percent of the plots where insecticides were applied to the soil and to the foliage. It was of interest to find that in plots where insecticides had been applied only to foliage, the toxicity of the insecticide residue in the soil was sufficient to eliminate first-instar grubs in about half of the plots.

The bioassay of the soil of certified nursery plots to which the chlorinated hydrocarbon insecticides had been applied in previous years, using *Drosophila* as the test insect, was authorized in 1961 to determine whether there was sufficient toxicity in the soil to continue the plots in a certified status. During the following 2 years the toxicity was determined in 469 certified nursery plots in Connecticut, Indiana, Maryland, New Jersey, New York, Ohio, Pennsylvania, Rhode Island, and Virginia. The results of these bioassays are summarized in table 41. The toxicity was inadequate in 17 percent of the plots, but was at such a level in the other plots that 5 percent of them could be continued in a certified status for 1 more year and 78 percent of them for 2 more years.

TABLE 41.—*Bioassay during 1962–63 of nursery plots treated in previous years with chlorinated hydrocarbon insecticides, using* Drosophila *as test insect*

Insecticide	Plots	Plots with toxicity in soil		
		Not adequate	Adequate for—	
			1 year	2 years
	Number	*Percent*	*Percent*	*Percent*
Aldrin	22	14	9	77
Chlordane	55	31	9	60
Chlordane + DDT	3	0	0	100
Chlordane + dieldrin	6	0	0	100
Chlordane + DDT + dieldrin	6	0	0	100
Chlordane + heptachlor	55	0	3	97
DDT	122	35	20	45
DDT + dieldrin	2	50	0	50
DDT + malathion	4	25	0	75
Dieldrin	161	3	6	91
Heptachlor	28	43	10	47
Not identified	5	0	0	100
Total or average	469	17	5	78

Chemical Analysis Versus Bioassay

Fleming et al. (*1962*) collected samples of soil from experimental turf plots to which the chlorinated hydrocarbon insecticides had been applied for control of Japanese beetle grubs. The toxicity in each sample was determined by bioassay with

third-instar grubs, and the equivalent amount of insecticide was calculated by interpolation of the mortality data. The organic chlorine in each sample was determined and the equivalent amount of insecticide residue was calculated by multiplying the organic chlorine by an appropriate factor. The data were arranged in groups and the average residue for each group was calculated. The biological and chemical determinations are summarized in table 42.

The relationship between the biological and chemical determinations remained fairly constant throughout the sampling periods with chlordane and DDT, but as the other insecticides weathered, there was a trend for larger amounts of these insecticides to be recovered by chemical analysis than by bioassay. Kiigemagi et al. (*1958*) also found good agreement initially between the organic chlorine method and bioassay with larvae of *Culex quinquefasciatus* Say in the analysis of soils containing aldrin, dieldrin, and heptachlor. However, after the soils had weathered for several months, larger amounts of these insecticides were recovered by chemical analysis than by bioassay. Terriere and Ingalsbe (*1953*) usually recovered more aldrin, benzene hexachloride, chlordane, DDT, heptachlor, and toxaphene by the organic chlorine method than by assay with culicine larvae.

Other investigators have found discrepancies in the results obtained by colorimetric chemical methods and bioassays with *Drosophila*. Young and Rawlins (*1958*) found higher amounts of heptachlor in mineral soils by colorimetric analysis than by bioassay. Lichtenstein and Polivka (*1959*) found more chlordane and less aldrin and heptachlor in soil by colorimetric analysis than by bioassay. Edwards et al. (*1957*), Lichtenstein and Schulz (*1959*), and Lichtenstein et al. (*1960*) also found less aldrin in soil by colorimetric analysis than by bioassay. Lichtenstein et al. (*1960*) found more lindane by colorimetric analysis than by bioassay.

The decision to use a biological or a chemical procedure to evaluate insecticide residues in soil is dependent on the type of information desired about the residues. The identification and the quantity of the residues in a soil can be determined only by chemical analysis, but the toxicity in a soil can be established only by bioassay. In a determination of whether an insecticide residue in a soil is sufficient to eliminate newly hatched grubs, it is more important to know the level of toxicity than to have information on what residues are in the soil and how much of each is present.

TABLE 42.—*Amounts of chlorinated hydrocarbon insecticide residues per 3-inch acre in soil during weathering as determined by bioassay and chemical analysis*

Insecticide and years residue weathered	Plots	Residues determined by—		Difference
		Bioassay	Chemical analysis	
	Number	*Pounds*	*Pounds*	*Pounds*
Aldrin:				
0.5	6	1.77	1.07	−0.70
2	8	.43	.64	+ .21
3	5	.26	.92	+ .66
5	11	.10	.90	+ .80
Chlordane:				
0.5	9	5.8	5.3	− .5
1	12	3.3	3.9	+ .6
2	13	1.5	2.0	+ .5
5	11	.7	.5	− .2
DDT:				
0	8	24.5	21.1	−3.4
4	7	17.8	14.0	−3.8
6	12	11.6	10.3	−1.3
7	16	7.5	9.0	+1.5
9	3	2.5	3.8	+1.3
Dieldrin:				
0	5	3.04	3.66	+0.62
1	4	2.10	2.60	+ .50
2	4	1.78	2.38	+ .60
3	7	1.13	1.61	+ .48
4	7	.83	1.81	+ .98
5	4	.55	1.35	+ .80
Heptachlor:				
0	2	2.15	1.85	− .30
1	4	.35	1.80	+1.45
2	4	.20	.55	+ .35
3	4	.10	1.25	+1.15
Toxaphene:				
0	3	30.5	33.1	+2.6
1	3	16.6	18.2	+1.6
3	1	13.1	10.5	−2.6
5	3	7.9	8.2	+ .3
6	7	3.9	9.8	+5.9

SUMMARY

Federal quarantines 40 and 48, which became effective in 1920, regulated the interstate movement of all kinds of farm products and plants from the area infested by the Japanese beetle (*Popillia japonica* Newman). The infested States imposed quarantines to regulate the intrastate movement of these products and plants. The general policy in the enforcement of these quarantines was to permit unrestricted movement of all agricultural products within the regulated area and to restrict shipments beyond this area to uninfested products and plants.

When these quarantines were promulgated there was no precedent for treating the many agricultural products to kill the insect. Progress in the development of treatments was slow because farm products and plants were often less tolerant of insecticides than the insect, but eventually many treatments were developed and authorized.

Quarantine on Farm Products

The quarantine on farm products was in operation from the first emergence of the adult beetle until none could be found in the area, approximately 3 months of the year.

Inspection

The inspection of farm products was not satisfactory because it was difficult to find all the beetles in packages of fruits and vegetables, and except for the first few years sufficient manpower to examine each package was lacking.

Nonchemical Treatment of Fruits and Vegetables

The commercial grading of apples and peaches eliminated beetles hiding among the fruit. A mechanical device was developed for separating beetles from string beans. A high frequency electrostatic field, ultraviolet light, vacuum and pressure, centrifugal force, refrigeration, and heat were either not effective or impractical.

Fumigation of Fruits and Vegetables

Fumigation with carbon disulfide or ethylene oxide killed beetles in boxes and crates of blackberries, blueberries, and raspberries without injuring the berries.

Hydrocyanic acid and calcium cyanide were used successfully to fumigate carloads of green bananas at the ports. Hydrocyanic acid was not always effective in killing beetles in baskets of apples, peaches, and vegetables.

Methyl bromide killed beetles in packages of fruits and vegetables, usually without injuring the products.

Dusts

A dilute DDT dust applied before and after loading killed beetles hiding in refrigerator cars and trucks and clinging to the outside of sacks of potatoes.

Quarantine on Nursery and Greenhouse Stock

Safeguarding the movement of nursery and greenhouse plants was the most important part of the Japanese beetle quarantine because the immature stages of the beetle are in the soil throughout the year.

Preventing Infestation

The infestation of greenhouses and coldframes was prevented by screening and following sanitary procedures.

Soil Removal

Many species of herbaceous and deciduous plants were not seriously injured by washing all soil from their roots with water, but the narrowleaf and broadleaf evergreens were seriously retarded or killed.

Nonchemical Treatments

A high frequency electrostatic field did not kill all the immature stages in the soil of potted plants and it was very injurious to plants.

All grubs were killed by reducing the temperature of the soil from 50° to 12° F. within a few hours, but most of the narrowleaf evergreens were seriously injured or killed.

Immersing the soil of balled and potted plants in water at 112° F. killed all stages of the beetle. Many species of dormant and semidormant herbaceous and deciduous plants were not injured, but the evergreens were killed.

Fumigation in Chamber

Of all the fumigants that killed the immature stages in soil, methyl bromide was the least injurious to plants. The insects

usually died within 3 weeks after exposure to the vapor of methyl bromide. Most species of plants were not injured by the fumigant.

Insecticide Dips

Immersion of the dormant roots of herbaceous plants in a dilute wormseed oil emulsion killed all grubs in masses of soil not larger than 1 cubic inch without injuring the plants, but not all grubs were killed in larger masses of soil.

Grubs in the soil of balled and potted plants were killed by immersing the soil in a dilute emulsion of aldrin, carbon disulfide, ethylene dichloride, or heptachlor. All immature stages were killed by immersing the soil in a dilute emulsion of ethylene dibromide or an ethylene dibromide-chlordane mixture. Herbaceous and deciduous plants tolerated these treatments, but sometimes the evergreens were injured.

Insecticide Emulsions and Solutions Applied to Soil Surface

Grubs in potting soil were killed by applying to the surface of the soil a dilute emulsion of ethylene dibromide, ethylene dibromide-chlordane, or ethylene dichloride, or an aqueous solution of methyl bromide.

Grubs in the soil of potted plants were killed by pouring on the surface of the soil a dilute emulsion of aldrin, carbon disulfide, ethylene dibromide, ethylene dibromide-chlordane, or ethylene dichloride. Most plants tolerated these treatments.

Grubs in the soil of field plots were killed prior to planting by applying to the surface of the soil a dilute emulsion of Bedrench, D-D, ethylene dibromide, or Vapam, or an aqueous solution of methyl bromide.

Before digging plants in the field, the grubs in the soil among the plant roots were killed by applying to the surface of the soil a dilute emulsion of carbon disulfide, ethylene dibromide, ethylene dibromide-chlordane, or ethylene dichloride, or an aqueous solution of methyl bromide.

Insecticide Liquids Injected Into Soil

All immature stages were killed in potting soil by injecting into the soil carbon disulfide, chloropicrin, or methyl bromide.

Grubs in the soil of unplanted nursery plots were killed by injecting carbon disulfide, D-D, Dorlone, isobenzan, or methyl bromide into the soil.

Most of the insecticide liquids injected into the soil of balled or potted plants did not kill all of the grubs or they seriously injured the plants. A dilute alcoholic solution of ethylene dibromide or ethylene dichloride injected into the soil killed grubs without injuring the plants.

Residues of Plant Origin Mixed With Soil

Derris, hellebore, mowrah meal, or pyrethrum mixed with soil had little effect on the grubs.

Naphthalene Mixed With Soil

Naphthalene mixed with potting soil killed all immature stages in the soil. The chemical disappeared rapidly, largely because of decomposition by soil micro-organisms. Before naphthalene disappeared, the soil was injurious to plants. After it had disappeared, plants grew normally in the soil.

Mixing naphthalene with the upper 2 inches of soil of cultivated plots at intervals during the summer did not prevent oviposition in the soil. All grubs in unplanted plots were killed in the early fall by mixing naphthalene with the upper 3 inches of soil.

p-Dichlorobenzene

p-Dichlorobenzene mixed with potting soil killed all grubs, but it seriously injured or killed plants potted in the soil.

When balled and potted plants were plunged for 5 days into soil with which *p*-dichlorobenzene had been mixed, the grubs were killed and many species of plants were not seriously injured.

Inorganic Residual Insecticides Mixed With Soil

In contrast to the fumigants that persist for only a short time in soil, some residual insecticides may be effective for several years in killing successive generations of grubs hatching in the soil. This type of treatment made it possible to treat plots of nursery stock en masse, instead of individual plants, and to dig and prepare the plants for shipment in the usual manner.

Grubs were killed in potting soil by mixing lead arsenate with the soil. Lead arsenate mixed with the upper 3 inches of soil in planted and unplanted nursery plots before the eggs hatched killed the grubs by early fall. It was effective for 1 to 4 years, depending on the type of soil. Many herbaceous, deciduous, and evergreen plants were grown successfully in soil treated with this arsenical.

Lead arsenate was less toxic to grubs than barium, dicalcium, magnesium, manganese, tricalcium, and zinc arsenates, equivalent in toxicity to aluminum and ferric arsenates, and more toxic than basic lead arsenate. Ferric and lead arsenates mixed with soil were the least injurious of the arsenates to plants.

Arsenious oxide and arsenious sulfide were more toxic to grubs than lead arsenate, but they seriously injured or killed plants.

Paris green and some of its homologs were more toxic to grubs than lead arsenate. All of them were injurious to plants.

The inorganic borates of calcium, lead, magnesium, nickel, sodium, strontium, and zinc were only slightly toxic to grubs but very injurious to plants.

The inorganic fluorides of aluminum, barium, calcium, copper, lead, magnesium, strontium, and zinc were only slightly toxic to grubs. They did not affect the growth of plants.

The hexafluorosilicates of potassium and sodium were more toxic to grubs than lead arsenate. Barium hexafluorosilicate was equivalent in toxicity to lead arsenate, and magnesium hexafluorosilicate somewhat less toxic. Calcium hexafluorosilicate was only slightly toxic to grubs. The hexafluorosilicates lost more than half of their insecticide effectiveness during weathering for 6 months. Plants tolerated the hexafluorosilicates in the soil.

Residual Chlorinated Hydrocarbon Insecticides Mixed With Soil

Since 1943 many new chlorinated hydrocarbon insecticides have become available. In contrast to the halogenated hydrocarbons used previously, these chlorinated hydrocarbons were less volatile and more persistent in soil. Only a small amount of these chemicals was needed to kill grubs in the soil.

DDT was the first of these chlorinated hydrocarbon insecticides to be tested as a substitute for lead arsenate. A small amount of the chemical in potting soil killed grubs. When mixed with the upper 3 inches of soil in planted and unplanted nursery plots before the eggs hatched, DDT eliminated by early fall six to eight annual broods of grubs that hatched subsequently in the soil. Over 500 species of nursery and greenhouse plants were grown successfully in DDT-treated soil.

Methoxychlor and TDE, chemicals closely related to DDT, were much less toxic to grubs.

Toxaphene was very similar to DDT in its toxicity to grubs, persistence in soil, and low phytotoxicity.

Chlordane was more toxic to grubs than DDT. A small amount in potting soil killed grubs. When mixed with the upper 3 inches

of soil in planted and unplanted nursery plots before the eggs hatched, chlordane eliminated by early fall three or four annual broods of grubs that hatched subsequently in the soil. Most nursery and greenhouse plants were not affected by chlordane in the soil.

Aldrin was more toxic to grubs than chlordane. A very small amount of aldrin mixed with potting soil killed grubs. When mixed with the upper 3 inches of soil in planted and unplanted nursery plots before the eggs hatched, aldrin eliminated by early fall three annual broods of grubs that hatched subsequently in the soil. Most nursery and greenhouse plants were not affected by aldrin in the soil.

Dieldrin was less toxic than aldrin and more toxic than chlordane to grubs. Its persistence in soil approached that of DDT. A small amount in potting soil killed grubs. When mixed with the upper 3 inches of soil in planted and unplanted nursery plots before the eggs hatched, dieldrin eliminated by early fall five annual broods of grubs that hatched subsequently in the soil. Most nursery and greenhouse plants were not injured by dieldrin in the soil.

Heptachlor was very similar to aldrin in its toxicity to grubs, persistence in soil, and low phytotoxicity.

Preliminary tests were made with other chlorinated hydrocarbon insecticides. Benzene hexachloride lost most of its insecticide effectiveness within 1 year and it retarded the growth of plants. Endrin had about the same persistence in soil as DDT and it did not affect the growth of most plants. Endosulfan disappeared from soil in about 5 months. Isobenzan and isodrin were much more toxic to grubs than aldrin; tests with these chemicals were discontinued when they were withdrawn from the market. Kepone was less toxic to grubs than chlordane. Lindane was less toxic to grubs than benzene hexachloride.

By 1955 many nursery plots had residues of chlordane and DDT, chlordane and dieldrin, or DDT and dieldrin because the insecticide applied initially did not have to be used in re-treatment to continue plots in a certified status. DDT plus aldrin, chlordane, dieldrin, endrin, heptachlor, or toxaphene was more toxic to grubs than DDT or the other components of these binary mixtures. Toxaphene plus aldrin, endrin, or heptachlor was more toxic than toxaphene or the other components of the mixtures. There was no definite change in the toxicity of most of the other binary mixtures.

Carbamate Insecticides Mixed With Soil

In preliminary tests the toxicity of Hercules 5727 and Landrin to the grubs was about the same as that of dieldrin. Propoxur and Shell SD–8786 were three-fifths and carbaryl three-tenths as toxic as dieldrin. The other carbamates were less toxic. Tests on their persistence in soil indicated that carbamates applied before eggs hatched would be effective in eliminating only one brood of grubs.

Organic Phosphorus Insecticides Mixed With Soil

In preliminary tests Stauffer N–2788 was slightly more toxic to grubs than dieldrin. Bayer 37289 and Zytron were equivalent in toxicity to dieldrin. Akton ™, Union Carbide 8305, Diazinon, and Shell SD–8803 were slightly less toxic than dieldrin. The other phosphorus compounds were even less toxic to the grubs. These insecticides disappeared rapidly from soil. An application of the organic phosphorus insecticides to soil before the eggs hatched would not be effective in killing first-instar grubs throughout the summer.

Quarantine on Turf

The production and distribution of turf became an important industry in the quarantined area after World War II. Topdressing turf with residual insecticides was not adapted to the requirements of the turf industry because turf treated in the spring could not be certified for shipment until the fall. Many emulsions and solutions were applied to eliminate grubs in turf, but only two of them were authorized. Turf could be dug and shipped 24 hours after applying a dilute emulsion of ethylene dibromide to the surface. The turf was in a certified status until adult beetles appeared in the vicinity. It also could be shipped 24 hours after applying a dilute emulsion of ethylene dibromide-chlordane, but this turf could be in a certified status for several years.

Determining Residues of Persistent Insecticides in Soil

The certification of plants grown in nursery plots treated with lead arsenate or the chlorinated hydrocarbon insecticides was based on the knowledge that sufficient residue was in the soil to kill grubs hatching during the summer.

The variation in the types of soil, cultural practices, and environmental conditions throughout the quarantined area made it necessary to determine how much residue remained in a plot after weathering. The residues were determined by chemical analyses and bioassays. The method of sampling soil and the chemical and biological methods for assaying the residues are discussed in this bulletin.

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