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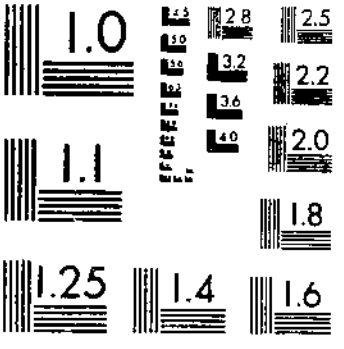

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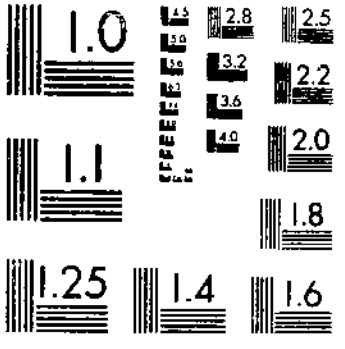

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BIOLOGICAL CONTROL

Of The

JAPANESE BEETLE

By Walter E. Fleming

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BIOLOGICAL CONTROL Of The JAPANESE BEETLE

By WALTER E. FLEMING, *collaborator, Entomology Research Division, Agricultural Research Service*

During a routine inspection in mid-August 1916, E. L. Dickerson and H. B. Weiss of the New Jersey Department of Agriculture collected about a dozen beetles in a nursery of Henry A. Dreer between Riverton and Moorestown, N.J. These beetles were later found to be the same as specimens of the Japanese beetle (*Popillia japonica* Newman) in the collection of the National Museum in Washington, D.C. Prior to its discovery in New Jersey the beetle was known to occur only on the main islands of Japan—Honshu, Kyushu, Shikoku, and Hokkaido. Other members of the genus are widely distributed in the Far East. It is not known definitely how and when the beetle came from Japan to New Jersey. It was probably transported in the grub stage in soil about the rhizomes of Japanese iris. The abundance of the beetle in 1917 indicated that the insect had been in the nursery for at least 5 years. (Clausen et al. *1927;* Dickerson and Weiss *1918;* Howard *1918;* Smith and Hadley *1926;* Weiss *1918, 1918a*[1])

A large part of the main islands of Japan is heavily forested mountainous country. Much of the land is rolling prairie covered with persistent bamboo grasses at the foot of high mountain ranges. These conditions are not favorable for the beetle. The natural, agricultural conditions in Hokkaido more nearly approach those of the Eastern United States. The beetle is most abundant in the northern half of Honshu and in all of Hokkaido where grasslands occur, but its population density never reaches that in the Eastern United States, probably because its natural enemies keep it under control. Nowhere in Japan is the beetle considered to be an economically important pest. Damage to crops sometimes occurs in Hokkaido, but the preference of the insect for certain weeds growing along the roadsides usually prevents serious injury to the plants (Clausen et al. *1927*).

Recognizing the potential threat of the beetle to American agriculture, the former U.S. Bureau of Entomology established the

[1] The year in italics after the author's name is the key to the reference in Literature Cited, p. 70.

Japanese Beetle Laboratory in 1917 near Riverton, N.J., to study the life history and habits of the insect in its new environment and later to develop biological, chemical, and cultural methods for its control (Agee *1918;* Davis *1920, 1920a;* Hadley *1922;* Headlee *1918;* Howard *1918*). In 1927 the Laboratory was moved to Moorestown, N.J., where it has remained.

In New Jersey the beetle found a generally favorable climate, large areas of permanent turf for the development of the immature stages, almost 300 species of plants to satisfy its voracious appetite, and no important natural enemies (Fleming *1963*). It spread rapidly. The infestation covered 2½ square miles in 1917, 6½ square miles in 1918, 48 square miles in 1919, 103 square miles in 1920, and 270 square miles in 1921 (Smith and Hadley *1926*). By 1962 the beetle had spread over more than 100,000 square miles in 14 eastern States and the District of Columbia (Fleming *1963*). In its new environment it soon became a threat to American agriculture. It seriously damaged orchard crops, certain field crops, and ornamental trees and shrubs. The grub destroyed large areas of turf in lawns, golf courses, and pastures and damaged the roots of some cultivated crops.

Although preliminary studies had been made earlier, the investigation of biological control of the beetle was undertaken in 1920. Two entomologists were sent that year by the former U.S. Bureau of Entomology to Japan to study the predators and parasites of the beetle in its native land. The program in the Far East was successful. Five species of parasitic insects from the Far East were colonized and became established in the infested area in the United States.

The studies of diseases of the beetle were undertaken in 1922, when sick grubs found in the field were discovered to be infected mainly by bacteria and fungi, although the organisms causing the infections were not identified specifically. One of the most impressive achievements of the Japanese Beetle Laboratory was the development of the knowledge of pathogens attacking the beetle to a point where it was feasible to mass-produce and colonize some of these organisms in the field to control this destructive insect.

Some phases of the investigation of biological control were conducted cooperatively with other Federal agencies, State agricultural experiment stations, State departments of agriculture, and universities within the area infested by the beetle. Reports on the progress of the investigation appeared from time to time in Federal and State publications and in various scientific journals, but much additional information is found in the unpublished progress reports by H. W. Allen, J. W. Balock, M. H. Brunson, R. W. Burrell, T. N. Dobbins, S. R. Dutky, H. Fox, T. H. Frison, T. R. Gardner, H. B. Girth, H. A. Jaynes, H. C. Hallock, I. M. Hawley, J. K. Holloway, J. L. King, T. L. Ladd, P. J. McCabe, L. B. Parker, G. E. Spencer, H. Tashiro, G. F. White, R. T. White, and H. J. Willard and in the unpublished quarterly and annual reports of the Japanese Beetle Laboratory by C. H. Hadley and W. E. Fleming on file at the Laboratory. I have reviewed these published and unpublished records and have prepared a digest so that information on

the biological control of the Japanese beetle from 1920 to 1964 might be more available to other entomologists and the general public.

PREDATORS AND PARASITES FOR CONTROL OF BEETLE

Native Predators and Parasites

The insect-feeding birds, small terrestrial mammals, and the few predaceous and parasitic insects indigenous to the Eastern United States were not able to cope with the twentyfold to thirtyfold reproductive potential of the Japanese beetle. Although these natural enemies from time to time have reduced beetle populations within limited areas, their efforts have been too sporadic and too restricted to have much effect on the population in a region.

Insectivorous Birds

Some native birds feed readily on adult beetles. Smith and Hadley (*1926*) reported that during 1919 and 1920 E. A. Chapin and C. W. Leister of the former U.S. Bureau of Biological Survey had examined the stomach contents of 31 species of birds in the infested area and found remains of beetles in 16 of them. The birds that fed on beetles were the bobwhite (*Colinus virginianus*), the eastern kingbird (*Tyrannus tyrannus*), the crested flycatcher (*Myiarchus crinitus*), the crow (*Corvus brachyrhnchos*), the European starling (*Sturnis vulgaris*), the red-winged blackbird (*Agelaius phoeniceus*), the meadowlark (*Sturnella magnam*), the common grackle (*Quiscalus quiscala*), the English sparrow (*Passer domesticus*), the vesper sparrow (*Pooecetes gramineus*), the songsparrow (*Melospiza melodia*), the cardinal (*Richmondena cardinalis*), the catbird (*Dumetella carolinensis*), the brown thrasher (*Toxostoma rufum*), the wood thrush (*Hylocichla mustelina*), and the robin (*Turdus migratorius*).

There was no indication that beetles had been eaten by the little blue heron (*Florida caerulea*), the mourning dove (*Zenaidura carolinensis*), the sparrow hawk (*Falco sparverius*), the yellow-billed cuckoo (*Coccyzus americanus*), the yellow shafted flicker (*Colaptes auratus*), the eastern wood pewee (*Contopus virens*), the brown-headed cowbird (*Molothrus ater*), the orchard oriole (*Icterus spurius*), the American goldfinch (*Spinus tristis*), the swamp sparrow (*Melospiza georgiana*), the chewink (*Pipilo erythrophthalmus*), the red-eyed vireo (*Vireo olivaceus*), the ovenbird (*Seiurus aurocapillus*), the tufted titmouse (*Parus bicolor*), or the eastern bluebird (*Sialia sialis*).

The grackle ate more beetles than did any other bird. All 29 grackles examined had eaten beetles and 66 percent of the contents of their stomachs consisted of fragments of beetles. After the grackle in importance as predators of the beetle were the meadowlark, starling, cardinal, and catbird.

Other observers reported that the English sparrow and the robin feed on beetles (Bell *1930;* Cory and Langford *1955;* Fleming *1958;* Hadley and Hawley *1934*).

Pheasants are known for their fondness of beetles of all kinds. A pair of ring-necked pheasants (*Phasianus colchicus*), furnished by the New Jersey State Fish and Game Commission, showed a great liking for both living and dried beetles. Since colonizing these birds in the infested area might be a factor in the control of the beetle, pairs of pheasants were liberated at several places. The New Jersey Legislature passed a law prohibiting the killing of pheasants in certain designated areas for a period of 5 years (Davis *1920;* Smith and Hadley *1926*).

Chickens, turkeys, ducks, and guineas feed readily on beetles. An analysis of the beetles showed 67.4 percent moisture, 2.1 percent fat, 22.1 percent protein, 6.6 percent crude fiber, 1.5 percent ash, and 0.3 percent nitrogen free extract, indicating that the insects were good feed for poultry (Davis *1920;* Hadley and Hawley *1934;* Smith and Hadley *1926*).

The domestic fowl, the European starling, the common grackle, the crow, and the gulls (*Larus* spp.) dig up and devour large numbers of grubs in heavily infested areas, especially when fields are being plowed and the grubs are close to the surface in grasslands. The most important of these are the European starling, which has increased in numbers and extended its range in the Eastern States, and the gulls. Whenever grubs are feeding close to the surface of the ground, flocks of starlings may be seen on lawns and in pastures digging up grubs with their long pointed bills. In lawns the small circular holes made by these birds are sometimes not more than 2 inches apart. The starlings reduced the grub population in one lawn from about 100 per square foot to 5 or 6 within a few days. The crows, instead of extracting the grubs through small holes, frequently pull up small pieces of turf and scatter them over a lawn. (Cory and Langford *1955;* Fleming *1958, 1963;* Hadley and Hawley *1934;* Polivka *1950;* White *1941*)

Toads

The common toad (*Bufo lentiginosus*) eats a great many beetles. An examination of seven toads in a heavily infested area showed that 22 percent of the stomach contents consisted of remains of beetles. However, toads are not sufficiently abundant to be a factor in the control of the beetle (Smith and Hadley *1926*).

Mammals

For many years hogs have been known to gorge themselves on white grubs in heavily infested land. Davis (*1920*) recommended using swine to reduce grub populations in pastures.

Sim (*1934*) investigated several small mammals as possible destroyers of grubs. The common mole (*Scalopus aquaticus* L.), the star-nosed mole (*Condylura cristata* L.), the short-tailed shrew (*Blarina brevicauda* (Say)), and the skunk (*Mephitis mephitis* P. & B.) fed readily on grubs while in captivity. The common mole

is abundant in the sandy soil of the higher parts of the Coastal Plain. It works back and forth, just beneath the surface of the soil, while hunting for grubs in a lawn, and eventually may cover practically the entire lawn. The star-nosed mole has similar habits, but it is more prevalent in lower ground along streams and ponds where the soil is constantly moist.

The short-tailed shrew is the largest and the most generally distributed of its kind in southern New Jersey. In captivity it eagerly attacked grubs. Each shrew ate 10 to 20 grubs for a meal. Within an hour it was eating again. In view of its great abundance, this species undoubtedly destroys a large number of grubs in the field.

The largest and most diligent of the quadrupedes that prey on grubs is the skunk. Although practically omnivorous, the skunk has long been recognized as a hunter of scarabaeid larvae. One skunk was observed digging almost daily in a grub-infested lawn. The holes were numerous, but scarcely larger than those made by the starling and not conspicuous enough to disfigure the turf. Friend (*1942*) reported that destruction of grubs by skunks had increased in Connecticut in 1941.

Although chiefly herbivorous, several species of mice fed on grubs to some extent while in captivity, including the pine mouse (*Pitymys pinetorum scalpsoides* (Audubon & Bachman)), the Pennsylvania field mouse (*Microtus pennsylvanicus* (Ord.)), the red-backed mouse (*Cletrionomys gapperi rhoadsi* (Stone)), the deer mouse (*Peromyscus leucopus* and var.), the house mouse (*Mus musculus* L.), the jumping mouse (*Zapus hudsonius* and var.), and the Lemming mouse (*Synaptomys cooperi* Baird). However, with the possible exception of the pine mouse, which lives almost entirely underground and has somewhat molelike modifications in structure, the usefulness of mice as destroyers of grubs is limited (Sim *1934*).

Predaceous Insects

Smith and Hadley (*1926*) reported that larvae of *Calosoma calidum* Fab., *Poecilus lucublandus* Say, *Harpalus caliginosus* (Fab.), *Staphylinus mysticus* Er., and *Tabanus sulcifrons* Macq. fed readily on the immature stages of the beetle. Hallock (*1929*) observed larvae of *Tabanus quinquevittatus* Wied., *Psilocephala haemorrhoidalis* (Macq.), *Harpalus pennsylvanicus* DeG., *Effenia aestuans* (L.), and *Ommatius marginellus* (Fab.) feeding on grubs. Adults of *Carabus nemoralis* Müll., *Harpalus vagans* Lec., and *Chlaenius sericeus* Först. would also feed on grubs (Smith and Hadley *1926*).

White (*1940*) observed ants occasionally attacking living grubs and adult beetles in the field. Several species of ants were involved, including *Formica pallide-fulva nitidiventris* Emery, *F. fusca* L., *F. rubicunda* Emory, *F. integra* Nylander, *F. subintegra* Emery, *F. neogagates* Emery, *Solenopsis molesta* (Say), *Acanthomyops claviger* (Roger), *A. interjectus* (Mayr.), *Lasius alienus* (Foerster), *L. neoniger* Emery, *Pheidole morrisi* Forel, *P. pilifera* (Roger), *P. bicarinata vinelandica* Forel, *Crematogaster cerasi* (Fitch), *Tetra-*

morium caespitum (L.), *Tapinoma sessile* (Say), *Monomorium minimum* (Buckley), *Prenolepis imparis* (Say), *Aphaenogaster* sp., and *Myrmica* spp. These ants had little effect on populations of the immature stages of the beetle. *F. fusca* was observed overpowering adults of *Tiphia vernalis* Rohwer and *T. popilliavora* Rohwer, parasites of the beetle grubs. One ant, *S. molesta,* consistently damaged a small percentage of the cocoons of these parasites.

A few predaceous insects occasionally attack the adult beetle. Davis (*1920*), Bromley (*1946*), and Moul (*1945*) saw the wheel bug (*Arilus cristatus* (L.)) attacking beetles. Bromley (*1945*) observed robber flies (*Laphria grossa* (Fab.), *Protacanthus nigriventris* Macq., and *P. philadelphicus* Macq.) preying on beetles. Davis (*1920*) saw the spined soldier bug (*Podisus maculiventris* (Say)) attacking beetles. The praying mantis (*Paratenodera sinensis* Sauss.) occasionally captures beetles. These predaceous insects feed not only on beetles but also on other insects. However, native predaceous insects are not normally numerous enough to markedly reduce the beetle population.

Parasitic Insects

Frison (unpublished) studied the native Tiphiidae as parasites of *Popillia* grubs. In laboratory tests a few native *Tiphia* species deposited eggs to a limited extent on the grubs, but none of the developing larvae were reared to the cocoon stage. He induced *Elis obscura* Fab. and *E. quinquecincta* Fab., parasites of *Phyllophaga,* to deposit eggs occasionally on *Popillia* grubs, but none of the parasitic larvae developed sufficiently to form cocoons. Brunson (unpublished) conducted tests with *Tiphia intermedia* Mall. Thirty-five females parasitized 796 grubs, but only 31 of the parasitic larvae formed cocoons and only 15 of them emerged as adults. Jaynes and Gardner (*1924*) studied a *Tiphia* sp. normally parasitic on *Cyclocephala immaculata* Olivier. A few eggs were deposited on *Popillia* grubs, but only one larva developed to the cocoon-spinning stage and it subsequently died. Occasionally other Scoliidae species have been observed attacking the grubs. More recently King and Parker (unpublished) found a native dipteron, tentatively identified as *Ptilodexia* sp., parasitizing *Popillia* grubs.

Hallock (*1929a*) found that the flesh fly (*Boettcheria latisterna* (Park.)) did not attack living *Popillia* grubs, but larvae placed on freshly killed grubs developed rapidly, and after feeding for 4 or 5 days wandered away and pupated in the soil. This species is probably more of a scavenger than a parasite.

The results with native parasitic insects have not been encouraging. They appear to have only a minor part in controlling the beetle.

Foreign Predaceous and Parasitic Insects

Plans were made by the former U.S. Bureau of Entomology in 1919 to study the predaceous and parasitic insects attacking the beetle and closely related species in the Orient and to ship the most

effective species to the United States for colonization in the beetle-infested areas. It was hoped that biological control of the beetle could be attained ultimately in its new environment.

Tillard (*1922*) reported that the lacewing (*Ithone fusca* Newman) was predaceous on various soft-bodied insects, particularly larvae of the Scarabaeidae in Australia. A consignment of eggs of this species was forwarded to New Jersey late in 1921 for tests with *Popillia* grubs. Several of the eggs hatched, but the larvae died before reaching the second instar (Smith and Hadley *1926*). More recent investigations have shown that larvae of *Ithone* are unable to feed on other insects and probably derive their food from plant roots (Clausen *1940*).

The colonization of foreign parasites in the United States was stimulated by the apparently successful introduction of the Scolia wasp (*Scolia manilae* Ash.) into Hawaii to control the oriental beetle (*Anomala orientalis* Waterhouse). Although *S. manilae* is not a true parasite of the Japanese beetle and it is a tropical species native to the Philippine Islands, there was a possibility that it might be of some value in controlling the beetle in the United States. O. H. Swenzey forwarded a small consignment of *Scolia* to New Jersey in 1919 and T. F. Illington made larger shipments in 1922 and 1923. This wasp parasitized *Popillia* grubs readily, but it did not survive the winter in the temperate climate of New Jersey. (Hadley unpublished; King unpublished; Smith and Hadley *1926*)

Explorations

C. P. Clausen and J. L. King were sent by the former U.S. Bureau of Entomology in 1920 to study the predators and parasites of the beetle in Japan. The investigation was confined for the first 2 years to the Japanese islands. In 1922 it was extended to Korea where *P. japonica* was not found, but the presence of other species of the same genus gave promise of additional parasites of value. By 1923 the explorers had found two species of Tachinidae that were parasitic on the adult beetle and in addition two species of Tachinidae, one species of Scoliidae, and three species of Tiphiidae that were parasitic on the grubs. A predator, one of the Carabidae, also was found. (Clausen and King *1924*; Clausen et al. *1927*)

When it was discovered that parasites of other species of *Popillia* would attack *P. japonica*, it was possible to extend the search to other areas where the genus *Popillia* was known to occur. Without interrupting the investigation in Japan and Korea, the exploration was extended elsewhere. During 1923–24 a Russian entomologist, who was not identified, explored the Crimean region of Russia, but found no parasites of *Popillia*. J. F. Illington and H. A. Jaynes explored the coastal region of China from Peiping southward to Amoy and up the Yangtze River Valley as far inland as Ichang during 1923–26. The main centers of collection were in the outlying districts about Shanghai and Foochow. *P. japonica* was not found in China, but there were several other species of the genus and several other Scarabaeidae. The adult beetles were almost entirely free from attack by parasites, but several parasites, some of which occurred in Japan, were found attacking the grubs.

The investigation in China was discontinued in 1926 because of the unsettled political situation there.

The Khasi Hills in Assam, India, where the altitude is high enough to give a temperate climate, were explored by C. P. Clausen and L. B. Parker during 1925–29. *P. japonica* was not found there, but other species of *Popillia* and of Scarabaeidae were abundant. Several additional parasites were found in India. R. W. Burrell searched for parasites in southeastern Australia during 1930 and 1931. The genus *Popillia* is not represented in the fauna of Australia, but other Scarabaeidae species are very abundant. A preliminary study was made of the common parasites. In addition to the main fields of exploration, special trips were made to Formosa, the Philippine Islands, Java, Malaya, Burma, and Ceylon. After 1931 the investigation was confined largely to Japan and Korea. The Japanese Beetle Laboratory discontinued active participation in the foreign parasite investigations in 1934, when all these investigations in the Bureau were grouped in a new division. (Burrell unpublished; Clausen et al. *1933;* Gardner and Parker *1940;* Hadley unpublished; King unpublished)

The search for parasites during 1920–33 in Japan, Korea, China, India, Formosa, and Australia disclosed that (1) adult beetles of the genus *Popillia* and related Scarabaeidae were parasitized by 7 species of Tachinidae and 1 species of Pyrgotidae; (2) the grubs of these species were parasitized by 2 species of Tachinidae, 2 species of Scoliidae, and 52 species of Tiphiidae; and (3) both stages were attacked by 1 of the Carabidae (Burrell unpublished; Clausen et al. *1927, 1933;* Gardner and Parker *1940*). Most of the parasites were new species. They have been described by Aldrich (*1923, 1928*), Allen and Jaynes (*1930*), Parker (*1935, 1937*), Roberts (*1930*), and Rohwer (*1924, 1927*). Allen (*1934*) also described 12 new native *Tiphia* species so that they could be distinguished from the foreign species. Allen (*1935, 1961, 1962, 1965*) and Allen and Krombein (*1964*) continued the taxonomic study. Allen (*1966*) published the first monograph on the Tiphiinae of eastern North America. The parasites discovered, with the countries where they were found and the species of Scarabaeidae attacked by them, are listed in table 1.

Biology of Important Parasites and a Predator in Far East

The life history and habits of the more important parasites and a predator of *P. japonica* and related species in the Far East were studied to evaluate their potential effectiveness in controlling the beetle in the United States.

Parasites of Adult Beetle

Eutrixopsis javana.—In 1923 and in 1928 a few adults of *Eutrixopsis javana* were reared at the Japanese Beetle Laboratory from shipments of beetles parasitized by *Hyperecteina aldrichi*, which had been forwarded from Sapporo, Japan. Although efforts were made to obtain information on this tachinid, nothing was known about its life history and habits in Japan. Apparently

TABLE 1.—*Some parasites and a predator of* Popillia *and other Scarabaeidae in Japan, Korea, China, India, Formosa, and Australia*

Species	Native land	Normal hosts
PARASITES OF ADULT BEETLES		
TACHINIDAE		
Erythrocera genalis (Aldrich)[1]		(1).
Eutrixopsis javana Townsend	Japan	*Popillia japonica* Newman, *Anomala* sp.
Hamaxia incongrua Walker	Japan, Korea, China, India.	*Popillia japonica*, *P. quadriguttata* (Fab.), *P. chinensis* Friv., *P. cyanea* Hope, *P. cupricollis* Hope, *P. mulans* Newm., *P. pustulata* Fairm., *Anomala orientalis* Waterh., *Adoretus tenuimaculatus* Waterh.
Hyperecteina aldrichi Mesnil[2]	Japan	*Popillia japonica*.
Hyperecteina unicolor (Aldrich).	Korea	*Anomala sieversi* Heyd., *Phyllopertha pubicollis* Waterh.
Palpostoma spp. (mixture of *P. testacea* R. D. and *P. subsessilis* Malloch).	Australia	*Anoplognathus chloropygus* Drapiez, *A. flavipennis* Boisd., *A. olivieri* Dalm., *Repsimus aeneus* Fab., *Anodontonyx hirticeps* Blackb., *Scitala rugosiceps* Blanch., *S. sericans* Er., *Metanastes vulgivagus* Olliff.
Trophops clauseni Aldrich	Japan	*Popillia japonica*, *Anomala rufocuprea* Mots.
PYRGOTIDAE		
Adapsilia flaviseta Aldrich	India	*Popillia cupricollis*, *P. cyanea*, *P. macclellandi* Hope.
PARASITES OF GRUBS		
TACHINIDAE		
Dexilla ventralis Aldrich	Korea, Manchuria.	*Phyllophaga* spp., *Popillia quadriguttata*, *P. atrocoerulea* Bates, *Miridba koreana* N. & K., *Anomala* spp., *Phyllopertha* spp., *Serica* spp.
Prosena siberita (Fabricius)	Japan, Korea, India.	*Popillia japonica*, *P. cupricollis*, *Popillia* spp., *Anomala* spp., *Adoretus* sp., *Serica* spp.
SCOLIIDAE		
Campsomeris annulata Fabricius	Japan, Korea, China.	*Holotrichia diomphalia* (Bates), *Popillia* spp., *Anomala* spp.
Scolia japonica Smith	Japan, Korea	*Popillia japonica*, *Anomala* spp.

[1] *Erythrocera genalis* (Aldrich) was reared at the Japanese Beetle Laboratory from adults of *P. japonica* collected in central Japan in 1925 and shipped for rearing of *Hamaxia incongrua* Walker; since then it has never been encountered in this study.

[2] *Centeter cinerea* Aldrich, a synonym of *Hyperecteina aldrichi* Mesnil, was used in Japanese beetle publications.

TABLE 1.—*Some parasites and a predator of* Popillia *and other Scarabaeidae in Japan, Korea, China, India, Formosa, and Australia*—Continued

Species	Native land	Normal hosts
PARASITES OF GRUBS—continued		
TIPHIIDAE		
Tiphia agilis Smith	Japan, Korea, China.	*Maladera orientalis* Mots., *Serica* sp.
Tiphia antigenata Allen & Jaynes.	China	
Tiphia asericae A. & J.	Japan, Korea, China.	*Serica* spp.
Tiphia assamensis A. & J.	India	*Popillia cupricollis.*
Tiphia autumnalis Rohwer	Japan, Korea	*Popillia* spp.
Tiphia bicarinata Cam.	Japan, Korea, China.	*Anomala sieversi* Heyd., *Phyllopertha pubicollis.*
Tiphia bisceulata A. & J.	Japan	*Anomala schönfeldti* Ohaus, *A. orientalis.*
Tiphia brevicarinata A. & J.	China	
Tiphia brevilineata A. & J.	Japan, Korea	*Anomala sieversi, Phyllopertha pubicollis, Popillia mutans.*
Tiphia brevistigma A. & J.	India	
Tiphia burrelli Parker	Japan	*Anomala cuprea* Hope, *A. rufocuprea, A. schönfeldti, Mimela testaceipes* (Mots.).
Tiphia capillata A. & J.	India	
Tiphia castaneaevora Parker	Japan	*Maladera castanea* (Arrow), *Serica* spp.
Tiphia cilicincta A. & J.	China	
Tiphia clausseni A. & J.	India	*Popillia* sp.
Tiphia communis A. & J.	China	*Miridiba trichophorus* Fairm., *Popillia formosana* Arrow, *Autoserica* spp.
Tiphia compressa Smith	China, Philippines.	
Tiphia fossata A. & J.	Korea	
Tiphia frater Parker	China	*Adoretus* sp., *Serica* sp.
Tiphia fukiensis A. & J.	----do	
Tiphia homoncularis Parker	Japan	*Serica* sp., *Maladera castanea.*
Tiphia inconspicua A. & J.	China, Japan	
Tiphia isolata Parker	Japan	*Serica* sp., *Maladera castanea.*
Tiphia koreana Rohwer	Korea	*Anomala sieversi, Popillia atrocoerulea, Phyllopertha pubicollis.*
Tiphia latistriata A. & J.	Korea, China	
Tiphia levipunctata A. & J.	India	
Tiphia longilegulata A. & J.	China	
Tiphia lyrata Magretti	Burma, China	
Tiphia malayana Cam.	Borneo, China, Korea.	*Adoretus* sp., *Popillia chinensis, Serica* sp.
Tiphia matura A. & J.	India	*Popillia cupricollis.*
Tiphia minutopunctata A. & J.	China	
Tiphia nana A. & J.	----do	
Tiphia nervidirecta A. & J.	----do	
Tiphia notopolita A. & J.	China, Japan, Korea.	*Popillia chinensis, P. formosana.*
Tiphia notopolita alleni Roberts	Japan, Korea, China.	*Phyllopertha conspurcata* Har., *P. pallidipennis* Reitter, *Anomala orientalis, A. schönfeldti.*

TABLE 1.—*Some parasites and a predator of* Popillia *and other Scarabaeidae in Japan, Korea, China, India, Formosa, and Australia*—Continued

Species	Native land	Normal hosts
PARASITES OF GRUBS—continued		
TIPHIIDAE—continued		
Tiphia ovidorsalis A. & J.	Korea	*Serica* sp.
Tiphia ovinigris A. & J.	do	
Tiphia phyllophagae A. & J.	Japan, Korea, China.	*Phyllophaga* spp.
Tiphia pigmentata A. & J.	China	
Tiphia popilliavora Rohwer	Japan, Korea, China.	*Popillia japonica, P. atrocoerulea, P. quadriguttata, P. chinensis, P. formosana.*
Tiphia pullivora A. & J.	India	*Popillia cupricollis, Popillia* spp.
Tiphia rufomandibulata Smith	China	
Tiphia satoi Parker	Korea	*Serica* spp.
Tiphia singularis A. & J.	China	
Tiphia sternata Parker	Japan	*Serica* spp.
Tiphia sternocarinata A. & J.	China	
Tiphia sternodentata A. & J.	Korea	
Tiphia tegitiplaga A. & J.	Japan	*Anomala schönfeldti, A. rufocuprea, A. orientalis, Phyllopertha conspurcata.*
Tiphia totopunctata A. & J.	China, Korea	*Anomala sieversi, Phyllopertha pubicollis.*
Tiphia vernalis Rohwer	Japan, Korea, China.	*Popillia japonica, P. quadriguttata, P. chinensis, Popillia* spp.
Tiphia sp. (Taiwan 5)	Formosa	*Popillia quadriguttata, P. chinensis.*
Tiphia sp. (Taiwan 2)	do	*Anomala* sp.
PREDATOR OF ADULT BEETLES AND GRUBS		
CARABIDAE		
Craspedonotus tibialis Schaum	Japan	*Popillia japonica, Anomala* spp., *Serica* spp., other insects.

Eutrixopsis at times parasitizes *P. japonica* in the absence of some other host. This parasite was considered to be of little importance in the control of the beetle in Japan. (Gardner and Parker *1940*)

Hamaxia incongrua.—This species is a parasite of the adults of many Scarabaeidae species and is widely distributed throughout the Asiatic region. In Japan where *Hamaxia incongrua* is a parasite of *P. japonica,* it has two and possibly three generations a year. Being of crepuscular habit, it is active during the early morning hours and in the evening.

Although the exact method of parasitization is not known, it is presumed that the female fly deposits fully developed larvae either on the beetle or on nearby foliage and that these larvae enter the beetle through the softer parts of the body. Parasitized beetles were found in central and western Japan from the middle of June to the end of August, but at no locality was the parasitization more than 20 percent, and it usually was less than 5 percent.

In areas where adults of *P. japonica* are present in the field for 2 months or longer, *Hamaxia* is able to develop two generations on this host, but in areas where this does not occur, other scarabaeid beetles are necessary to preserve the species. In Korea many female parasites were in the field during late September and early October when it was cool and no beetles of any species were available for attack. Possibly part of the *Hamaxia* population may pass the winter in the adult stage. (Clausen et al. *1927, 1933;* Gardner and Parker *1940*)

***Hyperecteina* spp.**—*Hyperecteina aldrichi* is the most effective parasite of the adult beetle in northern Japan. On the island of Hokkaido, where there is a partial 2-year cycle of the host so that beetles are abundant every other year, 20 to 90 percent of the beetles are parasitized each year. On the island of Honshu, where the beetle has one generation a year and the number of beetles is fairly constant from year to year, about 50 percent of the beetles are parasitized each year. *H. aldrichi* has one generation a year. The appearance of the fly in the fields is well synchronized with that of its host. The flies feed on aphis honeydew and at the nectar glands of various plants, particularly *Polygonum reynoutria* Makino.

Oviposition usually takes place on the female beetles of mating pairs, since they are not alarmed as readily as single beetles. The egg is usually placed dorsally on the thorax. The female fly does not discriminate between parasitized and unparasitized beetles. The deposition of eggs is governed by chance, so that more than one female may deposit an egg on a beetle. Some female beetles were found with as many as 14 eggs on the thorax. The parasitic larva is fully mature within 36 to 48 hours after the egg is deposited. While still inside the eggshell, it drills downward through the shell into the body of the beetle, where it consumes the contents of the thorax and the abdomen. The beetles usually die within 5 days. The parasitic larva then pupates within the skeleton of its host. The pupal period lasts about 10 months. The mature fly then breaks the cap of the puparium and works its way through the soil to open air. (Clausen et al. *1927, 1933;* Gardner and Parker *1940*)

Hyperecteina unicolor, a parasite of *Anomala sieversi* and *Phyllopertha pubicollis* in Korea, has habits similar to those of *H. aldrichi* (Parker *1934*).

***Palpostoma* spp.**—Two tachinids, *Palpostoma testacea* and *P. subsessilis,* are the most important parasites of Scarabaeidae adults in southern Australia. The combined parasitization by these two

species of flies ranges up to 25 percent on *Anoplognathus olivieri*, the preferred host, in the vicinity of Woy Woy, New South Wales. The parasitization was lower on the other species of beetles. The size of the host beetles ranged from smaller than *P. japonica* to as large as *Cotinis nitida* (L.) and *Pelidnota punctata* (L.) in the United States. There are probably six or seven generations from October through May, but they are not distinct. The flies are nocturnal. Their most active period begins at dusk and lasts about 3 hours.

The female fly probably deposits young larvae directly on the host beetle. The larvae enter the host through the softer chitin connecting the segments or near the base of the wings, and feed on the thoracic and abdominal tissues. The beetles die 5 or 6 days after being parasitized. The parasitic larvae mature 5 or 6 days after the death of the beetle and pupate within the skeleton of the host. The flies emerge from the puparia 8 to 11 days later. It was not determined how *Palpostoma* overwinters in the field. (Burrell unpublished)

Trophops clauseni.—This species is a parasite of adults of *P. japonica* and *Anomala rufocuprea* in western Japan. *Trophops clauseni* is apparently nocturnal because the fly has never been observed in the field. The female fly deposits living larvae, but the exact manner in which it attacks the beetle or the manner that a larva penetrates its host has not been determined. The parasitization of *P. japonica* in the field is low, averaging less than 2 percent. About two-thirds of the beetles parasitized were males. There are at least two generations a year, the first on *P. japonica* and the following one or more on available *Popillia* and *Anomala* species. (Clausen et al. *1933;* Gardner and Parker *1940*)

Adapsilia flaviseta.—This insect was found parasitizing adults of *Popillia cupricollis* and occasionally *P. cyanea* and *P. macclellandi* in a forested area a few miles from Shillong, India, where there was a fairly dense undergrowth of brush at an elevation of 5,000 feet. It was determined experimentally that *Adapsilia flaviseta* would accept *P. japonica* as a host. The correlation between the emergence of *A. flaviseta* and that of the beetles in India was not always as close as might be desired. In 1928 most of the beetles emerged about 10 days prior to extensive oviposition by the parasite.

In parasitizing a beetle, the female remains quiescent on the foliage near a feeding beetle until it takes flight. Then the fly immediately pounces upon the beetle from above and quickly inserts an egg through a long, slender, sharp ovipositor into the middorsal region of the abdomen near the junction with the thorax. The eggs hatch in about 3 days and the larva begins to feed on the contents of the abdomen. The larva pupates 12 to 15 days later. The beetle is killed 3 or 4 days before the parasite pupates. There is a single generation a year. The winter is passed in the puparium in the body of the host beetle. (Clausen et al. *1933;* Gardner and Parker *1940*)

Parasites of Grub

Dexilla ventralis.—This species is the most common of the Tachinidae in Korea. *Dexilla ventralis* has three complete generations a year on a variety of hosts, including *Miridiba koreana, Phyllopertha* spp., *Phyllophaga* spp., *Anomala* spp., *Serica* spp., and *Popillia* spp. *P. japonica* does not occur in Korea, but it was determined experimentally that *D. ventralis* would accept grubs of this species. The fly is crepuscular in habit; it is active at dawn and in the evening. The adult flies have not been observed feeding in the field, but they probably feed on honeydew or nectar because the mouth parts are short with broad flat labella.

The adult female scatters living larvae promiscuously over the surface of the soil. The first-stage larva burrows at once into the soil in search of a host, but apparently does not discriminate in the choice of a grub other than its physical characteristics. In a short time it enters a grub. The parasitic larva has three instars, the first of which lasts only a few hours. After the first molt a spiracular funnel is formed. The larva feeds on the tissues of the grub for 12 to 16 days and consumes everything except the integument and the head capsule. The larva then draws away from its spiracular funnel, tears an irregular hole in the posterior ventral segments of its host, moves a short distance away, and forms a puparium. The fly emerges from the puparium 15 or 20 days later. The last generation of larvae develops until it has completed growth in the second stage. The development then ceases and the parasitic larva overwinters within its host. In the spring the larva resumes activity and completes its development within the host.

With *Popillia* as the winter host only two generations are produced each year, but three generations are completed with *Miridiba* as the winter host. In Korea there is a different species of grub for each generation of the parasite. (Burrell *1931;* Clausen et al. *1927, 1933;* Gardner and Parker *1940*)

Prosena siberita.—Another tachnid, *Prosena siberita,* which is generally distributed through the Asiatic regions, is an important parasite of *P. japonica* in northern Japan. It is capable of developing on many species of scarabaeid grubs. The adult fly is somewhat crepuscular in habit; it is active on cloudy days and about sunset on bright days. *P. siberita* has one generation a year. The species is primarily a parasite of *P. japonica* in Japan, normally parasitizing 10 to 15 percent of the grubs.

The female fly deposits larvae promiscuously on the soil. The young larva burrows about in the soil in search of a grub. When a grub is found, the larva seeks out a crevice or suture in the integument and commences penetration. This is accomplished in 1 or 2 hours. The first-stage larva lies within the body of the host surrounded by fluids and masses of fatty tissues. No permanent connection for respiratory purposes is made at this stage. Early in the second stage, however, the caudal spiracles are attached to a main tracheal trunk near the thoracic or first abdominal spiracles of the host. The body of the larva is directed caudad and feeding takes place largely in the midabdominal region. The second molt

occurs usually in the early spring. The development of the larva in the final stage is very rapid. The body of the host grub becomes more translucent with the consumption of the fat bodies. The grub remains alive and active until practically all the body contents, except the vital organs, are consumed. After the death of the host, the larva tears a hole ventrally in the integument of one of the distal segments of the abdomen, severs its connection with the respiratory funnel, reverses its position, and completes feeding in the thoracic region. Pupation takes place in the soil 1 or 2 inches below the carcass of the host. (Clausen et al. *1927, 1933;* Gardner and Parker *1940*)

Campsomeris annulata.—The larvae of two large solitary wasps, *Campsomeris annulata* and *Scolia japonica*, are external parasites of the larger grubs of the Scarabaeidae in Japan, Korea, and China. *C. annulata* is the more prevalent. Although it normally parasitizes species of *Anomala* and at times *Holotrichia diomphalia*, it was determined experimentally that the parasite would accept third-instar grubs of *P. japonica*. The adult feeds exclusively on the nectar of various blossoms, particularly those of thistle, wild carrot, whiteclover, goldenrod, and the Negundo chaste-tree (*Vitex negundo* L.) and never on secretions of insects. There are apparently three generations a year in Japan and Korea, and there may be a fourth brood in China. The winter is passed in the pupal stage in the cocoon.

The female wasp burrows extensively in soil searching for grubs. When a grub is located in its cell, the wasp curls its body transversely about it and inserts its stinger several times into the thoracic region, causing complete and permanent paralysis. It may sting many more grubs than it is capable of parasitizing successfully. However, grubs that are stung but do not receive an egg never recover. The wasp then burrows deeper into the soil, dragging the paralyzed grub behind it, and forms a cell within which the grub is placed.

The egg is attached lightly on the ventral surface of the grub so that it stands vertically on its posterior end. At hatching the chorion is broken over the head and thoracic segments and the forepart of the body of the larva emerges. The larva bends forward and is soon able to make a feeding puncture on the median line of the segment preceding that bearing the egg. Very shortly thereafter the larva moves entirely out of the eggshell and lies either longitudinally or diagonally on the body of its host. At first only the head is embedded in the feeding puncture, but after the first molt, the larva penetrates farther until the thoracic segments are within the body of the host. The puncture at this time is slightly less than 2 mm. in diameter, and there is a marked exudation of body fluids, which appear as a ring about the neck of the larva. The early feeding is largely in the anterior part of the body, but later the position is reversed and the entire contents of the host are consumed. The larva spins a cocoon and pupates in the cell with the remains of its victim. (Clausen *1940;* Clausen et al. *1927, 1932, 1933*)

Tiphiidae.—The family Tiphiidae is the dominant group of hymenopterous parasites attacking grubs of the Scarabaeidae in the Far East. All species are solitary wasps and develop as external parasites, usually on the final larval stage of the host, although if necessary they will attack smaller grubs. The many species of the genus *Tiphia* are widely distributed in the Far East. Most of them occur in the temperate regions and have only one generation a year. Species such as *T. asericae, T. matura,* and *T. vernalis,* which emerge in the spring, hibernate as adults in the cocoon, whereas those such as *T. bicarinata* and *T. popilliavora,* which emerge in the summer and fall, hibernate in the larval stage. Pupation of the summer and fall species occurs only a short time prior to the appearance of the adults.

The spring species almost invariably subsist on honeydew secretions of aphids, scale insects, leafhoppers, and other homoptera; however, *T. vernalis* and *T. malayana* also feed at the blossoms of forsythia. During the late summer and fall, honeydew is largely unavailable. The species occurring at that time feed instead at the blossoms of various plants, especially those of the Umbellifera and Polygonacea and on the nectar from various glands associated with foliage.

Usually each species of *Tiphia* parasitizes several species of grubs in the same genus, but not grubs of other genera. *T. vernalis* has as its normal host *Popillia quadriguttata* in Korea, *P. chinensis* in China, and *P. japonica* in Japan, whereas *T. popilliavora* normally parasitizes *P. atrocoerulea* in Korea, *P. chinensis, P. formosana,* and others of that genus in China, and *P. japonica* in Japan. *T. biseculata* has as its normal hosts *Anomala orientalis* and *A. schönfeldti,* whereas *T. burretti* has *A. cuprea* and *Mimela testaceipes. T. castaneaevora, T. isolata,* and *T. homoncularis* have as their normal hosts *Maladera castanea* and species of *Serica. T. phyllophagae* normally parasitizes species of *Phyllophaga.*

It is usually necessary to have suitable grubs near the feeding grounds when *Tiphia* adults appear in the field. *T. matura* is an exception to this general requirement. This species travels several miles in India to aphid-infested plants where it feeds and then returns for oviposition. The lack of sufficient food for the adults and of suitable grubs in the field limits the effectiveness and distribution of *Tiphia* in the Far East. (Clausen *1940;* Clausen et al. *1927, 1932, 1933;* Gardner and Parker *1940;* Jaynes and Gardner *1924*)

Clausen et al. (*1927, 1933*) and Gardner and Parker (*1940*) studied the life history and habits of the many species of *Tiphia* in the Far East. There is little difference in the biology of the various species occurring in different countries or in widely separated areas of the same country, other than in the time of the year that the adults appear in the field. The manner of attack by the females of all species on grubs is relatively uniform and corresponds to that described for *T. popilliavora.*

The female of *T. popilliavora* burrows into the soil and gains access to the cell occupied by a grub. The parasite first crawls over the dorsum of the grub from the rear, curls its abdomen downward

and around the side, and inserts its stinger into the ventor of the thorax, usually between the first two segments. The stinging is repeated until the grub is quiescent. The wasp then turns to the abdomen and kneads the ventral surface. When this is completed, it grasps the lateral margin with its mandibles and coils its body transversely over the dorsum of the grub to the ventral surface, forming almost a complete circle, and applies the tip of its abdomen to the groove between the fifth and sixth abdominal segments and moves it backward and forward several times to widen the groove. During this preparation any egg or young larva that may be present as a result of earlier oviposition is rubbed off or broken. The egg is finally extruded and is firmly attached by a mucilaginous material. The position in which the egg is placed on the body of a grub is virtually constant with each species of *Tiphia*. The variation between species is with respect to the segment where the egg is attached ventrally, laterally, or dorsally and the orientation of the egg with respect to the median line of the grub.

After depositing the egg *T. popilliavora* may bite or chew one of the legs of the grub and then imbibe the fluid that exudes from the wound. The grub recovers from the effect of the sting in 20 to 40 minutes. The hatching of the egg is effected by a longitudinal split of the chorion over the head and thorax of the larva. The head is extruded and a feeding puncture is made. Each successive instar makes a new feeding puncture a short distance in front of the previous one. In the last part of the fifth instar, suctorial feeding is abandoned and the entire body of the grub, except the head and legs, is consumed. Death of the grub does not occur until the parasite is in its fifth larval stage. The larva then spins a cocoon in the cell of its victim and pupates.

Clausen et al. (*1933*) and Gardner and Parker (*1940*) found that there was a marked difference in the time that *T. popilliavora* appeared in the field in Japan, Korea, and China. It emerges about the middle of August at Koiwai in northern Japan, about 2 weeks earlier than at Suigen, Korea, although the temperatures during the summer are higher in Korea than in Japan. At Nanking, China, near where *T. popilliavora* is found, the monthly temperatures are higher throughout the year than at Suigen, but the parasite emerges 3 weeks later there than in Korea. These biological differences would normally indicate that more than one species is involved. However, the anatomical differences in the specimens from those countries have not been sufficiently constant to demonstrate that there is more than one species. This also was the opinion of Allen and Jaynes (*1930*). In view of the biological differences, individuals from these countries are considered to be separate strains or races.

Predator of Adult Beetle and Grub

A carabid, *Craspedonotus tibialis*, occurs abundantly in the sandy areas near Miho, a small seacoast village about 50 miles south of Yokohama, Japan. It is predaceous on both the larval and adult stages of many insect species, including the Scarabaeidae.

There is one generation annually. Hibernation takes place apparently in the last larval stage. The adults live in deep tubular burrows, 10 to 18 inches deep, which enter the soil at an angle of 45°. The eggs are laid singly in small cells, about one-half inch deep, which angle off in a downward direction from the main burrow. Apparently the entire egg-laying period of the female is passed in the burrow, except for periodic forays for food. (Clausen et al. *1927*)

Hyperparasites in Far East

As opportunity afforded, Clausen (*1927*) and Clausen et al. (*1927, 1932*) studied the insect enemies of *Tiphia* in the Far East. It was not possible to make extensive collections of *Tiphia* cocoons in Japan for rearing hyperparasites because very little wasteland was available for extensive digging, but one natural enemy, *Palarus saishiuensis* Okam., was found in Korea and others in India. *P. saishiuensis*, one of the hunting wasps, stores its underground nest with various adult bees and wasps. About two-thirds of these were species of *Tiphia*. Most of the *Tiphia* were females.

The velvet ants (*Mutilla attenuata* Spin. and *M. stephani* Magr.) parasitize a small proportion of the cocoons of *Tiphia matura* in the field. An examination of the parasitized cocoons showed that the development of the hyperparasite had been completed on the mature larvae.

Several adults of *Perilampus* sp. emerged from cocoons of *Tiphia pallivora* that had been collected in the field. The cast skins of the *Perilampus* planidia were found attached to the larval skin of the host, although development had been completed on the pupae. *Perilampus* is not considered to be a normal parasite of *Tiphia*. The few instances of parasitism represent stray planidia, which in some manner had gained access to the larvae, either in the feeding stage or after formation of the cocoon, and once there they were able to develop to maturity.

The smaller species of *Tiphia* in India are generally and often heavily attacked by rhipiphorid beetles. Twenty-eight percent of the cocoons of *T. pallivora* collected in the field were parasitized by *Macrosaigon pusillum* Gerst. This hyperparasite killed the mature *Tiphia* larvae in the cocoon and emerged slightly earlier than the normal time for its host. The life history of this group of beetles in the role of parasites of *Tiphia* has not been studied and there is considerable doubt as to the exact manner in which the primary larvae gain access to the host.

Four species of Bombyliidae, the well-known bee flies, were found parasitizing cocoons of *Tiphia*. The most important, *Velocia oenomanus* (Rond.), occurred in the field in India in large numbers and parasitized 55 to 60 percent of the cocoons of a large red-legged *Tiphia* that was not definitely identified. It also attacked cocoons of *Scolia* sp. and *Campsomeris* sp. The adult flies appear in the field immediately after the period when the adult hosts are on the wing, and presumably deposit eggs on the surface of the ground. The young larvae find their way into the cocoons and develop as external parasites of the *Tiphia* larvae. A few *Tiphia*

cocoons were parasitized by *Exoprosopa sipho* Cole, *Aphoebantus clauseni* Ald., and *A. serratus* Ald.

An occasional cocoon of *Tiphia* collected in the field in India was parasitized by a nematode, *Eomermis tenuissima* Cobb. The nemas emerged from the cocoons early in July, shortly after the formation of the cocoon by *T. matura* and immediately preceding the normal emergence time of *T. pullivora*.

A large shipment of puparia of *Hyperecteina aldrichi* from Japan in 1921 was infested by several species of chalcid hyperparasites. The most important one was *Spalangia* sp. About 10 percent of the puparia were parasitized.

Shipping Parasites and Predators to United States

The parasites that appeared most promising for control of the beetle in the United States were collected in their native habitats in the Far East, or reared there, and shipped to the Japanese Beetle Laboratory. Little information was available on the handling of the different species. Methods had to be developed for rearing them and containers had to be devised for shipping them. The dipterous parasites with a long pupal period were shipped in the pupal stage, but those with a short pupal period were forwarded as larvae within their living host. The hymenopterous parasites were shipped in the cocoon and adult stages. The coleopterous predator was sent in the adult stage.

One of the important problems in the 1920's and 1930's was the long journey from the Orient to the eastern seaboard of the United States. In the present "Jet Age" a trip from the east coast to California can be completed in 5 hours, one to Japan in 24 hours, and one to Hong Kong in 27 hours, but at that time consignments from Japan went by ship to the west coast and then across the continent by train; they were usually en route for 18 days. Shipments from China were usually en route for 22 days. The ocean voyage from India or Australia to the eastern seaboard required 50 to 60 days. Even when the mortality of the insects was low on arrival, the long trip was unfavorable and tended to reduce their vitality. (King et al. *1927*)

Hamaxia incongrua.—Large numbers of adults of *P. japonica* were collected in the field in Japan where *Hamaxia incongrua* was abundant and forwarded without delay to the Japanese Beetle Laboratory. Usually less than 5 percent of the beetles were parasitized. In 1923 the cases containing the beetles were sent by cool storage from Yokohama by ship and on arrival in Seattle were packed in iced containers for the journey across the continent by train. All the beetles and the parasites were dead on arrival. In 1924 only part of the shipment was iced at Seattle. Again no flies emerged from the puparia subjected to the low temperature, whereas the noniced shipment arrived in good condition. The subsequent shipments were not iced at Seattle. (Clausen et al. *1927, 1933;* Gardner and Parker *1940;* King and Hallock *1925*)

Hyperecteina aldrichi.—Large numbers of adults of *P. japonica* bearing eggs of *Hyperecteina aldrichi* were collected in the field by

natives in Japan and were held in boxes with foliage of grape or *Polygonum* for 6 days. The beetles that were alive at the end of that period were destroyed. A beetle alive at that time was evidence that the eggs had not hatched or the parasitic larva had died. Great care was taken to eliminate the dead unparasitized beetles in order to reduce decomposition during transit. The dead beetles containing the puparia of the parasites were packed in moist sphagnum moss for shipment. The mortality en route was usually very low. (Clausen et al. *1927, 1933;* Gardner and Parker *1940*)

***Palpostoma* spp.**—The adults of *Anoplognathus olivieri,* one of the favored hosts of *Palpostoma* spp. in Australia, were collected in the field and held in cages until they were dead. The dead beetles were placed in moist sand and examined 5 or 6 days later. The puparia of the parasite were removed and packed in moist sphagnum moss for shipment. Fifty percent of the parasites were dead on arrival at the Japanese Beetle Laboratory. No flies emerged during the following 3 months from the remaining puparia. (Burrell unpublished; Gardner unpublished)

Trophops clauseni.—A few puparia of *Trophops clauseni* were shipped from Japan in 1932. Most of the pupae were dead on arrival at their destination and no flies emerged. (Gardner and Parker *1940*)

Adapsilia flaviseta.—Large numbers of adults of *Popillia cupricollis,* the favored host of *Adapsilia flaviseta* in India, were collected in areas where 1 to 2 percent of the beetle population was parasitized. The beetles were placed in heavy cardboard boxes with sliced apples as food. Twelve days later the dead beetles were examined and those not parasitized were discarded. The puparia in the bodies of the dead beetles were packed in moist sphagnum moss for shipment. The first consignment in 1926 was a total loss. It was en route more than 3 months. The later shipments arrived at the Japanese Beetle Laboratory in 50 to 60 days. About 70 percent of the pupae in these shipments were alive on arrival. (Clausen et al. *1933;* Gardner and Parker *1940*)

Dexilla ventralis.—No shipments of *Dexilla ventralis* to the United States were made prior to 1925 because *P. japonica* does not occur in Korea, and it was considered to be too risky to ship the parasitic larvae in the living grubs of other species of *Popillia.* The grubs of *P. japonica* could not be sent to Korea for parasitization, but in 1925 female parasites were shipped to Japan to parasitize the grubs there. Female parasites were caged over soil containing grubs, and the grubs were inoculated by placing planidia dissected from gravid female parasites upon them. That year only 850 parasitized grubs were obtained for shipment to the United States because most of the grubs were too far advanced in development for satisfactory parasitization.

The parasitized living grubs were packed in moist fumigated soil in tin-lined boxes, 1 foot square and 6 inches deep, which were divided into 500 compartments, each about 1 cubic inch in volume, with one grub in each compartment. The living grubs must be shipped in soil because they have cannibalistic tendencies and the

individuals must be isolated. The duration of the life cycle of the parasite is such that most of the flies were in the pupal stage en route and were ready to emerge on arrival at their destination. However, the emergence from that first shipment was only 3 percent.

In 1926 and 1927 arrangements were made to ship the parasitic larvae to the United States in the bodies of living grubs of the *Popillia* species that were native to Korea. Special precautions were taken in packing and in handling at destination to prevent the escape of any grubs. The first consignment in 1926 consisted of grubs containing second-generation *D. ventralis* larvae. On arrival most of the parasites had left their host and were in the larval and pupal stages. About 36 percent of this shipment arrived in good condition. The second consignment in 1926 contained third-generation parasitic larvae. Only 3 percent of this shipment arrived in good condition. However, all the subsequent shipments arrived with 30 percent or more of the parasites in good condition. In spite of the precautions taken, it was considered to be too risky to continue to ship living grubs of other species of *Popillia* to the United States, and at the close of the 1927 season the practice was discontinued.

During 1929–31, field-collected females of *D. ventralis* were again shipped from Korea to Japan to parasitize grubs of *P. japonica* there. About 30 percent of the parasites shipped during these years arrived in good condition. (Burrell *1931;* Clausen et al. *1927, 1933;* Gardner and Parker *1940;* King et al. *1927*)

Prosena siberita.—Grubs of *P. japonica* were parasitized in large numbers with first-stage larvae taken from the ovisacs of gravid *Prosena siberita* females. A consignment of inoculated grubs from Japan was received at the Japanese Beetle Laboratory in April and another in October 1922. Only a few flies emerged from those shipments. During the following 5 years naturally parasitized grubs were collected in the field in Japan. A consignment of field-collected grubs, 10 percent of which were parasitized, was received in mid-July 1923. Flies emerged from 24 percent of the parasitized grubs. Another consignment of grubs, 13 percent of which were parasitized, was received in June 1924. Over 80 percent of these parasitized grubs produced flies. After 1927 the grubs were parasitized in the laboratory in Japan. In subsequent shipments a high percentage of the parasites arrived in good condition. (Clausen et al. *1927, 1933;* Gardner and Parker *1940;* King and Hallock *1925*)

Campsomeris annulata.—Grubs of *P. japonica* were parasitized by confining females of *Campsomeris annulata* over soil containing grubs. The parasitized grubs were placed in individual clay cells with a small amount of moist sphagnum moss as soon as the eggs of the parasite hatched. The cells were packed in moist sphagnum moss and shipped immediately from Japan to the United States. In 1923 all the parasites were dead on arrival because of excessive loss of moisture in transit. This situation was corrected in subsequent shipments, and about 75 percent of the immature parasites in these shipments were alive on arrival.

Adults of *C. annulata* were also collected in the field in Japan, Korea, and China and shipped in special containers with food, water, and soil. In the earlier shipments an aqueous solution of honey or sugar was provided as food. In the later shipments a semisolid mixture of agar and honey or sugar sirup was used and finally a mixture of sugar and honey. About 14 percent of these Scoliidae adults arrived in good condition. (Clausen et al. *1933;* Gardner and Parker *1940;* King and Hallock *1925*)

Tiphiidae.—Different species of *Tiphia* were shipped to the United States in the cocoon and adult stages. Cocoons of the different species were obtained by confining the female wasps over soil containing grubs. Grubs of *P. japonica* were used in Japan and grubs of other species of *Popillia* in China, Korea, and India. After parasitization the grubs were placed in individual cells with soil in cross-section trays. A mixture of soil and sod was used in the trays in Japan and Korea and a mixture of soil and sphagnum moss in India. There was considerable variation in the proportion of parasitic larvae that pupated, depending on the temperature, condition of the soil, and other factors. The cocoons were packed in moist sphagnum moss for shipment. These shipments were not satisfactory in that usually less than 10 percent of the parasites completed their development and emerged as adults.

It was more satisfactory to ship the adults of those species that had a longevity of 30 days or more. The female wasps were collected in fields where abundant and shipped immediately in special containers with food, water, and soil. They were released immediately on arrival. During 1926–30 about 70 percent of the *Tiphia* adults arrived in good condition and during 1930–33 more than 80 percent. (Allen and Jaynes *1928;* Balock *1934;* Clausen et al. *1927, 1933;* King et al. *1927*)

Craspedonotus tibialis.—Adults of *Craspedonotus tibialis* were collected in the field and individual beetles were placed in small wooden boxes containing a little damp sphagnum moss. The shipments were made in cool storage from Japan to the United States and then at ordinary temperatures across the continent. About 50 percent of the beetles were alive on arrival. (Clausen et al. *1927*)

Number of Parasites and Predators Shipped to United States.—Allen and Jaynes (*1928*), Clausen et al. (*1933*), Gardner and Parker (*1940*), and Hadley (unpublished) tabulated the number of parasites and predators shipped from Japan, Korea, China, Formosa, India, Hawaii, and Australia to the United States. During 1919–36 a total of 1,771,340 parasites and predators were shipped, including 1,185,963 Tachinidae, 483,593 Tiphiidae, 62,972 Scoliidae, 21,462 Pyrgotidae, 16,450 Carabidae, and 900 Ithonidae. A total of 1,610,847 of these insects were shipped for control of *P. japonica*, 154,258 for control of *Maladera castanea*, and 6,235 for control of *Anomala orientalis*. Information on each species shipped is given in table 2.

TABLE 2.—*Shipments of foreign parasites and predators to United States to control Japanese beetle and other foreign Scarabaeidae species, 1919–36*

Species	Source	Years shipped	Number shipped
TACHINIDAE			
Dexilla ventralis	Korea	1925–31	50,324
Entrixopsis javana	Japan	1922–27	288
Hamaxia incongrua	do	1923–28	300,400
Hyperecteina aldrichi	do	1920–33	637,158
Palpostoma spp.	Australia	1932	5,056
Prosena siberita	Japan	1921–31	192,500
Trophops clausseni	do	1932	237
Total			1,185,963
TIPHIIDAE			
Tiphia agilis [1]	Korea	1925–26	197
Tiphia asericae [1]	do	1928–29	73,465
Tiphia bicarinata [2]	do	1925–26	5,720
Tiphia biseculata	Japan	1924–32	24,376
Tiphia brevilineata	Korea	1925–26	91
Tiphia burrelli	Japan	1932	61
Tiphia castaneaevora [1]	do	1933–34	11,126
Tiphia communis	China	1925–26	1,587
Tiphia frater [1]	do	1925–26	1,506
Tiphia homonecularis [1]	Japan	1932	264
Tiphia isolata [1]	do	1932	79
Tiphia koreana	Korea	1925	10
Tiphia matura	India	1925–29	35,270
Tiphia notopolita	China	1925–26	33
Tiphia notopolita alleni	Japan, Korea	1925–32	1,375
Tiphia ovidorsalis [1]	Korea	1925–26	99
Tiphia phyllophagae	China, Korea	1925	1,409
Tiphia popilliavora	China, Korea, Japan	1920–36	44,442
Tiphia pulliveora	India	1925–28	93,815
Tiphia satoi [1]	Korea	1931–33	132
Tiphia sternata [1]	Japan	1931–36	67,390
Tiphia tegitiplaga	do	1932	1,180
Tiphia totopunctata [2]	Korea	1925–26	476
Tiphia vernalis	China, Korea	1924–33	119,072
Tiphia sp. (Taiwan 5)	Formosa	1929	379
Tiphia sp. (Taiwan 2) [2]	do	1929	39
Total			483,593
SCOLIIDAE			
Campsomeris annulata	China, Korea, Japan	1925–26	37,824
Scolia manilae	Hawaii	1919–23	25,148
Total			62,972

[1] Shipped for control of *Maladera castanea* (Arrow).
[2] Shipped for control of *Anomala orientalis* Waterhouse.

TABLE 2.—*Shipments of foreign parasites and predators to United States to control Japanese beetle and other foreign Scarabaeidae species, 1919–36*—Continued

Species	Source	Years shipped	Number shipped
PYRGOTIDAE			
Adapsilia flaviseta	India	1926–29	21,462
CARABIDAE			
Craspedonotus tibialis	Japan	1920–21	16,450
ITHONIDAE			
Ithone fusca	Australia	1921	900
Grand total			1,771,340

Rearing Imported Parasites in United States

All the Tachinidae, Pyrgotidae, and some of the Scoliidae and Tiphiidae were shipped from the Far East in their immature stages and had to be held at the Japanese Beetle Laboratory until the adults emerged and conditions were suitable for their liberation in the field. The success of the undertaking depended to a large extent on being able to rear these parasitic insects to maturity.

Eutrixopsis javana.—This tachinid had attacked some of the beetles bearing eggs of *Hyperecteina* in Japan and was unwittingly shipped to the United States. Two hundred and ten *Eutrixopsis javana* parasites emerged along with the *Hyperecteina* parasites and were segregated for liberation in the field. (King and Hallock *1925*)

Hamaxia incongrua.—When the consignments of *Hamaxia incongrua* reached the Japanese Beetle Laboratory, the dead beetles were removed from the shipping boxes and placed in large battery jars where they were covered with 2 inches of sterile moist sawdust. The flies were collected as they emerged and transferred to a large cloth-walled cage for mating before being released in the field. (King and Hallock *1925*)

Hyperecteina aldrichi.—The puparia of *Hyperecteina aldrichi* had to be held over winter for emergence the following June. In a preliminary study of methods of hibernating the puparia, King and Hallock (*1925*) put 83 percent of the boxes of the October 1923 shipment in a chamber held at 35° to 45° F. and the remainder in cages out of doors, where they were covered with 1 inch of soil and 2 inches of leaves. The following June, 67 percent of the flies emerging from puparia had wintered under natural conditions. Thereafter, the temperature in the chamber was modified according to the monthly mean temperature of the soil in the field at a

depth of 3 inches. The temperature was gradually increased at 15-day intervals from 35° in January and February to 76° the last half of July, and then it was gradually decreased to the low temperature in January. With the hibernating temperatures controlled in this manner, 50 to 70 percent of the imported pupae completed their development and emerged.

After importation of *H. aldrichi* ceased in 1933, parasitized beetles were collected in areas where the parasite had become established in order to rear flies for further colonization. The parasitized beetles were placed with foliage of sassafras in wooden boxes, the bottom and top of which were covered with a fine mesh screen. The sassafras provided food for the beetles until they were killed by the parasite. Three weeks later the puparia were removed and packed in boxes with sand and sphagnum moss for hibernation in the controlled temperature chamber. The following June the boxes containing the puparia were transferred to a large cloth-walled cage out of doors and the flies were collected as they emerged. Several thousand *H. aldrichi* parasites were reared in this manner. (Balock unpublished; King, Parker, and Willard unpublished; King and Willard unpublished; Parker unpublished; Parker and Willard unpublished)

Adapsilia flaviseta.—The puparia of *Adapsilia flaviseta* were removed from the shipping boxes and packed in boxes with moist sand and sphagnum moss in the same manner as *H. aldrichi*. The boxes were held in the controlled temperature chamber until June and then transferred to a large cloth-walled cage for emergence of the flies. (King unpublished)

Dexilla ventralis.—The larvae and puparia of *Dexilla ventralis* received from the Far East were transferred from the shipping containers to boxes and packed in moist sand and sphagnum moss. The boxes were placed in a large cloth-walled cage for emergence of the flies. Usually about 85 percent of the parasites emerged. (Burrell *1931*)

D. ventralis was not imported after 1931, but in subsequent years several thousand flies were reared in the laboratory for distribution in the field. In the initial propagation experiments Burrell (*1931*) used field-collected female parasites, but later he used reared females. The parasites were confined over soil containing grubs. The parasitized grubs were transferred to cross-section trays containing soil in which wheat had been sown and were held there until the flies emerged. He induced mating by confining 70 to 100 flies in a large cage, the top of which was covered with white muslin and the sides with black mosquito netting. The second generation of *D. ventralis* was numerically six times that of the original stock, but few of the third generation reached maturity.

Parker (unpublished) was more successful in propagating the flies. In his experiments seven first-brood females collected in the field parasitized 227 grubs; 59 male and 66 female parasites emerged from those grubs. After mating in the large cage, 58 females parasitized 1,649 grubs, from which 536 males and 498 females of the third brood emerged. One hundred mated females of the third brood parasitized 953 grubs. The grubs parasitized by

the first and second broods of flies were held in cross-section trays with soil at 75° F., but those parasitized by the third brood were placed in a chamber where the temperature over the winter was adjusted according to the temperature of the soil in the field. A total of 71 males and 72 females emerged from the 415 grubs that survived the long period in storage. Although it was possible to maintain a stock of *D. ventralis* throughout the year in the laboratory, it was more practical to use field-collected females for parasitizing grubs.

Prosena siberita.—The grubs parasitized by *Prosena siberita,* which were received from the Far East, were handled in the same manner as those parasitized by *Dexilla ventralis.* (King unpublished)

Tiphiidae.—The emergence of *Tiphia* from cocoons in the earlier shipments from the Far East was so low that sufficient adults for colonization were not obtained. The cocoons were removed from the sphagnum moss in the shipping boxes and repacked in layers with moist soil, making a cell-like depression in the soil for each cocoon. The emergence of *T. popilliavora, T. pullivora,* and *T. vernalis* was less than 10 percent; 14 to 20 percent of *T. biseculata* and *T. totopunctata* emerged, and 36 to 56 percent of *T. asericae* and *T. bicarinata.* The situation was not improved by substituting for the boxes shallow trays in which the cocoons were embedded in cells of moist sphagnum moss or by placing the cocoons in individual cells in plaster of paris blocks or in vials with plaster of paris bottoms. The mortality of the most desirable species was always high when the cocoons were torn from their original cells and placed in artificially made cells of soil or moss or were subjected to long exposure on the surface of a moist earthy substratum. (Allen and Burrell *1934*)

In nature the cocoon stage of *Tiphia* is always passed in a small subterranean cell where the temperature fluctuates slowly and the relative humidity is continuously high. Allen and Burrell (*1934*) duplicated the natural environmental conditions in large chambers. The temperature in one chamber was varied as the monthly mean temperature of the soil changed at the normal depth of the cocoons in their natural habitat. It was gradually increased at 15-day intervals from 35° F. in January and February to 76° in the last half of July and then gradually decreased to the low temperature in January. Another chamber was kept 5° higher to expedite the emergence of those species that tend to be unduly retarded at normal temperatures. The relative humidity was maintained at approximately 90 percent by flooding the floors of the chambers with water. Later Burrell (*1930*) devised a humidifier in which the air was blown over wet screens. The cocoons were placed in 2-dram homeopathic vials, which were closed with stoppers of fine copper-wire screen and so arranged in trays as to make frequent inspection convenient. This arrangement of the vials in trays was an adaptation of a similar arrangement used in breeding parasites at the European Corn Borer Laboratory, then at Arlington, Mass.

Under these conditions in 1928, emergence was increased 28 percent for *T. popilliavora,* 87 percent for *T. pullivora,* 21 percent

for *T. vernalis,* and 61 percent for *T. biseculata.* The emergence was also better synchronized with the optimum periods for releasing the adult parasites in the field. Since that time this method has been used almost exclusively for rearing *Tiphia.* King (*1937*) has discussed further the methods for rearing the Tiphiidae and Scoliidae species.

Colonization of Foreign Parasites and Predators in United States

The establishment of a foreign parasite or predator in a new environment in the United States is a slow tedious process extending over several years. Some species were not able to adapt themselves to their new environment. Every effort was made to liberate a species in an area favorable for its welfare. There must be an adequate supply of food for the adults and an adequate population of *P. japonica.* The site selected for liberation should be one where these requirements may be satisfied for several years. Usually suitable sites were found on golf courses and in parks, cemeteries, estates, permanent pastures, and occasionally in housing developments. The practice of selecting such sites for liberation of the parasites and predators does not imply that they were extremely selective in their requirements. It was an attempt to provide them with every advantage to facilitate their establishment.

Species Not Known To Be Established

Although a diligent search was made at the sites where several species of foreign parasites and predators had been liberated a year previously, none of them were found at these sites or in the general vicinity. After several surveys had been made in subsequent years with negative results, it was concluded that these insects had failed to become established in their new environment.

About 400 adults of *Adapsilia flaviseta* were released in 1927 near Moorestown, N.J. This species was never recovered in the field. (Hadley unpublished; King unpublished)

About 190 adults of *Eutrixopsis javana* were released near Riverton, N.J., in 1923. Collections of adult beetles at this site in subsequent years did not have a single beetle parasitized by *E. javana.* (Allen and Jaynes *1928*)

About 4,000 adults and puparia of *Hamaxia incongrua* were released at two sites near Riverton during 1924–28. In subsequent years about 48,000 beetles were collected at these sites, but none of them were parasitized by *H. incongrua.* (Allen and Jaynes *1928;* King et al. *1927*)

Over 4,000 adults of *Campsomeris annulata* were released near Riverton during 1923–26. This scoliid failed to become established. (Allen and Jaynes *1928;* Hadley unpublished; King unpublished)

Another scoliid, *Scolia manilae,* was also released near Riverton, a few in 1919 and almost 11,000 in 1922 and 1923. The parasite was seen in the field during the summers it was released, but apparently it was not able to survive the winter. (Allen and Jaynes *1928;* King unpublished)

Several species of *Tiphia*—*biseculata* from Japan, *communis* from China, *matura* from India, *notopolita* from China, *phyllophagae* from Korea and China, *pullivora* from India, and *tegitiplaga* from Japan—were released at one or more sites during 1926–33, but even though in some cases more than 11,000 adults were liberated, none of them became established. (Hadley unpublished; King unpublished)

Craspedonotus tibialis, a predaceous carabid from Japan, was released in 1921 in a large field plot that was covered with a cage in order to observe the insect in its new habitat. About 7,000 were liberated. The beetles formed burrows, they deposited eggs, and larvae developed normally during the summer, but they did not survive the winter. (King unpublished)

Species Known To Have Been Established

About 700 adults of *Hyperecteina aldrichi* were first released in 1922 near Moorestown, N.J. No parasitized beetles were found that summer, but in 1923, shortly after liberating an additional 6,400 flies at that site, many parasitized beetles were found in the field. Surveys in subsequent years showed that this colony had spread over 12 square miles by 1924, 48 square miles by 1925, and 60 square miles by 1926. During the following 26 years colonies of *H. aldrichi* were each released at 54 additional sites in 12 States and the District of Columbia. This colonization is summarized in table 3. Surveys of 22 sites, or colonies, 2 years of age or older in 1937 showed that *H. aldrichi* was active at 59 percent of them. A 1950 survey showed that the parasite was present at 43 percent of the sites. (Allen and Jaynes *1928;* Hadley *1938;* King *1931, 1937, 1939;* King and Hallock *1925;* King et al. *1927, 1951;* Parker and Willard unpublished; Smith *1928, 1930*)

TABLE 3.—*Liberation and recovery of colonies of* Hyperecteina aldrichi *2 years of age or older in United States, 1922–50*

State	Colonies released in			Total released	Colonies in 1937		Colonies in 1950	
	1922–31	1932–41	1942–50		Scouted	Recovered	Scouted	Recovered
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Pennsylvania	10	3	3	16	13	7	12	3
New Jersey	3	4	6	13	5	4	6	3
Connecticut	2	1	1	4	3	1	2	2
District of Columbia	0	1	0	1	1	1	------	------
Massachusetts	0	1	5	6	------	------	1	1
New Hampshire	0	1	0	1	------	------	------	------
Virginia	0	1	0	1	------	------	------	------
Delaware	0	0	2	2	------	------	------	------
New York	0	0	5	5	------	------	1	1
Ohio	0	0	2	2	------	------	------	------
Rhode Island	0	0	1	1	------	------	1	0
Maryland	0	0	2	2	------	------	------	------
West Virginia	0	0	1	1	------	------	------	------
Total	15	12	28	55	22	13	23	10

About 1,528 adults of *Dexilla ventralis* were first released in 1926 at Haddonfield, N.J., and more in subsequent years. The most encouraging development in 1927 was to find the progeny of the flies liberated in 1926, showing that the parasite had passed the winter successfully. By 1949 *D. ventralis* had been released at four sites in New Jersey, five in Pennsylvania, four in Illinois, two in Maryland, one in New York, and one in Connecticut. All these sites were scouted diligently in the years following the release of the flies, but the parasite was recovered only at Haddonfield. (Allen and Jaynes *1928;* King *1931, 1937a, 1939;* King et al. *1927, 1951*)

Several hundred adults of *Prosena siberita* were released at Moorestown in 1923. Additional flies were liberated there in 1924, 1926, and 1927 to strengthen the colony and to enhance its chances for survival. Two puparia and a fertilized female of *P. siberita* were recovered at that site during the summer of 1927. Subsequent surveys indicated that the colony, though small, was in the first stages of establishment. The parasite was released at four additional sites in southern New Jersey and southeastern Pennsylvania during 1925–30. No other liberations of *P. siberita* were made. The Moorestown site was the only place where the parasite has been recovered. The colony remained weak for several years and then disappeared. No recoveries of the parasite have been made there for several years prior to 1950. (Allen and Jaynes *1928;* King *1931, 1939;* King et al. *1927, 1951;* Smith and Hadley *1926*)

The Japanese strain of *Tiphia popilliavora* was liberated first in 1921 and 1922 at Cinnaminson, N.J. The exact number released was not recorded, but most likely it was less than 50 individuals of both sexes. In 1926 many *T. popilliavora* parasites were observed on the flowers of wild carrot (*Daucus carota* L.) and parasitized grubs were found at that site. During August 1927 the adults of both sexes occurred in surprising numbers and the colony had spread over 1 square mile. The second liberation was in a pasture about a mile from the first site. Two mated females, which had deposited some eggs in captivity, were released there in 1921 and six females in 1923. Two males were found at this site in 1927. The third liberation was at a pond, 2 miles from the first site and 1 mile from the second site, where 45 mated females were liberated in 1922 and 9 more the following year. By 1927 this colony had spread over an area of approximately one-half square mile. The three colonies merged in 1929 and together covered 3½ square miles.

Nine hundred females of *T. popilliavora* were collected there in 1927 and were released in lots of 100 each at six other sites in New Jersey and at three sites in Pennsylvania where the ground was well drained, the grub population was at least 1 per square foot, and wild carrot was abundant. This was the beginning of the colonization program with the Japanese strain. By 1950 this parasite had been released at 716 sites in eight States.

The colonization program is summarized in table 4. A survey of 378 sites, or colonies, in 1937 showed that the parasite was established at 51 percent of the sites. Another survey of 523 colonies in 1950 showed that the parasite was at 47 percent of the sites. (Allen and Jaynes *1928;* Hadley *1938;* King *1931, 1937, 1939;*

King and Hallock *1925;* King and Holloway *1930, 1930a;* King et al. *1927, 1951;* Smith *1928, 1930;* Smith and Hadley *1926*)

In 1927, 45 mated females of the Korean strain of *Tiphia popilliavora* were released at Moorestown. Since then this strain has been liberated at 50 additional sites in eight States. The last liberation was in 1948. The colonization is summarized in table 5. A survey of 39 of the colonies in 1950 showed that this strain was established at 36 percent of the sites. (Allen and Jaynes *1928;* King *1937a, 1939;* King et al. *1951*)

In 1927, 232 mated females of the Chinese strain of *Tiphia popilliavora* were released at Woodbury, N.J. During the next 2 years, 4,130 mated females were released at 22 sites in New Jersey and southeastern Pennsylvania. A few adults were recovered at one site in 1929 and again in 1930, but this parasite failed to become established in its new environment. (Allen and Jaynes *1928;* King *1939*)

TABLE 4.—*Liberation and recovery of Japanese strain of colonies of* Tiphia popilliavora *2 years of age or older in United States, 1921–50*

State	Colonies released in—			Total released	Colonies in 1937		Colonies in 1950	
	1921–30	1931–40	1941–50		Scouted	Recovered	Scouted	Recovered
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
New Jersey	67	157	0	224	140	56	199	58
Pennsylvania	80	228	0	308	230	133	283	177
Maryland	0	85	0	85	1	0	3	0
New York	3	31	0	34	3	0	5	2
Delaware	0	31	0	31	4	2	22	2
Connecticut	1	23	0	24	------	------	11	5
Virginia	0	3	5	8	------	------	------	------
Ohio	0	0	2	2	------	------	------	------
Total	151	558	7	716	378	191	523	244

TABLE 5.—*Liberation and recovery of Korean strain of colonies of* Tiphia popilliavora *2 years of age or older in United States, 1927–50*

State	Colonies released in—			Total released	Colonies in 1950	
	1927-30	1931–40	1941–50		Scouted	Recovered
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
New Jersey	1	12	0	13	13	5
Pennsylvania	0	20	1	21	19	9
Maryland	0	1	4	5	3	0
Connecticut	0	0	2	2	1	0
Delaware	0	3	0	3	3	0
Ohio	0	0	1	1	--------	--------
Virginia	0	0	4	4	--------	--------
North Carolina	0	0	2	2	--------	--------
Total	1	36	14	51	39	14

In 1925, 30 adult females of *Tiphia vernalis* were released near Moorestown. To strengthen the colony 4,300 parasitized grubs were placed in the sod in 1926. This first attempt was a failure, probably because the site was too wet for the normal development of the parasite. In 1927, 16,600 parasitized grubs and 4,107 mated females were released at three well-drained sites where grubs were abundant—Merchantville, N.J., Berlin, N.J., and Philmont, Pa. By 1932 the parasite was so well established at Philmont that the female wasps could be collected readily for liberation at other sites; 4,600 females were collected there in 1932 and 5,400 in 1933. Two other sites in Pennsylvania where females had been released in 1929 had such a marked increase in population that in 1933 female parasites were also collected at those sites. After 1933 no further importations of *T. vernalis* were made from Korea. The established colonies were adequate to supply mated females for the colonization program. The domestic females were better than the imported females in that they had not been weakened by a long journey and they deposited more eggs.

The large-scale colonization program was initiated in 1933. One hundred field-collected females were liberated at each site where as nearly as possible the following conditions were satisfied: (1) An abundance of *Popillia* grubs, (2) the proximity of plants where the adult parasites could feed on honeydew, and (3) high and low ground to assure the continuance of the grub population in both wet and dry years. By 1953 over 240,000 females of *T. vernalis* had been liberated at 2,027 sites in 14 States and the District of Columbia.

This colonization program is summarized in table 6. Surveys of 351 sites, or colonies, in 1937 and 423 sites in 1950 showed that the parasite was well established at 64 and 63 percent of them, respectively. In addition, the cooperating State agencies developed their own colonization programs and liberated the parasite at many additional sites within their borders. (Adams *1944;* Allen and Jaynes *1928;* Balock *1934;* Cory and Langford *1944;* Friend *1940, 1942;* Gardner *1934;* King *1931, 1937a, 1939;* King and Parker *1950;* King et al. *1927, 1951;* Parker and Willard unpublished; Smith and Daniel *1942*)

By 1940 *T. vernalis* had become so abundant in southern New Jersey that it was a common insect. The public sent many specimens for identification to the Japanese Beetle Laboratory and to the New Jersey Department of Agriculture. As opportunity afforded, general surveys were made in other areas to obtain information on the dispersal of the parasite. It was found at many places several miles away from where it had been liberated. It was generally distributed in 1950 and 1951 in Pennsylvania throughout Bucks, Montgomery, Chester, Delaware, Northampton, Lehigh, Berks, Lancaster, Lebanon, and York Counties. It was generally distributed in 1952 in New York throughout Rockland, Westchester, and Putnum Counties, but more localized in its distribution in Nassau and Suffolk Counties on Long Island. It was found throughout Fairfield and New Haven Counties in Connecticut. It was generally distributed in 1953 in Delaware throughout Kent and

TABLE 6.—*Liberation and recovery of colonies of* Tiphia vernalis *2 years of age or older in United States, 1926–53*

State	Colonies released in—			Total released	Colonies in 1957		Colonies in 1950	
	1926–35	1936–45	1946–53		Scouted	Recovered	Scouted	Recovered
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
New Jersey	190	168	0	358	130	75	110	52
Pennsylvania	285	247	2	534	210	139	198	136
Maryland	2	373	17	392	2	2	44	35
New York	2	226	37	265	1	1	18	14
Delaware	11	49	0	60	5	5	11	9
Connecticut	0	119	32	151	------	------	5	5
Massachusetts	2	4	13	19	2	1	6	0
Rhode Island	0	25	3	28	------	------	14	4
West Virginia	0	2	25	27	------	------	------	------
North Carolina	0	2	0	2	------	------	------	------
Virginia	0	49	82	131	------	------	6	6
Ohio	0	17	14	31	------	------	------	------
New Hampshire	0	3	0	3	------	------	3	2
District of Columbia	1	8	0	9	1	0	8	4
Vermont	0	0	17	17	------	------	------	------
Total	493	1,292	242	2,027	351	223	423	267

New Castle Counties and in Maryland throughout Baltimore, Caroline, Cecil, Harford, and Queen Annes Counties. The distribution was less general in Somerset, Wicomico, and Worcester Counties of Maryland on the Eastern Shore.

This survey program was disrupted in 1953, when all the research on parasites and predators of the beetle was discontinued. Although the project has not been reactivated, another survey was made in Virginia in 1958. *T. vernalis* was generally distributed throughout the northwestern part of Loudoun and over most of Fairfax Counties. It was present in the remainder of these counties and in Clarke, Warren, Fauquier, and Prince William Counties, but it was not found in Frederick, Shenandoah, Rockingham, Augusta, Albermarle, Henrico, Page, or Rappahannock Counties. (King et al. *1951;* Parker unpublished; Parker and Willard unpublished)

Since *Hyperecteina aldrichi, Tiphia popilliavora,* and *T. vernalis* could adapt themselves to their new environment on the Atlantic seaboard, plans were made to colonize these parasites more extensively in the area infested by the beetle and to extend the program as the beetle invaded new areas. In cooperation with State agencies the Japanese Beetle Laboratory released 2,859 colonies of these parasites in 14 States and the District of Columbia, but the colonization program did not keep pace with the expanding beetle population. After 1940 the program was curtailed progressively and finally was discontinued in 1953; 23 percent of these colonies were released during 1921–30, 67 percent during 1931–40, and only 10 percent after 1940.

Effect of Milky Disease on *Tiphia*

The milky disease of *Popillia* grubs, caused by *Bacillus popilliae* Dutky and *B. lentimorbus* Dutky, does not affect larvae of *Tiphia popilliavora* and *T. vernalis* directly. After the parasitic larvae have spun cocoons in infected grubs, the recently voided meconium in the posterior end of the cocoons often is laden with spores of these pathogens, but the larvae show no evidence of infection. Some of the larvae fail to complete their development, owing to the death of the grubs from infection. The greatest loss of parasitized grubs occurs when the infection is well advanced at the time of parasitization. This is not an important factor with *T. vernalis*, which oviposits during May when the soil temperature in southern New Jersey rarely exceeds 65° F., a temperature not favorable for the development of these pathogens. However, in August when *T. popilliavora* is ovipositing and the soil temperature is generally above 70°, the disease is more likely to be well advanced at the time of parasitization.

No doubt some populations of *Tiphia* have been reduced somewhat by the effect of milky disease on the host. In general, however, these biological control agents are compatible. On the other hand, adult *Tiphia*, while searching for grubs in infected soil, may become contaminated with the spores of these bacteria and thus serve as a vector in the dispersal of the pathogens. (White *1943*)

Effect of Insecticides on Parasites

The treatment of soil with highly toxic insecticides such as DDT, chlordane, or dieldrin was detrimental to *Tiphia* developing and emerging in that soil. However, subsequent generations of *Tiphia* were not affected by a chemical treatment at a site, because with the Japanese beetle grubs destroyed by the chemical the parasite did not enter the soil for oviposition. Since the areas where insecticides have been applied to soil to control grubs constitute only a small part of the beetle-infested terrain, the use of insecticides in this manner probably has little effect on the population of the parasite. (King and Parker *1950*)

The application of sprays containing DDT, methoxychlor, or carbaryl to plants where *Hyperecteina* is parasitizing beetles or where *Hyperecteina* and *Tiphia* are feeding probably killed the parasites on the plants. After the spray had dried and the beetles on the plants had been destroyed, *Hyperecteina* would not be attracted to the plants, but *Tiphia* might still come to the plants to feed on honeydew or blossoms. However, the use of insecticides to protect plants from attack by the beetle probably has little effect on the population of parasites in an area.

Status of Foreign Parasites in United States

The establishment and survival of the five most important parasites of the beetle in the United States—*Hyperecteina aldrichi*, *Dexilla ventralis*, *Prosena siberita*, *Tiphia popilliavora*, and *T. vernalis*—are dependent to a large extent on having available a

suitable supply of food for the adult flies and wasps and an adequate population of grubs. The adults of *H. aldrichi, D. ventralis,* and *T. vernalis* feed on honeydew and nectar, whereas adults of *T. popilliavora* feed at the blossoms of wild carrot (*Daucus carota*) and *P. siberita* adults at various blossoms. *H. aldrichi, T. popilliavora,* and *T. vernalis* parasitize only *Popillia japonica* in this country. *D. ventralis* and *P. siberita* having a variety of hosts in the Far East might parasitize some other species of Scarabaeidae here.

In the 270 square miles in southern New Jersey and southeastern Pennsylvania occupied by the beetle in 1921, the annual beetle populations increased in magnitude and importance until by 1929 it was estimated that there were more than 500 million beetles per square mile. Some turf areas had a grub population of 150 per square foot. The density of the annual populations then declined until by 1945 the beetle was found only at isolated sites throughout the area. It has persisted at many of these sites, but usually the populations are relatively small. This cycle of the rise and decline of beetle populations has been repeated with modifications as the beetle invaded new areas. (Fleming *1963*)

Profound changes have occurred in the economics of this area, particularly since 1945. Pastures, orchards, and fields of asparagus, tomatoes, corn, and other crops on many farms have been replaced by large housing developments and industrial parks. Many hedgerows on farms where wild carrot grew profusely have been eliminated to make better use of modern agricultural implements.

Hyperecteina aldrichi.—In southern New Jersey and southeastern Pennsylvania *Hyperecteina aldrichi* parasitized only the first beetles of the season. The peak emergence of the fly was 3 or more weeks in advance of that of the beetle. About 20 percent of the parasite population was available to attack about 30 percent of the beetle population. The parasitization reached 28 percent in some local areas, but it declined rapidly as the season advanced and the beetles became more numerous. In spite of this situation the parasite persisted for many years in this area.

In attempting to find areas where the life cycles of the fly and beetle were better synchronized, *H. aldrichi* was released northward in Connecticut, Massachusetts, Rhode Island, New Hampshire, and New York, southward in Delaware, Maryland, Virginia, West Virginia, and the District of Columbia, and westward in Ohio. It was possible to survey only a few of these sites. The parasite was recovered 2 or more years after being released in Connecticut, Massachusetts, and the District of Columbia, but little information is available on the status of these colonies. The life cycles of the fly and the beetle in these areas have not been studied to determine whether they are better synchronized there. (King *1931, 1937a;* King et al. *1951;* Parker and Willard unpublished)

Dexilla ventralis.—This parasite has three generations a year in southern New Jersey. The first generation is on the wing in June, the second in midsummer, and the third in the fall. The beetle may be parasitized in any of its larval stages and occa-

sionally in the early pupal stage, but parasitism is infrequent in other than the third-instar grub. The first brood has available some third-instar grubs, but mostly prepupae and pupae; the second brood has mostly first- and second-instar grubs; and the third brood has third-instar grubs. The scarcity of third-instar *Popillia* grubs and the lack of suitable grubs of other species during the summer have seriously limited the otherwise excellent control potential of *Dexilla ventralis*.

This parasite became established in New Jersey only at a site where the soil was low and moist so that the development of the grubs was retarded and some third instars were available for the second brood. It was liberated in Pennsylvania, Illinois, Maryland, New York, and Connecticut with the expectation that other species of Scarabaeidae would be available for the second brood of flies, but there has been no indication that any of these colonies became established. An area where grubs of *Phyllophaga, Anomala,* or *Serica* were abundant to serve as an alternate host for the second brood had not been located by 1950. (Burrell *1931;* Hadley *1938;* King *1931, 1939;* King et al. *1951*)

Prosena siberita.—The peak emergence of *Prosena siberita* in southern New Jersey is about July 20; the peak of larviposition occurs about 10 days later. At that time the *Popillia* population in the soil is mostly first-instar grubs with a few second and third instars. Although *P. siberita* does not parasitize first-instar grubs readily, it will accept second instars, but it prefers third instars. The scarcity of suitable grubs and the tendency of the fly to disperse over a large area tended to retard the development of a colony. The last liberation of this fly was in 1930. It is possible that *P. siberita* would become better established in New Hampshire and Vermont, where some of the *Popillia* grubs require 2 years to complete their development and third instars are more prevalent during the summer, but the possibilities of the parasite in this area were not explored. (Hadley *1938;* King *1931, 1939;* King and Hallock *1925;* King et al. *1951*)

Tiphia popilliavora.—The Japanese strain of *Tiphia popilliavora* is on the wing in southern New Jersey and southeastern Pennsylvania during August and the first week in September. During the first 10 days of this period 80 percent of the *Popillia* population in the soil is made up of first- and second-instar grubs, but during the last 10 days 42 percent are second instars and 53 percent third instars. The parasite oviposits only on second and third instars, preferring the larger grubs. The progeny developing on second-instar grubs was 94 percent male, whereas those developing on third instars were 67 percent female. The sex ratio of the progeny was not changed by transferring parasite eggs from second instars to third instars, or vice versa, showing that the sex of the progeny was determined at the time the egg was placed on the grub.

Although much of the potential effectiveness of the Japanese strain was dissipated, it is a worthwhile parasite. The continued effectiveness of this parasite is dependent on having an adequate grub population available. It was abundant at those sites where there were 10 to 20 grubs per square foot, fairly abundant when

the grub population was 5 to 10 per square foot, but relatively scarce when there were less than 5 grubs per square foot. (Brunson *1934, 1937, 1938;* Hawley *1944;* King *1937a;* King et al. *1951*)

The life cycle of the Korean strain is better synchronized with that of *P. japonica* than the Japanese strain, in that the adults are on the wing in September when most of the grubs in the soil are third instars. However, surveys at sites where it had been colonized indicated that it did not become established any more readily in its new environment. The density of parasite populations also was modified by the abundance of grubs in the soil. (King et al. *1951*)

The Chinese strain never became established in southern New Jersey and southeastern Pennsylvania because the temperature was too low in October when the adults were on the wing. The optimum temperature for mating, 65° to 75° F., occurred only during the first few days of the month. The temperature of the soil was below 60°, the minimum for the development of the larvae and the formation of cocoons. This strain of the parasite is adapted to more southerly areas. (Holloway *1931*)

Tiphia vernalis.—*Tiphia vernalis,* the most promising of the foreign parasites, was colonized the most extensively in the area infested by the beetle. The adults are on the wing in May and June when the *Popillia* grubs are third instars. The female usually parasitizes grubs near plants when feeding on honeydew. When only a few such plants are in an area, the efficiency of the parasite is decreased. The denser the grub population the higher was the percentage of parasitization. The females parasitized 57 percent of a grub population with 6 per square foot, 46 percent with 5 per square foot, 31 percent with 2 per square foot, and less than 20 percent with 1 per square foot. The continued survival of the parasite is dependent on having an adequate grub population in the soil.

In many parts of the infested area where *T. vernalis* was colonized, the beetle population has declined to a low level. In southern New Jersey and southeastern Pennsylvania, beetles have been found since 1960 at only isolated sites. In a survey of 29 sites in New Jersey and Pennsylvania where the parasite had been very abundant, it was found in 1963 at only one site. Although the conditions in that area have not been favorable for the parasite, no doubt it has persisted in small numbers at many sites, even though the populations were too small to be found readily. (Balock *1934;* Gardner *1938;* Hadley *1938;* King and Parker *1950;* King et al. *1951;* Ladd unpublished)

ENTOMOGENOUS DISEASES OF BEETLE

Some diseased grubs were found in the field in New Jersey as early as 1921 (Smith and Hadley *1926*). Many of the grubs dug from the field in the fall and stored in a cool place in boxes of soil for experimental work during the winter developed symptoms of infection in 1922 and in subsequent years. Disease became so rampant among grubs in storage that sometimes 50 percent or more were lost (Hawley and White *1935*). Although the organisms causing these diseases were not identified specifically, the patho-

gens were determined in 1922 as mainly bacteria and fungi (Spencer unpublished).

A survey in 1933 and 1934 showed that diseased grubs were present in certain parts of the area then infested by the beetle in New Jersey, Delaware, and Pennsylvania (Hadley and Hawley *1934*). Hawley and White (*1935*) classified the various maladies according to the gross appearance of the affected grubs into three groups—black, white, and fungus. The grubs in the white group were filled with a micro-organism in nearly pure culture. This was the first recognition of the malady later called milky disease.

The early work on the diseases of the beetle, however, was limited in scope and not conclusive. Intensive study of the pathogens of the beetle was not undertaken until 1933. Dutky (*1963*) and Fleming (*1958a, 1963*) have summarized some of the later investigations of entomogenous diseases of the beetle.

Protozoan and Amebic Diseases

Kowalczyk (*1938*) found that the adult beetle as well as the pupa lacked a protozoan fauna, but the hindgut of the grub was commonly filled with a mass of soil particles among which lived a variety of micro-organisms, mostly bacteria and protozoa. Most of the latter appear to have a commensal or symbiotic relationship to their host, feeding on the bacteria, but some of them are pathogenic. He found in the hindgut of the grub five flagellate protozoans —*Retortamonas phyllophagae* (Travis and Becker), *Monocercomonas mackinnoni* Kow., *Monocercomonoides melolonthae* (Grassi), *Tetratrichomastix mackinnoni* Kow., and *Polymastix melolonthae* (Grassi), two entomophilic amebas—*Endamoeba* sp. and *Endolimax* sp., and one sporozoan—*Actinocephalus* sp. Steinhaus (*1947*) reported that *P. melolonthae* and related species inhabit the intestines of *Melolontha melolontha* (L.), *Popillia japonica* Newman, *Anomala orientalis* Waterhouse, and other insects. This group of organisms has not been studied further for control of the beetle.

In a survey at Cape Charles, Va., in 1939, Dutky and White (*1940*) found several grubs of *Strigoderma pygmaea* (F.) and *Phyllophaga gracillis* (Burm.) with a microsporidian infection of the fat bodies. The microsporidian seemed to be identical in both species. Attempts to produce infection artificially in grubs of these species and in grubs of *P. japonica* at this time were not successful. In 1951 a few *Popillia* grubs infected by a microsporidian were found at Warrenton, Va., but since then no cases of this disease have been found there or elsewhere within the area infested by the beetle. This microsporidian was classified tentatively as a species of the genus *Plistophera*. Of the several hundred grubs injected with suspensions of this organism, only two became infected. None of the grubs introduced into soil inoculated with suspensions of the organism became infected. This microsporidian seems to be of little importance in the control of the beetle. It was estimated that only 1 grub in 1,000 was infected naturally at Warrenton (Dutky unpublished).

Nematode Diseases

Neoaplectana glaseri Steiner

In the spring of 1929 Glaser and Fox (*1930*) found *Popillia* grubs infected by the nematode *Neoaplectana glaseri* at Haddonfield, N.J. The grubs were flaccid and ocherous brown; they contained many of the nematodes. Later that year infected pupae and adult beetles were found in that area. Although a search for this nematode was made in other parts of New Jersey and in Pennsylvania, it was not found at that time in any other area, but several species of saprophytic nematodes were found in dead grubs at several places. Steiner (*1929*) classified the nematode as a new parasitic species. It is probable that the nematode had some other insect as its normal host and had adapted itself to the beetle.

Hosts.—Glaser (*1931*) and Glaser et al. (*1940*) found that *N. glaseri* parasitized several species of insects in addition to *P. japonica,* including larvae of several species of *Phyllophaga, Maladera castanea* (Arrow), *Macrodactylus subspinosus* (F.), *Cyclocephala borealis* Arrow, *Cotinis nitida* (L.), *Xyloryctes satyrus* (F.), *Catalpa* sp., *Graphognathus* spp., and *Ostrinia nubilalis* (Hübner). Attempts to parasitize armyworms, silkworms, and house fly larvae were not successful, indicating a degree of specificity for the nematode.

Glaser (*1931, 1932*) found that the nematode was not pathogenic to plants. The soil and plants of greenhouse-reared tomatoes, cabbage, lettuce, corn, and wheat were treated with heavy doses of *N. glaseri,* but in no case were the plants affected. Field tests demonstrated that the nematode did not injure wheat, rye, and other grasses but probably furthered their growth by destroying the grubs. Since many nematodes attack plants, it was necessary to demonstrate that *N. glaseri* was not phytophagous.

Life Cycle.—The nematode passes through five stages in its life cycle—a small, slender, transparent first-stage larva; an active, slender, rather opaque, except the esophageal and caudal regions, second stage; an ensheathed third-stage larva; a preadult; and the adult male and female nemas. If the second-stage larva is in an environment favorable for further development, such as a living grub, the third larval stage is suppressed and the nematode completes its life cycle with three ecdyses, but if the environment is not suited for further development, such as a cadavar already exhausted of available food, the larva ceases to grow, empties the alimentary tract, and forms a new cuticle with the third-stage form within. The ensheathed larva is a resistant, truly infectious form.

After becoming mature the adult nematodes copulate. The female must be fertilized by the male to produce offspring. The females are viviparous. Larvae are born 1 or 2 days after mating. The female usually produces about 15 young. However, abnormally large gravid females were occasionally observed in grubs. One of these produced 1,420 young. Under optimum conditions the life cycle may be completed within 5 to 7 days.

Probably most of the larvae live free in the soil during the winter, though some may be in host insects during that period. The third-stage larvae parasitize the grubs during the warm periods of the year. Their activity is limited at 50° F. but becomes noticeable at 64° F. (Glaser et al. *1940;* McCoy et al. *1938*)

Mode of Infection.—The infective third-stage nemas enter the grub through the mouth with soil and food and develop rapidly into preadults and adults in the alimentary tract of the host. In most cases the grub dies by the time the first generation of the second-stage larvae has developed. Further development continues in the cadaver. At that time the nematode must be considered to be a saprozoic organism rather than a parasite. *N. glaseri* appears to be a species in transition from the saprozoic mode of existence to a parasitic one. As many as three generations may occur within one host, two of them appearing after the death of the grub. As many as 2,400 infective-stage larvae, 486 adult females, 502 adult males, some preadults, and some first-stage larvae have been removed from a grub. Usually about 1,500 infective-stage larvae are produced. Adult beetles are readily parasitized when they enter the soil for oviposition. Beetles also may become infected between completion of metamorphosis and escape from the pupal cell. (Glaser *1931, 1932;* Glaser et al. *1940*)

Natural Dispersion.—The nematode is spread slowly through the soil by its own movement and by nema-infected grubs. Other soil-inhabiting organisms probably assist in its dispersion. It may be carried long distances by parasitized beetles and by other insects, birds, and small mammals, and by the movement of soil by wind, water, or man. (Girth et al. *1940;* Glaser et al. *1940*)

Conditions for Establishment.—Glaser (*1932*) demonstrated that *N. glaseri* can be established in an area where it does not occur naturally and when so established can cause a high mortality of the grubs. He applied a water suspension of nematodes to established turf by spraying the suspension over the surface and washing the nemas from the foliage and by introducing the suspension into shallow holes approximately 4 inches square at 5-foot intervals and replacing the turf. The most consistent results were obtained with the subsurface application of 25,000 ensheathed nematodes per hole.

In additional experiments Glaser and Farrell (*1935*) and Girth et al. (*1940*) obtained 0.3- to 81.5-percent parasitization among the grubs, depending on the soil moisture, soil temperature, density of grub population, and nematode dosage. The optimum conditions for the establishment of the nematode are (1) a soil temperature of 60° F. or above at a depth of 1½ inches, (2) a soil moisture of 20 percent or higher without flooding, (3) turf or other permanent cover, and (4) a dense grub population. The most critical environmental factor is moisture. Moisture is essential during the dispersal of the nematode. A film of moisture is necessary for movement and respiration. Girth et al. (*1940*) and Dutky (*1959*) found that ensheathed nematodes cannot withstand desiccation.

Girth et al. (*1940*) reported that *N. glaseri* had maintained itself under natural field conditions for 6½ years with a grub population of less than five per square foot for most of that period. Later he (unpublished) found it had maintained itself for 14 years under these conditions. It survived for 24 years when the grub population was maintained by periodic restocking and for 1½ years in the absence of insect hosts.

Culture on Artificial Media.—During the investigation of the life cycle of the nematode, Glaser (*1931, 1931a*) found it could be cultured under artificial conditions, using a dextrose-veal infusion agar on which a heavy growth of baker's yeast was established before introducing the nemas. This medium was used for maintaining a breeding stock of nemas because of its simplicity, but it was not well suited for the culture of large numbers of the organism. A potato culture medium (McCoy and Glaser *1936*) was first developed for the production of the nematode on a large scale, but this was replaced with a veal pulp medium (McCoy and Girth *1938*) when higher yields were obtained with the latter medium. The yield on the veal culture ranged from 9,000 to 12,000 nemas per square centimeter of culture surface. As many as 18 successive transfers on the medium have been made, with an approximate fifteenfold increase in numbers at each transfer, but it was customary to make not more than 10 successive transfers.

In the mass production of the nematodes, one or more associated species of organisms were usually present. However, Glaser (*1940*) and Stoll (*1959*) cultured the nematode in an axenic, or bacteria-free, liquid medium, showing that the presence of associated bacteria was not essential for the development of the nema. Stoll (*1953*) maintained an axenic culture for 7 years, during which 180 to 195 generations were cultured in vitro. At the end of that period the culture was still infectious for grubs.

Colonization.—With the development of methods of propagating *N. glaseri* in large quantities on artificial media, the New Jersey Department of Agriculture in cooperation with the U.S. Department of Agriculture and the Rockefeller Institute for Medical Research at Princeton, N.J., undertook in 1940 to establish colonies of the nematode at 3½-mile intervals in New Jersey (Girth et al. *1940*). About 5 million ensheathed infective-stage nemas were placed in established turf at each site. This program was completed in 6 years. A similar colonization program was carried out at 100 sites in Maryland (Cory and Langford *1944, 1955*). This is one of the few instances in which nematodes have been colonized over a large area for the control of an insect pest.

N. glaseri is not effective under so wide a range of conditions as the bacterial diseases and the parasitic insects, but in a suitable environment it is a worthwhile parasite of the beetle. No information is available on the present status of this nematode in New Jersey and Maryland.

Neoaplectana chresima Steiner

During 1937–40 Glaser et al. (*1942*) found another nematode parasitizing the grubs at 14 localities in New Jersey and at 1 place

in Maryland. *Neoaplectana chresima,* another new parasitic species, has a life cycle similar to that of *N. glaseri,* but the average number of young produced by viviparous females is 250 to 400 instead of 15. A method for culturing this species on fresh sterile rabbit kidney was developed. No growth was ordinarily obtained when contaminants were present. Although this nematode was not colonized, it has been encountered from time to time in surveys throughout the area infested by the beetle.

Neoaplectana alloia Steiner

The soil in a greenhouse at the Japanese Beetle Laboratory became heavily infested with *Neoaplectana alloia* in 1938. A high mortality of the grubs introduced into the soil occurred and disrupted the experiments underway with other pathogens. This nematode was also found in the field at Rhoadstown, N.J. The highly infectious soil from the greenhouse was applied to turf at Plainfield, N.J., but the nematode failed to become established there. (Dutky and White unpublished)

Other Nematodes

Sick and dead grubs infected by many other parasitic nematodes were encountered from time to time in field surveys. Although these nematodes were not identified, they probably belong to the genus *Neoaplectana.* Bacteria were often associated with the nematodes. Dutky (*1937*) was the first to demonstrate that nematodes could be vectors of bacterial pathogens affecting grubs. The blood of many dead nematode-infected grubs was swarming with a peculiar oval-shaped bacterium containing spindle-shaped refractile bodies. Healthy grubs inoculated with this bacterium died within 2 days. The nematode seemed to serve as a vehicle to introduce the bacterium into the body cavity of the grub.

A nematode found at Arlington, Va., in 1947 had a red pigmented bacterium, *Serratia* sp., associated with it. Dutky (unpublished) found that a pure culture of the bacterium and of the nematode could be grown in a peptone-dextrose agar medium with autoclaved cubes of pork kidney. When a suspension of the infective-stage nemas was treated with sodium hypochlorite to destroy superficial contaminants, most of the nematodes survived the treatment. Five days after inoculating the medium with that culture, a good growth of the bacterium in nearly pure culture had developed and the nemas had changed to adults and were feeding on the bacterial growth. When the suspension was treated with weak formaldehyde, there was no growth of the bacterium and the nematodes did not develop to the adult stage. Grubs injected with a suspension of the bacterium died within 24 hours.

When the nematodes were introduced into soil, the degree of infection among the grubs varied greatly. At the time of death of the infected grubs, a few nemas were within their bodies, but the blood was swarming with the bacterium. Four or five days later many larvae and mature nemas were in the cadavers. This nematode was colonized at Buck Hill Falls, Pa., in May 1951, using 2,000 nemas per square foot. Two grubs infected by this nematode

were found 10 days later, but no other cases of infection developed during the following year. The indications are that the nematode failed to become established there.

Fungus Diseases

Metarrhizium anisopliae (Metsch.) Sorok

Metarrhizium anisopliae, the green muscardine, was found attacking grubs in the field in New Jersey in 1921 (Smith and Hadley *1926*). Since then, grubs infected by this fungus have been discovered from time to time throughout the beetle-infested area. Although *M. anisopliae* is widely distributed naturally, the incidence of this disease among the grubs is usually very low (Hawley and White *1935*).

Dutky (*1937*) grew the fungus on a variety of simple media, but the best growth and sporulation were obtained on a dextrose-peptone agar medium and on a dextrose-peptone-potassium phosphate liquid medium. The liquid medium was better suited for the production of large numbers of spores. Widemouthed 500-ml. Erlenmeyer flasks, filled with approximately 50 grams of excelsior wetted with 100 ml. of the liquid medium and sterilized, yielded about 18 billion spores per flask after incubation for 1 month at 86° F. *M. anisopliae* maintained on the dextrose-peptone agar medium was still infectious after 6 months but lost its pathogenicity after being held for a year. In preparing the spores for inoculation in the field, Dutky (unpublished) harvested the spores, dried them for 3 hours at 122°, mixed them with an equal weight of talc, and then further diluted the mixture with dry sand to provide sufficient bulk for application with a fertilizer distributor.

Eggs of the Japanese beetle hatched normally in soil containing 500,000 spores per gram, but the newly hatched grubs soon became infected and seldom survived more than 2 days. There was a definite relationship between the density of the spores in soil and the rate of infection among third-instar grubs. The average time of infection at 86° F. was 14 days with 50 spores per gram of soil, 10 days with 500 spores, 7 days with 5,000 spores, 5 days with 50,000 spores, and 2 days with 500,000 spores. Third-instar grubs dusted with spores all became infected within 2 days at 86°, within 4 days at 72°, and within 7 days at 60°. Only 50 percent of them became infected within 40 days at 54° and none of them developed infection at 50°. A temperature of 54° seemed to be about the minimum for the development of the fungus. The infection at 86° progressed rapidly when the air was saturated with moisture, but it was retarded at lower humidities. The rate of infection was also affected by the extent to which soil particles adhered to the body of the grubs. (Dutky *1937, 1959*)

M. anisopliae was colonized to a limited extent in New Jersey, New York, and Pennsylvania during 1936–38. In most cases the spores were applied to established turf with a fertilizer distributor at the rate of 600 million per square foot. Samples of soil were taken periodically from those plots to determine the status of the

fungus. The results were not promising. Although some grubs introduced into the samples of soil became infected, the incidence of infection was very low. The fungus appeared to be so fastidious in its environmental requirements that artificial dispersion was impractical. There is no doubt that the sporadic but small and continuous outbreaks of this disease among the grubs in the field are a factor in the biological complex that tends to suppress beetle populations, even though *M. anisopliae* may never be an important factor. (Dutky and White unpublished)

Metarrhizium glutinosum Pope

Dutky (unpublished) found *Metarrhizium glutinosum* to be pathogenic to the grubs. This fungus seemed to have about the same limitations as *M. anisopliae*.

Isaria densa Auct.

Isaria densa is a pathogen infecting *Melolontha* spp. in France. In laboratory tests this fungus killed 40 percent of the third-instar *Popillia* grubs. It was colonized in the field during the fall of 1923. The following spring 48 percent of the grubs in the plot were infected. However, *I. densa* did not persist under the climatic conditions of southern New Jersey, and further experiments with the pathogen were abandoned. (Hawley and White *1935;* Smith and Hadley *1926*)

Beauveria globulifera Speg.

Dutky (unpublished) recovered the white fungus *Beauveria globulifera* from a dead peach tree borer (*Sanninoidea exitiosa* (Say)). A pure culture of the fungus was obtained from the dried-out larva on malt extract agar. Inoculations of the borers by injection or merely dusting with the spores produced infection consistently and rapid death. Similar tests with second- and third-instar *Popillia* grubs gave variable results. The pathogenicity of this fungus to the grubs was usually low.

Beauveria bassiana (Balsamo) Vuill.

Interest in *Beauveria bassiana* as a possible parasite of the Japanese beetle was stimulated by the pathogenicity of the organism to the European corn borer (*Ostrinia nubilalis* (Hübner)) and to the Colorado potato beetle (*Leptinotarsa decemlineata* (Say)). Lefebvre (*1931*) readily killed larvae of the corn borer by inoculating them with the spores. Bartlet and Lefebvre (*1934*) killed up to 70 percent of the borers in cornfields by dusting with flour containing spores. Bradley (*1952*), in summarizing the experiments with the fungus against the borer, pointed out that although the infected borers had been recovered shortly after dissemination of the fungus, the pathogen had failed to become established as a natural control measure.

Rex (*1940*) reported that *B. bassiana* was infecting larvae of the Colorado potato beetle in New Brunswick, Canada. Timonin (*1939*) definitely established the pathogenicity of the fungus to these larvae. Schaerffenberg (*1957*) obtained 91-percent control of

the Colorado potato beetle in Austria within 3 weeks by dusting the field with the spores, and in Germany 80 percent of the larvae became infected within 4 days.

B. bassiana grows on the common culture media, but such media are not well suited for the production of the spores in abundance. McCoy and Carver (*1941*) obtained large quantities of the spores by culturing on autoclaved moistened wheat bran. The yield was about 22 grams of spores per pound of bran. After drying, the spores were separated from the bran by an air-separation procedure.

In preliminary experiments Rex (*1940*) dusted and sprayed *Popillia* grubs with spores and introduced the grubs into soil inoculated with the spores. *B. bassiana* appeared to have little pathogenicity to the grubs. When adult beetles were dusted with the spores, a high incidence of infection developed within a few days. Beetles also became infected by feeding on foliage sprayed with a dilute suspension of the spores or by coming into contact with infected beetles. The foliage of plants favored by the beetle in several heavily infested areas in New Jersey was sprayed with an aqueous suspension of the spores. Many infected beetles were found subsequently in the sprayed areas. However, the fungus failed to become established as a natural control agent.

Bacterial Milky Disease

Milky disease was discovered in central New Jersey in 1933, when a few abnormally white grubs were found in the field. Microscopic examination showed that the blood of the infected grubs, normally clear, was filled with bacterial spores, which made it appear milky (Hawley *1952;* Hawley and White *1935*). This condition of the blood led to the designation of milky disease for the malady. This disease was prevalent in 1935 at most places where the beetle had been for several years, but it was not found in more recently infested areas (Hadley *1938*).

Surveys by White and Dutky (*1940*) in 1935 and 1936 showed that 92 percent of the infected grubs in the field were infected by the organisms that Hawley and White (*1935*) had classified as the white group. They demonstrated that the malady was caused by two closely related undescribed species of spore-forming bacteria, which grow and sporulate in the blood of the living grub, and they temporarily designated these bacteria as type A and type B milky disease bacteria. The type A bacteria were the most prevalent. The blood of 88 percent of the infected grubs contained that pathogen. Dutky (*1940*) described type A and type B bacteria and named them *Bacillus popilliae* and *B. lentimorbus,* respectively.

Origin.—The origin of the milky disease bacteria is not known. There is no evidence that the bacteria had been imported from Japan with the grubs. These bacteria are probably indigenous to the United States and obligate parasites of the grubs of the Scarabaeidae. Grubs of *P. japonica* are their most common host.

When other species of insects were inoculated by these bacteria, no infection developed. The milky disease bacteria did not affect earthworms, birds, warm-blooded animals, humans, or plants. (Dutky *1963;* Fleming *1961;* Hadley *1948, 1948a*)

During a search for pathogens of white grubs in Australia, Beard (*1956*) found two bacteria of the milky disease complex infecting grubs of *Sericesthis pruinosa* (Dalman) and *Heteronychus sanctae-helenae* (Blanch.). The symptoms produced by these organisms were similar to those caused by *Bacillus popilliae* and *B. lentimorbus* in grubs of *P. japonica*. He described and named these organisms *B. lentimorbus* variety *australis* and *B. euloomarahae*.

Hurpin (*1955*) reported milky disease among *Melolontha melolontha* grubs in France. Wille (*1956*) reported the disease in grubs of that species in Switzerland and named the organism *Bacillus fribourgensis*. Hurpin (*1959*) and Dutky (*1963*) considered this pathogen to be the *Melolontha* strain of *B. popilliae*.

Strains of Bacteria.—There are many strains of *Bacillus popilliae* and *B. lentimorbus*. A strain developed in grubs of one species may have a low or a high virulence to grubs of other species. *Phyllophaga hirticula* (Knoch) and *P. rugosa* (Melsh.) did not become infected in soil inoculated with spores of *B. popilliae* grown in the blood of *Popillia japonica*, but they developed infection in soil inoculated with these spores from a naturally infected grub of *P. hirticula* (Dutky and Dobbins unpublished). The incidence of infection among second-instar grubs of *Phyllophaga* sp. was not enhanced by the application to the soil of spores of *B. popilliae* grown in the blood of *Popillia* (Schwardt et al. *1943*). *Cyclocephala immaculata* (Olivier) did not become infected when injected with spores of *B. popilliae* from a *Popillia* grub (Dutky and Dobbins unpublished), yet grubs of that species infected by this pathogen were found in the field (White *1947*). Spores of the *Cyclocephala* strain, injected into the blood, infected grubs of *Cyclocephala borealis* Arrow, *C. immaculata*, and *P. japonica*.

The strains of *Bacillus popilliae* and *B. lentimorbus* produced in the blood of *Amphimallon majalis* (Razoumowsky) were more virulent to that grub than to grubs of *P. japonica* (Tashiro *1957*). The strains of *B. popilliae* produced in the blood of *Cyclocephala* sp., *Phyllophaga anxia* LeC., *P. fusca* (Froel.), and *P. hirticula* had a low pathogenicity to *Amphimallon* grubs, but after these strains had been passed several times through the blood of *Amphimallon*, their virulence to that grub increased (Tashiro unpublished). *B. popilliae* produced in the blood of *P. japonica* was initially equally infectious to that grub and to grubs of *A. majalis*, but 6 years after being colonized in an area where only *Amphimallon* occurred, it lost its potency against *Popillia* grubs and increased its virulence to *Amphimallon* grubs (Tashiro unpublished). The regular *Popillia* strain of *B. lentimorbus* had a low virulence to grubs of *A. majalis*, but the "Maryland type B" strain grown in the blood of *P. japonica* readily infected *Amphimallon* grubs (Tashiro and White *1954*).

Bacillus popilliae Dutky

Bacillus popilliae, the most common of the milky disease complex within the area infested by the beetle in 1935 (White and Dutky *1940*), has been investigated more extensively than the others.

Dutky (*1940*) described this bacterium. The vegetative form is a nonmotile Gram-positive rod measuring about 0.9 by 5.2 microns. The rods become swollen at sporulation, assuming first a spindle and then a pyriform shape. The spores are cylindrical and measure about 0.9 by 1.8 microns and are located centrally in the cell. In the broader pole of the cell is found a refractile body, which is about half the size of the spore and possesses staining reactions similar to those of the spore.

Cycle of Development.—This fastidious organism cannot develop in a dead insect. Dutky (*1940*) described the cycle of development in a living grub. Whether infection is induced artificially by injection or acquired naturally, the infection begins with the appearance of the vegetative cells in the blood. These rods are found in small numbers about 30 hours after the invasion of the blood at 86° F. The rods multiply rapidly at this temperature, reach extremely large numbers, and sporulate. Just what induces sporulation is not known, but it occurs when the vegetative forms become very numerous. Sporulation continues until all the bacteria have reached the definitive spore stage. By the end of the sixth day the spores are sufficiently numerous that the blood becomes turbid. Seven to ten days later when the number of spores reaches the maximum, the blood is distinctly milky in appearance.

Dutky (*1940*) estimated that the average number of spores produced in a grub is about 5 billion, whereas Beard (*1945*) and Langford et al. (*1942*) estimated about 2 billion. However, the nutritional state of the grub at the time of infection and incubation has a profound effect on the number of spores produced. A well-nourished grub will survive longer after infection and produce more spores than a grub in poor condition.

Temperature Limitation.—The lower limit of temperature at which *B. popilliae* can develop is about 60° and the upper limit about 97° F. Dutky (*1940*) injected *Popillia* grubs parenterally with 2 million spores per grub and held them at various temperatures. No infection developed at 60° or at 97°. Macroscopic symptoms of infection developed in 14 days at 63°, 11 days at 70°, 9 days at 77°, 6 days at 86°, and 4 days at 93°. No vegetative rods developed in the blood of grubs held for 29 days at 59°. When grubs held for 28 days at 48° were transferred to 86°, symptoms of infection developed after 5 days.

Beard (*1945*) obtained maximum sporulation by parenteral injection of *Popillia* grubs in 30 days at 66° F., 18 days at 70°, 11 days at 76°, and 7 days at 86°. Tashiro and White (*1954*) injected 100,000 spores into grubs of *Amphimallon majalis*. In 2 weeks 21 percent were infected at 70°, 85 percent at 80°, and 75 percent at 90°; in 3 weeks 45 percent were infected at 70°, 85 percent at 80°, and 97 percent at 90°; and in 6 weeks 5 percent were infected at

60°, 70 percent at 70°, 87 percent at 80°, and 100 percent at 90°. If the maximum temperature at which the bacterium can grow is below the body temperature of warm-blooded animals and man, *B. popilliae* can never be pathogenic to them.

The temperature limitation also has an important bearing on the organism's effectiveness in the field. When the temperature of the soil is below 70° F., a rapid buildup of the organism is impossible. The number of weeks in the growing season when the temperature of the soil is above 70° is a factor in the establishment and buildup of the bacterium. Southward at Cape Charles, Va., a definite buildup occurred within a year (White and Dutky *1940*), but northward in New York (Adams and Wheeler *1946*) and in Connecticut (Garman et al. *1942*) 3 to 4 years were required.

Resistance to Adverse Conditions.—The vegetative stage of the bacterium is killed by exposure to cold, heat, or sunlight, but the spores are very resistant to adverse conditions and may remain viable in the soil for many years, ready to infect successive generations of grubs. The spores endured the unusually cold winter of 1935–36, when the temperature of the soil without a snow cover dropped below 15° F. and many grubs were killed by the cold (White *1940a*). In contrast, the vegetative stage did not endure a temperature of 50° for 2 weeks (Dutky *1963*).

The spores are resistant to heat. They were not affected by an exposure for 80 hours to 140° (White *1946*) or for 10 minutes to 176° (Dutky *1940*), but they lost some of their potency by an exposure for 10 minutes at 194°, and most of them were killed at 212° (Beard *1945*). In contrast to the spores, no stage of the beetle survived an exposure for 1 minute in water at 128° (Fleming and Baker *1932*).

Neither excessively wet nor extremely dry soils impaired the effectiveness of the spores. Spores remained viable in air-dry soil for more than 10 years (White *1940a*). Spores in dried blood films also remained viable for several years (Dutky *1940, 1963;* Beard *1945*).

Spores were reduced in potency by exposure to ultraviolet light for 2 hours (Beard *1945*) or to sunlight for more than 8 hours (White *1946*). However, when spores were mixed with talc and chalk and exposed in a layer a thirty-second inch or more in depth, sunlight did not reduce their potency significantly during an exposure of 72 hours (White *1946*). Exposure for 16 hours to a dosage of 5,600 rads of gamma rays from a cobalt 60 source did not reduce the number of spores germinating and forming colonies on bran infusion agar or the potency of the spores. A dosage of 100,000 rads reduced the germination but did not modify the infectivity of the spores, whereas a dosage of 1 million rads killed most of the spores (Tashiro unpublished).

When the spores were suspended in a series of buffered solutions ranging in pH from 3.2 to 8.0 and then injected into grubs, all grubs developed infection with suspensions having a pH of 6.8 or more, but as the pH of the suspensions was lowered below 6.8, the incidence of infection among the grubs decreased progressively until it was only 10 percent with a pH of 3.2 (Beard *1945*). In

soil where the pH was adjusted progressively from 4.85 to 6.60 by the addition of hydrated lime, there was an indication that the potency of the spores was favored by the higher pH (Beard *1945*). Growth of the spores on artificial media under aerobic conditions was obtained only on freshly sterilized alkaline media. Under anaerobic conditions growth was obtained when the pH of the media was between 5.5 and somewhat less than 9.0 (Dutky *1963*).

Development of Infection in Grubs.—The spore stage of *B. popilliae* normally occurs in the soil. As the grubs work their way through soil, feeding on roots and humus, they may ingest the spores along with other material. Beard (*1945*) demonstrated that the normal method of invasion was by the mouth, rather than by penetration of the integument or the tracheal walls. Grubs with their mouths sealed with a synthetic resin were able to live in inoculated soil without becoming infected.

Hawley and White (*1935*) thought that the bacteria penetrated through open wounds, such as might be caused nipping each other under very crowded conditions, probably because Spencer (unpublished) in 1924 had reported that injured grubs acquired infection more readily than did normal grubs. This hypothesis is not tenable. Although grubs frequently bite each other when removed from soil, they rarely do so even under crowded conditions in the field, because in nature each grub is in its own individual cell in the soil. There is no record of more than one grub being found in a cell.

Beard (*1945*) found that the incidence of infection among wounded grubs was no higher than among normal grubs. However, if a healthy grub did bite an infected grub, thereby getting a mouthful of blood containing vegetative rods or spores, it might become infected. Since an isolated grub in inoculated soil may become infected and infection may be induced by oral injection of spores, there seems to be no doubt that the normal mode of invasion is by way of the alimentary tract.

The ingestion of spores by a grub does not always produce infection. The spores may pass through the alimentary tract and be discharged with the fecal matter, or they may germinate and produce infection. The first evidence of infection is the presence of vegetative rods, not spores, in the blood, indicating that the spores germinate in the alimentary tract and the bacteria reach the blood in the vegetative form. However, the wall of the alimentary tract is not a sievelike structure through which bacteria can pass readily. The gut, particularly the rectal sac, normally contains large numbers of soil micro-organisms, some of which are lethal when they enter the body cavity. An injury to the gut that permits these soil micro-organisms to enter the blood is usually fatal within 24 hours. Since some of these micro-organisms are more virulent in the blood than *B. popilliae* and many are smaller, the mechanism of infection is one that permits the passage of *B. popilliae* but excludes the other micro-organisms.

Beard (*1945*) endeavored to locate the region in the gut where penetration by *B. popilliae* occurred. It seemed evident from the low rate of infection of grubs in inoculated soil that there was a

restricted region of penetration. His tests demonstrated that the spores did not germinate and penetrate the wall of the rectal sac or the rectum. It was doubtful that the first, second, or third circle of gastric ceca or the ventriculus was the site. The most probable site of penetration was the Malpighian tubules, attached to the hindgut and extending anteriorly. In a few cases spores were detected in the tubules of grubs allowed to feed for a few days in soil containing a high concentration of spores.

It appeared that in passing slowly through the alimentary tract with particles of soil and other solid matter, some spores entered the tubules through their orifices and germinated there. There is no reason to believe that the spores in any way actively seek the point of germination, but are presumed to be passively carried to that point. If they failed to reach that location, they passed on through and out of the alimentary tract. The germinating spores apparently passed through the walls of the tubules into the blood, but there is no evidence that the vegetative form is excreted by way of the tubules. It is possible that the physiology of the germinating organism permits such penetration, whereas that of the dividing or sporulating organism does not. Thus, after the ingestion of spores by a grub, the development of infection is a probability phenomenon.

The three larval instars of the beetle appear to be equally susceptible to infection by *B. popilliae* (Beard *1945*). Infected adult beetles were produced experimentally by injecting spores into third-instar grubs and pupae (Dutky *1963;* Langford et al. *1942*). It is of interest that the bacterium could pass from grub to pupa, remain viable during histolysis and histogenesis, and produce an acute condition in the adult beetle. The natural incidence of infection among adult beetles in the field is very low. Langford et al. (*1942*) estimated the incidence as 26 per 10,000, but Dutky (*1963*) did not find a single instance of natural infection among several thousand beetles collected in areas where infection among grubs was high.

A close relationship exists between the development of the bacterium in the blood and the ability of the host to metamorphose. It is possible for an infected grub to molt before the bacterium has completed its development. If at the time of pupation the bacterium has not reached its final stage, metamorphosis proceeds in an apparently normal manner. After the blood becomes filled with spores, the grub does not develop further. However, the grub does not always die upon completion of the bacterial cycle. Many grubs live for weeks, or even months, after their blood has become loaded with spores. It is not uncommon for grubs that become infected late in the fall to overwinter, but they usually die soon after becoming active in early spring. Not only do infected grubs remain alive, but they continue to feed. As death approaches they become less active, then moribund. When the circulation of the blood stops, the bacteria settle to the bottom of the body cavity of the grub.

Susceptibility of Various Grub Species to Infection.—As different species of white grubs were collected in the field during

surveys or were made available, tests were conducted to determine their susceptibility to infection by *B. popilliae*. Dutky (*1941*) described the technique of such testing. Dutky and Dobbins (unpublished) made many tests to determine the susceptibility of other species to *B. popilliae* grown in the blood of grubs of *P. japonica*. Tests also were made by Adams (*1949*), Beard (*1956*), Carter (*1945*), Dumbleton (*1945*), Dutky (*1941a, 1957, 1963*), Hurpin (*1955, 1957, 1959*), Montgomery (*1943*), Tashiro and White (*1954*), and White (*1947*). The results of these tests are as follows:

Naturally infected in field:

Amphimallon majalis (Razoumowsky)
Anomala orientalis Waterhouse
Cyclocephala borealis Arrow
Cyclocephala immaculata (Olivier)
Maladera castanea (Arrow)
Phyllophaga anxia LeC.
Phyllophaga fraterna Harris (probably)
Phyllophaga fusca (Froel.)
Phyllophaga futilis (LeC.)
Phyllophaga hirticula (Knoch)
Phyllophaga inversa Horn
Popillia japonica Newman
Strigodermella pygmaea (F.)

Infected by feeding in inoculated soil:

Amphimallon majalis (Razoumowsky)
Anomala orientalis Waterhouse
Aphonus castaneus (Melsh.)
Cyclocephala sp.
Macrodactylus subspinosus (F.)
Melolontha melolontha (L.)
Odontria zealandica White
Phyllophaga anxia LeC.
Phyllophaga congrua (LeC.)
Phyllophaga ephilida (Say)
Phyllophaga fraterna Harris (probably) [1]
Phyllophaga futilis (LeC.) (from Illinois)
Phyllophaga hirticula (Knoch) [1]
Phyllophaga rugosa (Melsh.) [1]
Popillia japonica Newman

Infected by direct injection:

Adoretus sinicus Burmeister
Amphimallon majalis (Razoumowsky)
Amphimallon solstitialis (L.)
Anomala innuba (F.)
Anomala lucicola (F.)
Anomala oblivia Horn (probably)
Anomala orientalis Waterhouse
Aphodius howitti Hope
Aphonus castaneus (Melsh.)
Brachysternus sp.
Cetonia aurata (L.)
Cyclocephala borealis Arrow
Dichelonyx sp. [1]
Diplotaxis sp.
Heteronychus sanctae-helenae Blanch.
Hylamorpha elegans (Burm.)
Macrodactylus subspinosus (F.)
Maladera castanea (Arrow)
Melolontha melolontha (L.)
Melolontha vulgaris (F.)
Odontria zealandica White
Oryctes nasicornis (L.)
Pelidnota punctata (L.)
Phyllophaga anxia LeC.
Phyllophaga bipartita (Horn)
Phyllophaga congrua (LeC.)
Phyllophaga crassissima (Blanch.)
Phyllophaga crenulata (Froel.)
Phyllophaga drakei (Kirby)
Phyllophaga ephilida (Say)
Phyllophaga forbesi Glasgow
Phyllophaga forsteri (Burm.) (possibly)
Phyllophaga fraterna Harris
Phyllophaga fusca (Froel.)
Phyllophaga futilis (LeC.)
Phyllophaga glaberrima (Blanch.) (probably)
Phyllophaga gracilis (Burm.)
Phyllophaga hirticula (Knoch)
Phyllophaga hornii (Smith) (probably)
Phyllophaga implicita (Horn)
Phyllophaga inversa (Horn)
Phyllophaga marginalis (Lee) (probably)
Phyllophaga micans (Knoch)
Phyllophaga quercus (Knoch)
Phyllophaga rugosa (Melsh.)
Phyllophaga tristis (F.) [1]
Phytalus georgianus Horn
Popillia japonica Newman
Sericesthis pruinosa (Dalman)
Strigoderma arboricola (F.)
Trichiotinus sp.

Not infected by feeding in inoculated soil:

Amphimallon solstitialis (L.)
Cetonia aurata (L.)
Lichnanthe vulpina (Hentz)
Maladera castanea (Arrow)
Oryctes nasicornis (L.)
Phyllophaga fusca (Froel.)
Phyllophaga futilis (LeC.) (from Wisconsin)
Phyllophaga hirticula (Knoch)
Phyllophaga inversa (Horn)
Phyllophaga rugosa (Melsh.)

Not infected by direct injection:

Cotinis nitida (L.)
Cyclocephala immaculata (Olivier)
Dermolepida albohirtum Waterhouse
Lichnanthe vulpina (Hentz)

[1] Infected with spores recovered from naturally infected grub of *Phyllophaga hirticula*. In all other cases spores grown in blood of *Popillia japonica* were used.

Relationship Between Spore Concentration and Grub Infection. —The incidence of infection among the grubs is a graded response to the concentration of the spores in the soil. Information on this relationship has been published by Beard (*1944, 1945*), Dutky (*1937, 1963*), and Tashiro and White (*1954*). A large amount of unpublished data on the response of third-instar *Popillia* grubs to known concentrations of spores in soil was accumulated at the Japanese Beetle Laboratory while evaluating the potency of various lots of spore dust and in bioassays of soil from field plots containing unknown numbers of spores (Dobbins unpublished; Dutky unpublished; Fleming unpublished). Although the dosage response in each series was characteristic, the level of infection attained in the different series was variable. It was modified by the size, maturation, and nutritional state of the grubs, the temperature, and the potency of the spores.

A typical response of the grubs at 86° F. in soil inoculated with standardized spore dust is given in table 7. A population of about 60 million spores per kilogram of soil was required in these tests to produce a low level of infection among the grubs. The incidence of infection increased progressively with the increment in the spore population and the prolongation of the exposure. About the maximum incidence of infection was obtained with a population of 2 billion spores per kilogram of soil. Further increments in the spore population produced only a slight increase in the incidence of infection. About two-thirds of the grubs at each dosage level that became infected showed symptoms of infection by the 15th day of exposure. Only a few additional grubs became infected after an exposure of 30 days. The grubs that did not become infected were not necessarily resistant to the pathogen. It is more probable that by chance they did not ingest enough spores to become infected. Beard (*1944*) demonstrated that grubs failing to become infected during one exposure did not have any diminished susceptibility when introduced again into inoculated soil.

Probable Physiological Effects of Bacteria on Grubs.—The effects of *B. popilliae* on the grubs appear to be physiological. Beard (*1945*) found no necrosis of any organ system or tissues, and prior to the moribund condition no difference in the activity of infected

TABLE 7.—*Incidence of infection among third-instar* Popillia *grubs in soil inoculated with spores of* Bacillus popilliae *at 86° F.*

Spores per kilogram of air-dry soil (millions)	Grubs infected after—					
	10 days	15 days	20 days	25 days	30 days	35 days
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
None	1	4	4	5	6	6
62.5	4	12	15	16	16	16
125	10	24	28	29	30	30
250	14	32	38	41	42	43
500	19	42	50	56	58	60
1,000	28	54	64	68	72	75
2,000	42	68	78	82	84	86
4,000	52	74	81	83	85	87

and healthy grubs was apparent. A mechanical block of the blood by the spores was not observed. This block appeared to be remote because of the open circulatory system. When he injected living or dead spores, suspended in blood or in distilled water, all the grubs died within 12 days, but when heat-fixed blood minus the spores was injected, the mortality was only 40 percent during this period.

Dutky (*1963*) found that cell-free filtrates of cultures of *B. popilliae* injected into the blood in small amounts were lethal. The toxic substance was apparently heat labile, because injections of filtrates heated to 122° F. for 10 minutes did not injure the grubs. He found that injection of 20 micrograms of dihydrostreptomycin into a grub within 48 hours after the introduction of spores into the blood interrupted the development of the bacterium so that the grub remained healthy, but when the antibiotic was administered 72 hours or more after introducing the spores, the development of the bacterium was not inhibited. Penicillin G, Aureomycin hydrochloride, and sulfadiazine sodium did not prevent the development of the bacterium. It would appear from these tests that the development of toxic products in the blood was one cause of the death of the grub. Another cause could be the locking up of the nutrients in the blood in the vegetative cells and spores of the pathogen.

Possible Antibiotic Action of Bacteria.—Beard (*1946*) reported a phenomenon suggesting antibiotic activity of *B. popilliae* and *B. lentimorbus*. When a mixture of spores of these pathogens was injected into the same grub, either *B. popilliae* or *B. lentimorbus* developed, not both of them. The relative spore dosage largely determined which organism was successful. In most cases *B. popilliae* developed, but when the spores of *B. lentimorbus* greatly exceeded the number of spores of *B. popilliae*, *B. lentimorbus* might develop. Time was also a factor. When spores of *B. lentimorbus* were injected into a grub and 2 days later spores of *B. popilliae* were injected into the same grub, the growth of only *B. lentimorbus* occurred, except when large dosages of *B. popilliae* were used. In the latter case the time advantage was overcome and only *B. popilliae* developed. When *B. popilliae* was given the time

advantage, only that organism developed. In direct competition *B. popilliae* seemed to be the more potent pathogen. This antibiotic activity might explain the mutually exclusive development of these milky disease bacteria in the grubs.

Vertical Distribution of Spores in Field.—The spores are not uniformly distributed in the soil in the field, but are present in spots of high concentration where they have been released from decomposed grubs that had died of the infection. The spores become tightly bound to the soil particles and tend to remain where they have been deposited. However, the movement of the grubs and other creatures through the soil tends to distribute the spores. Since the grubs feed close to the surface of the soil and most of the infected ones die there, more spores occur in the upper few inches than at lower depths. The vertical distribution of the spores is affected not only by the movement of these insects but by tillage.

Beard (*1945*) introduced grubs into soil taken from turf where *B. popilliae* was well established. Infection developed in 90 percent of the grubs introduced into the upper inch of soil, in 54 percent of those in the second inch, and in 46 percent of those in the third inch of soil. Ladd (unpublished) found that the spores in cultivated fields were fairly uniformly distributed throughout the upper 6 inches of soil, the normal depth of tillage.

Natural Dispersion.—Field and laboratory studies have shown that birds and insects are important agents in the natural dispersion of the pathogen. Chickens and starlings fed infected grubs voided viable spores in the droppings, with no effect on either species (White and Dutky *1940*). Ants were observed dragging dead diseased grubs for distances up to 10 feet. The parasitic wasps *Tiphia vernalis* and *T. popilliavora* while searching for grubs in the soil may become contaminated with spores and carry them to other sites (White *1943*). Skunks and moles are probably carriers of spores. The movement of soil by wind, water, or man must also greatly affect the spread of the pathogen.

Compatibility of Milky Disease and Tiphia Wasps.—*B. popilliae* and the *Tiphia* wasps are in general compatible parasites of the grubs (White *1943*). The wasps oviposited on infected grubs, but the parasitic larvae feeding on the tissues did not develop infection. Some of the progeny of *Tiphia* failed to complete their development on infected grubs. When the development of the bacterium was well advanced at the time of oviposition, the grubs sometimes died before the parasitic larvae matured. This was not an important factor with *T. vernalis*, which is actively ovipositing in May when the soil temperature is too low for the rapid development of the bacterium. It is a more important factor with *T. popilliavora*, a species on the wing in August when the soil temperature is above 70° F. and favorable for the development of the pathogen. In a survey in an area where both *B. popilliae* and *Tiphia* were well established, 38 percent of the grubs were infected by the bacterium and 48 percent of them were parasitized by the wasp. Included in these percentages is 15 percent of the grubs that were both infected and parasitized.

Preservation of Cultures.—The different strains of *B. popilliae* have been preserved as dried blood films for future use. Dutky (*1942*) found that the dried blood films of infected blood retained their virulence and ability to germinate indefinitely. Usually a culture on a glass slide contained 2 to 3 billion spores. Most of the vegetative forms were killed during the preparation of the slide or died soon after. After about 6 months, suspensions prepared from dried films were very stable; they did not darken and showed little or no loss in infectivity.

In the preparation of dried blood films, the treatment of the grubs before bleeding to prevent clotting is most helpful. Dutky (*1963*) inhibited clotting by immersing the grubs for 5 minutes in 95 percent ethyl alcohol. The treatment did not kill the grubs. Blood withdrawn from grubs immediately after treatment still clotted, but within 10 minutes after removal from the alcohol, the clotting power was lost. The clotting power was regained after 24 hours. Beard (*1945*) accomplished this by immersing the grubs in water at 135° F. This treatment killed the grubs. More recently Haynes et al. (*1961*) showed that cultures can be preserved for at least 20 months by lyophilization.

Pathogen Propagation and Preparation for Distribution.—Many bacterial pathogens of man, animals, and plants can be grown in large numbers on artificial media, but *B. popilliae* grows poorly or not at all on most bacterial media. Since 1939 many studies have been conducted by Federal, State, and private laboratories to develop a practical method for propagating the bacterium on artificial media so that large numbers of spores could be produced for colonization of the organism (Hawley *1952*), but by 1964 the investigation had not been successful.

Dutky (*1947*) summarized the preliminary studies on the growth requirements of the bacterium. Later he (*1963*) discussed the effect of pH and the oxygen, carbohydrate, nitrogen, and mineral requirements of the organism for growth on artificial media. On media that supported a heavy growth of the vegetative stage, the bacterium failed to sporulate and the vegetative stage was short lived. On restrictive media, in which the organism grew poorly to a limited extent, the vegetative form was long lived. Steinkraus (*1957, 1957a*), Steinkraus and Provvidenti (*1958*), and Steinkraus and Tashiro (*1955*) produced some spores on solid medium by transferring the vegetative cells grown on a complete medium to a starvation medium or by raising the temperature of the medium to 113° F.

The virulence of the artificially produced spores was essentially the same as that of the natural spores when tested by injection into grubs, but it was only about one-fifth that of the natural spores in feeding tests with grubs in inoculated soil. However, this method is not practical for large-scale production of spores.

In 1959 the Northern Utilization Research and Development Division, U.S. Department of Agriculture, undertook studies to develop a practical method for producing the spores on artificial media. Phases of this study were conducted at Michigan State University and the University of Illinois as well as at the Division's

Fermentation Laboratory. At a conference in 1962 to discuss the progress of this investigation, it was evident that although much progress had been made on the propagation of the pathogen on artificial media, the large-scale production of spores had not been achieved.

In the absence of a practical method for the propagation of *B. popilliae* on artificial media, a process was devised in 1939 for producing the spores in the blood of the grubs and incorporating them in a dust for storage and distribution in the field. This process has been outlined in detail by Dutky (*1942a*). The essential features of this method were patented (Dutky *1941b, 1942*) and the patent was assigned to the Secretary of Agriculture.

In the procedure, the spores from dried blood films on stock slides are suspended in sterile distilled water and the number is adjusted to approximately 300 million per 1 ml. A hypodermic syringe filled with the standardized suspension is placed in a microinjector, a patented device developed by Dutky and Fest (*1942*). The microinjector is adjusted to deliver 0.033 ml. of the suspension. This volume of the suspension containing approximately 1 million spores is injected into each full-grown grub in the dorsal part of the suture between the second and third posterior abdominal segments. Care is taken not to puncture the gut.

The injected grubs are placed in moist soil containing one-half pound of grass seed in each 100 pounds of soil and incubated at 86° F. for 10 to 20 days until most of the grubs have developed macroscopic symptoms of infection. The dead and uninfected grubs are discarded. The living infected grubs are placed in jars of ice water and stored at 35° until enough of them have been accumulated for processing. The living infected grubs are then removed from the ice water and crushed in a meat chopper. A little water is used to wash out the chopper. The density of the spores per milliliter is determined. This standardized suspension is then added to U.S.P. precipitated calcium carbonate so that the mixture contains 1 billion spores per gram of dry material. After thorough mixing and drying, this concentrate is mixed with a sufficient amount of talc so that the final mixture contains 100 million spores per gram. If there is no loss in the preparation, 23 grubs each containing 2 billion spores would produce 1 pound of spore dust.

The final product is assayed to determine its potency. Twenty grams of the product containing 2 billion spores are mixed with each kilogram of soil and healthy third-instar grubs are introduced and incubated at 86° F. The development of infection with the test sample is compared with that produced by a standardized spore dust. During 1939–53 over 180,000 pounds of standardized spore dust were produced at the Japanese Beetle Laboratory (Fleming *1958a*). In addition, several thousand pounds of the dust have been manufactured for sale by licensees under the assigned patents. The spore dust has been held in storage for over 10 years without any noticeable deterioration (Hawley *1952*).

The preparation of spore dust in pellet form for distribution by autogiro and airplane was first investigated in 1941 (Dutky unpublished). Sufficient glycerin was added to the dust to make a

plastic mass, which was agitated in a rotating container until pellets formed. Stable pellets were produced by this method with 55 ml. of glycerin to each 200 grams of dust. Biological tests showed that the process did not impair the infectivity. However, the distribution of the material by autogiro or airplane at that time was impractical (Hadley unpublished).

Dobbins (unpublished) tested eight formulations of tablets prepared by a commercial company in 1945. Each tablet contained 200 million spores, an oil, and a dispersing agent, and in three formulations a binder was added. Biological tests indicated that the potency of the spores was not impaired in the tablets without the binder, but it was reduced by the addition of the binder. All these tablets disintegrated too slowly in the field to be satisfactory, and during exposure some of them lost up to 75 percent of their initial potency.

Beard (*1946a*) described a method for making soluble tablets containing spores. Macerated infected grubs were mixed with a 2:3 mixture of dextrose and sodium bicarbonate and standardized so that each pressed tablet contained 200 million spores. These tablets normally disappeared within 2 or 3 days on the ground, even when there was no rain. Tests showed that the potency of the spores in the tablets was about the same as in the regular spore dust.

In 1959 the Methods Improvement Laboratory, Gulfport, Miss., of the U.S. Plant Pest Control Division (unpublished) prepared a granular formulation of spores by tumbling 40 pounds of 16–30 mesh attapulgite clay with 5 pounds of spore dust assaying 1 billion spores per gram. After the ingredients were thoroughly mixed, the mixture was sprayed with 5 pounds of 50-percent aqueous glycerin as a sticker, deactivator, and humectant. The final product contained approximately 100 million spores per gram. Biological tests (Fleming unpublished) showed that there was considerable variation in the potency of the different batches, probably because the process at that time was not well standardized. However, most of the batches had a potency equivalent to that of the regular spore dust.

Development of Colonization Method.—White and Dutky (*1940*) demonstrated that spore dust, spore suspensions, inoculated grubs, or infectious soil could be used to establish infection among grubs developing naturally in the field in areas where the pathogen did not occur naturally. The application of spore dust was the most practical of these methods. Establishment of the organism resulted from the application of 25 million to 1.5 billion spores per square foot when applied continuously or in spots several feet apart.

Beard (*1945*) applied spores at the rates of 1.25, 2.5, and 5 billion per square foot to infested turf, containing 3, 18, and 48 grubs per square foot. Eight weeks later no infection had developed in the light population of grubs. The incidence of infection in the intermediate grub population ranged from 11 percent with the lowest dosage to 15 percent with the highest dosage, and in the high grub population from 28 to 37 percent, respectively.

Adams and Wheeler (*1946*) applied spore dust over turf in April at rates ranging from 10 million to 2 billion spores per square foot. Practically no infection developed among the brood of grubs before pupation, but infection began to appear among the new brood of grubs that hatched during the summer. By the following June the incidence of infection among a grub population of 13 per square foot ranged from 0.3 percent with the lowest dosage to 25 percent with the highest dosage, and among a grub population of 31 per square foot from 21 percent to 67 percent, respectively. The highest dosage gave adequate control of the grubs 18 months after application, but about 4 years elapsed before the lower dosages reached that level of effectiveness.

White (*1948*) applied 25 million spores per square foot over turf in October. The grub population of 11 per square foot was reduced to 27 per square foot by the following June, when the incidence of infection among the grubs was 8 percent. The next brood of grubs, averaging 22 per square foot in August, was reduced to 2 per square foot by the following June, and during that period the incidence of infection among the grubs increased from 11 to 70 percent. Infection developed more rapidly among the grubs in the tests by White because the plots were in Washington, D.C., where the soil was warmer and the growing season longer than in the tests by Adams and Wheeler in New York State.

White (*1940a*) demonstrated that *B. popilliae* could survive under adverse conditions in the field, and when the situation improved, it could become a factor in the control of the grubs. Turf at West Chester, Pa., was inoculated in October 1935, when the grub population averaged 27 per square foot. The low temperature during the winter of 1935–36 killed 81 percent of the grubs and the beetle did not become abundant again at that site until 1938. In September of that year the grub population in the inoculated plots averaged 39.5 per square foot and the incidence of infection 6 percent. By the following June the grub population had declined to 11 per square foot and the incidence of infection had increased to 13.9 percent. In addition, the bacterium had spread in all directions from the plots; infected grubs were found at a distance of 1,500 feet.

During 1938 and 1939 White and Dutky (*1940*) inoculated field plots in the region extending from Cape Charles, Va., northward to Staten Island, N.Y., and westward to Reading, Pa., where substantial grub populations occurred, to study the effectiveness of the pathogen under a wide variety of conditions. White (unpublished) established many additional plots during 1940 and 1941 in Connecticut, Delaware, District of Columbia, Maryland, New Jersey, New York, Pennsylvania, and Virginia. *B. popilliae* became established at practically all these sites, showing the adaptability of the organism to different types of soil and other environmental conditions. At most sites 2 or 3 years elapsed before the pathogen had built up to a level to control the grubs, but at Cape Charles the grubs were controlled the first year. The grub population there was 121 per square foot in July 1939 when the spores were applied. By September there were only six healthy grubs per square foot.

The various experiments showed that the density of the grub population was often a more important factor in the establishment and buildup of the pathogen to an effective level than was the dosage of spores applied. Regardless of the dosage applied, the pathogen soon became established under favorable conditions when the density of the grub population was 30 or more per square foot, but the establishment and buildup were slow when there were only one or two grubs per square foot. With a moderate to heavy population of grubs, control of the grubs was usually attained within 2 or 3 years by applying 2 grams of spore dust (200 million spores) per square foot. By raising the dosage to 20 grams per square foot, the time required to bring about satisfactory control was usually reduced to 1 or 2 years.

White and Dutky (*1942*) determined that the most practical method for colonizing the pathogen was to apply 2 grams of spore dust at intervals of 10 feet over established turf, using a hand corn planter of the rotary type so modified that the dosage was not deposited in a heap but was released over a larger area. The application of spore dust in this manner requires 1¾ pounds per acre.

Natural Establishment of Pathogen.—In the meanwhile the pathogen was becoming established naturally at many sites throughout the area infested by the beetle (White *1941*). The manner in which the soil became inoculated at those sites is not known. The development of the pathogen naturally at Springfield, N.J., Washington, D.C., and Perryville, Md., is typical of the situation at other sites.

There was no indication that *B. popilliae* was at Springfield in the fall of 1937. No infection was found among the several thousand grubs dug there at that time. Early in May 1939 about 0.2 percent of the grubs were infected. The population, which averaged 36 per square foot at that time, declined to 5 per square foot by July and the incidence of infection increased to 18 percent. The new brood averaged 40 per square foot in September. That population declined to 5.4 per square foot by the following June and the incidence of infection increased to 60 percent. Thus, the pathogen infected approximately 95 percent of that brood of grubs.

The presence of the pathogen in the District of Columbia was discovered in 1936, when one infected grub was found in the Benning Road section. A survey in 1940 showed that the distribution of the pathogen in the District was limited, and with the exception of one site the incidence of infection among the grubs was very low. It was evident that the organism was becoming slowly established naturally in that area.

White and McCabe (*1950*) studied the colony of the pathogen on the grounds of the Perry Point Veteran's Administration Hospital at Perryville, Md., over a 10-year period. In August 1939 the grub population averaged 37 per square foot and the incidence of infection was 1 percent. By the following June there were only 6.3 grubs per square foot and the incidence of infection had increased to 67 percent. This pattern of decline in the grub population and of increase in the incidence of infection was repeated with modifications with each of the following broods of grubs. In spite

of the movement of beetles each summer from the surrounding area into the grounds of the hospital, there was a trend for each successive brood of grubs to be less numerous than the previous one. It was estimated that the pathogen reduced the potential emergence of the broods by 86 to 91 percent.

Colonization of Pathogen.—The Japanese Beetle Laboratory in cooperation with various State and Federal agencies undertook in 1939 an extensive program of colonization to accelerate the spread and the buildup of *B. popilliae* throughout the area infested by the beetle. The spore dust was applied to pastures, lawns, golf courses, cemeteries, parks, and other areas of established turf. It was usually applied by a modified hand corn planter in spots at 10-foot intervals. About 2 grams of dust were deposited in each spot, about 1 square foot in area. Generally on Government reservations the dust was applied at 10-foot intervals in lines 20 feet apart. Usually two half-acre plots were established in each square mile of open country and one half-acre plot for each 10 acres in a golf course, park, or cemetery. In city areas 1 block in each 10 was treated, and 10 spots per property in approximately 20 properties per block (White and McCabe *1943*).

The Japanese Beetle Laboratory reported regularly on the progress of the colonization program (Hawley *1952*; White and Dutky *1942*; White and McCabe *1943, 1946, 1951*). Easter (*1947*) reported on the application of spore dust at 18 posts of the Second Army in Pennsylvania, Maryland, Virginia, and the District of Columbia. In addition, most of the cooperating State agencies reported on their progress in the program. The States were Connecticut (Garman et al. *1941, 1942, 1943*; Schread *1944, 1945, 1946, 1947*), Delaware (Chada et al. *1942*; Rice *1943*; Stearns et al. *1941*), Maryland (Cory *1940*; Cory and Langford *1944, 1950, 1955*), New York (Adams and Matthysse *1949*; Buchholz *1942, 1943*; Smith *1941*; Smith and Daniel *1942*; Wheeler *1943, 1943a*; Wheeler and Adams *1945*), Ohio (Polivka *1950, 1956, 1960*), Pennsylvania (Light *1943*), Rhode Island (Eddy *1943*), and Virginia (French *1941*; French et al. *1949*).

Most of the spore dust used in the colonization program was processed at the Japanese Beetle Laboratory. During 1939–53, 180,000 pounds of standardized dust were produced there. Several State agencies forwarded infected grubs for processing into standardized spore dust, other State agencies furnished grubs for injection and processing, and some State agencies were unable to supply any grubs. All the spore dust produced from the grubs supplied by the States was returned to them for distribution. The Laboratory produced over 43,000 pounds of dust for use by Federal agencies and to augment the dust available to the States. In addition, some State and Federal agencies obtained spore dust from commercial laboratories, which were licensed to produce the material under the assigned patents.

The cooperative colonization program was carried on as vigorously as possible. By 1942 over 64,000 pounds of spore dust had been applied to 33,500 acres at over 45,000 sites in 12 States and the District of Columbia (White and McCabe *1943*). Three years

later 113,500 pounds had been applied to 58,900 acres at over 73,000 sites in these States and the District of Columbia (White and McCabe *1946*). By 1949 over 147,700 pounds of spore dust had been applied to over 82,000 acres at 108,000 sites in 14 States and the District of Columbia (White and McCabe *1951*). By 1951 over 178,000 pounds of dust had been applied to over 101,000 acres at over 132,000 sites in these States and the District of Columbia (Hadley unpublished).

Further details on the colonization program are given in table 8. The program was disrupted in 1952, when the pathology laboratory was transferred from Moorestown, N.J., to Beltsville, Md. In 1953 the project was discontinued, even though the colonization program had not been completed. The data for 1952 and 1953 are incomplete because full reports were not received for those years from the cooperating State and Federal agencies. Fleming (*1961*) estimated that when the program was discontinued in 1953, almost 229,000 pounds of spore dust had been applied in the cooperative Federal-State program at 160,000 sites, and, in addition, 15,500 pounds had been used in treating properties owned or maintained by the Federal Government. Furthermore, private individuals had applied spore dust produced by commercial laboratories to many additional acres.

Status of Pathogen in Colonized Area.—When the project was terminated in 1953, the study of the status of *B. popilliae* in the various areas where it had been colonized was discontinued. The information then available indicated that the pathogen had become established in many of the areas and was becoming of increasing importance in the control of the beetle. However, within 5 years localized sporadic upsurgences of the beetle were reported in areas

TABLE 8. *Summary of milky disease colonization program, 1939–51*

State	Federal-State program			Government-owned or maintained areas		
	Sites	Acres	Dust	Sites	Acres	Dust
	Number	*Number*	*Pounds*	*Number*	*Number*	*Pounds*
Connecticut	2,993	2,182	4,080	3	192	807
Delaware	1,542	2,271	4,918	2	1,200	2,154
District of Columbia	14	46	84	80	2,243	2,828
Maryland	110,110	71,610	126,011	27	2,117	2,482
Massachusetts	23	216	661			
New Hampshire	7	3	43			
New Jersey	453	330	620	12	548	518
New York	5,527	4,776	10,361	23	2,960	3,839
North Carolina	14	549	1,715			
Ohio	550	1,900	1,981			
Pennsylvania	5,130	3,235	6,195	8	687	951
Rhode Island	136	153	393			
Vermont	2	4	28			
Virginia	1,903	2,055	4,356	23	1,132	1,753
West Virginia	708	719	1,339			
Total	132,112	90,049	162,795	187	11,169	15,332

where the pathogen was apparently well established and the beetle had not been troublesome for several years. It was possible to investigate some of these situations.

The upsurgence was particularly common in new large housing developments where there had been regrading and the upper infectious layer of soil had been removed or buried. The beetles were often numerous enough to damage ornamental trees and shrubs and injury to turf was common. Very little infection developed among grubs introduced into soil from those sites, indicating that at the time of the surveys the pathogen was of little importance in the control of the grubs. There was a possibility that *B. popilliae* would gradually become reestablished naturally from the surrounding undisturbed areas, but the application of spore dust to the turf would accelerate the reestablishment (Fleming unpublished).

During the summer of 1959 the beetle caused more damage to the ornamental plantings at the Naval Ordnance Laboratory at White-oak, Md., than it had done during any of the five previous summers. A survey in the fall showed that there were practically no grubs in the well-kept lawns, but a grub population up to 37 per square foot was found in the section of the golf course that had been previously a vegetable garden and in the turf of the new adjacent housing development. With the exception noted, *B. popilliae* was well established in the turf in the reservation. It was evident that the injury to the ornamental plantings had been caused largely by beetles migrating into the reservation from the housing development. A similar situation prevailed that year on the grounds of the Perry Point Veteran's Administration hospital at Perryville, Md. (Fleming unpublished)

Other situations where an upsurgence of the beetle occurred were more complex. The turf on three golf courses near Poughkeepsie, N.Y., had been inoculated with spores about 1945. During 1949–59 there had been no serious injury to the turf by the grubs, but in the fall of 1959 the grub population on these courses averaged 40 to 50 per square foot and damage to the turf was extensive and severe. An insecticide was applied to all the fairways and greens of one golf course, but on the two other courses the application of an insecticide was limited to the severely damaged areas. The question was raised whether the grubs had become immune to infection by *B. popilliae*. An assay of composite samples of soil from the two golf courses showed that the soil was highly infectious to grubs collected on the grounds, indicating that immunity was not a factor. Additional surveys showed that the lateral distribution of the organism was very heterogeneous.

An examination of the climatological data revealed that in contrast to the summer of 1959 the summers of the previous 10 years had been hot and dry, a situation that would tend to keep the grub populations at a low level. The summer of 1959 with abundant rainfall was very favorable for the development of the grubs. It was expected that the dense population of the 1959–60 brood would produce a more uniform dispersion of the spores. In August 1960 the new brood of grubs averaged 17 per square foot on one course

and 39 on the other, and the incidence of infection among them was low, but by June 1961 these populations had been reduced almost 90 percent and the incidence of infection at that time was in excess of 50 percent. It was evident that adequate dispersion of the spores had been attained. Since then *B. popilliae* has been effective in controlling the grubs on these courses. (Fleming unpublished)

A different situation was responsible for the upsurgence of the beetle at Waverly, N.Y., where the spores had been colonized in 1945 and no serious injury to turf occurred until 1959, a year favorable for the development of the grubs. When *Popillia* grubs were introduced into the soil from the site, none of them became infected, yet infection developed readily among *Amphimallon* grubs. When both species were introduced into soil inoculated with standardized spore dust, the infection of both species progressed at approximately the same rate. During the previous 14 years when *Popillia* populations had been very low, *B. popilliae* appeared to have lost temporarily its virulence to *Popillia* grubs and had developed a preference for *Amphimallon* grubs. (Fleming and Tashiro unpublished)

During 1960–63, assays were made of the soil at almost 100 sites to obtain more information on the status of *B. popilliae* in areas where the pathogen had been colonized about 20 years previously and in areas where the organism had not been colonized. Thirty assays were made of the soil from inoculated golf courses and pastures in Delaware, Maryland, New Jersey, New York, Pennsylvania, Virginia, and the District of Columbia. A more intensive study was made in New Jersey. Assays were made of inoculated turf at 17 sites in Atlantic, Camden, Gloucester, Hunterdon, Mercer, Middlesex, Monmouth, Ocean, Salem, and Warren Counties, and at 18 sites in Burlington County. In addition, assays were made of the soil in 19 uninoculated pastures and in 11 uninoculated cultivated fields within 1 or 2 miles of a colonization site.

The infectivity of the various soils, expressed as millions of spores per kilogram of soil, is summarized in table 9. About the same situation prevailed throughout the area assayed. Infection developed among grubs introduced into soil from all the inoculated sites, but the infectivity of the different soils varied greatly. It was estimated that the spore population in the upper 3 inches of the inoculated soils ranged from 1 million to over 1.6 billion per kilogram. The pathogen appeared to be well established at about one-third of the inoculated sites, as indicated by a population of 100 million spores per kilogram of soil. The pathogen had developed naturally in about one-fifth of the uninoculated pastures within 1 or 2 miles of an inoculated site to a level where adequate control of the grubs could be expected. It had become established naturally in 82 percent of the cultivated fields assayed, but the populations of spores were low. The pathogen probably would have been even better established if low populations of *Popillia* had not prevailed for several years. (Fleming and Ladd unpublished)

TABLE 9.—*Status of* Bacillus popilliae *about 20 years after colonization, 1960–63*

Colonization and area	Sites assayed	Sites with spore population per kilogram of soil of—							
		1 million	25 million	50 million	100 million	200 million	400 million	800 million	1,600 million
TURF COLONIZED	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
6 States and D.C.	30	100	53	40	30	20	17	10	3
11 N.J. counties	17	100	59	47	23	-----	12	6	-----
Burlington County, N.J.	18	100	67	44	33	28	22	-----	6
TURF NOT COLONIZED									
New Jersey	19	100	37	26	21	-----	-----	5	-----
CULTIVATED LAND NOT COLONIZED									
New Jersey	11	82	-----	-----	-----	-----	-----	-----	-----

Control of Isolated Beetle Colonies With Pathogen.—Since applying insecticides over a large area to control isolated beetle infestations has caused concern to the general public, wildlife enthusiasts, public-health officials, and others, the U.S. Plant Pest Control Division explored the possibility of applying a spore formulation as a substitute for insecticides under these conditions. A granulated formulation was applied in 1959 and 1960 at 50 and 100 million spores per square foot to established turf at Dahlonega, Ga., Wilmington, N.C., and Glenmor and Toledo, Ohio. Assays of the soil prior to treatment showed that the pathogen was not present at Dahlonega, but it was becoming established naturally at the other sites.

The results of the assays made periodically after the application of the spores were confounded to some extent by the presence of other pathogens that killed the grubs more rapidly than did *B. popilliae* and by insecticide residues in the soil at Wilmington, Glenmor, and Toledo. The dosage of spores applied did not seem to be the important factor. The application of the spores accelerated the buildup of the pathogen at Dahlonega, Glenmor, and Toledo, but it did not do so at Wilmington. Only at Glenmor did the pathogen increase sufficiently within 2 years to reduce the grub population to a low level.

In these tests the populations of the pathogen did not increase quickly enough to retard the spread of an isolated colony of beetles. However, even if the pathogen had been more effective, the application of spores at the rate of 100 million per square foot is not feasible at this time, because the spores have not been produced cheaply and in abundance on artificial media and the formulations

now available with spores produced in the blood of grubs are in short supply and too expensive to be applied at such a rate over a large area. (Fleming unpublished)

Use of Pathogen on Lawns.—There was much public enthusiasm in the use of spore dust to control the grubs in lawns on private premises, but misinformation about the pathogen was widely spread. To provide valid information for the public, Hadley (*1948*) and Fleming (*1961*) discussed the nature of the bacterium, the application of spore dust, and the control of the grubs that could be expected. It was pointed out that several factors were involved in the establishment and buildup of the organism and that several years might elapse before adequate control of the grubs was attained. Both these authors and Adams and Wheeler (*1946*) recommended that when grubs were very abundant and it was imperative to prevent further damage to turf, a quick acting insecticide such as dieldrin or chlordane should be applied to kill the grubs.

Bacillus lentimorbus Dutky

The vegetative stage of *Bacillus lentimorbus* is similar in appearance to that of *B. popilliae*, but the organisms are readily distinguished morphologically after sporulation. The refractile body so prominent in spores of *B. popilliae* is lacking and the spores are more nearly spindle shaped. The biology of both organisms is very similar, but *B. lentimorbus* has a more limited range of temperature for its development. The lower limit seems to be about 60° and the upper limit about 86° F. The spores of *B. lentimorbus* are also resistant to adverse conditions. (Dutky *1940*)

Infection of the Japanese beetle by *B. lentimorbus* is largely restricted to first- and second-instar grubs. Since the grubs usually molt before their development is arrested, a high incidence of infection may appear among third-instar grubs in the fall. At times 85 percent of the third instars were infected. However, the overall effect of this pathogen on the grub population is no greater than that of *B. popilliae*, which may infect only 15 to 30 percent of the grubs at that time. Since grubs infected by *B. popilliae* usually die before molting, the population of third instars infected by that pathogen had already been reduced by previous mortality among first- and second-instar grubs. In the spring the third-instar grubs are very resistant to infection by *B. lentimorbus*, and despite the large number of spores ingested, few grubs become infected. On the other hand, since third-instar grubs are highly susceptible to infection by *B. popilliae* in the spring, the disease caused by that organism may develop explosively and many grubs will show external symptoms of infection at the same time. (Dutky *1963*)

Grubs infected by *B. lentimorbus* in the late summer and fall have the same milky white appearance as those infected by *B. popilliae*, but appearance of the overwintering grubs in the spring is distinctly different. Grubs infected by *B. lentimorbus* collected in March were milky white, but when held at room temperature they darkened rapidly until 2 or 3 weeks later they became a choco-

late brown. Grubs infected by this organism usually were that color in the field during April and May. Microscopic examination of the blood showed that the darkening of the infected grubs was due to extensive formation of blood clots, which were brown or jet black. Although the chocolate-brown grubs were alive and active, the accumulation of these clots in the appendages blocked the normal circulation of the blood and produced a gangrenous condition that caused the affected parts to blacken. The death of the grubs is probably the result of gangrene. When healthy grubs were injected with the blood of chocolate-brown grubs and infection developed, the milky white condition developed rather than the brown color of the grubs used as inocula. (Dutky *1940*)

The susceptibility of other species of white grubs to infection by *B. lentimorbus* has been tested to a limited extent. Dutky and Dobbins (unpublished) infected *Anomala orientalis* Waterh., *Maladera castanea* (Arrow), and *Cyclocephala borealis* Arrow by injecting spores into the blood, and Beard (*1956*) infected *Sericesthis pruinosa* (Dalman) in that manner. Beard (*1956*) found that *Aphodius howitti* Hope and *Heteronychus sanctae-helenae* (Blanch.) were not affected by injection of spores. Montgomery (*1943*) had the same result with *Dermolepida albohirtum* Waterh. Tashiro and White (*1954*) found that *B. lentimorbus* had a low pathogenicity to *Amphimallon majalis* (Raz.) when spores were introduced by injection or ingestion. However, there are several strains of *B. lentimorbus*. The *australis* strain injected into the blood infected *Anomala orientalis*, *Aphodius howitti*, *Heteronychus sanctae-helenae*, *Popillia japonica*, and *Sericesthis pruinosa*, but not *Maladera castanea* (Beard *1956*). The *Amphimallon* strain had about the same infectivity to *Amphimallon majalis* by injection and ingestion as did the regular strain of *B. popilliae* (Tashiro and White *1954*).

Dust containing 100 million spores of *B. lentimorbus* per gram was prepared in the same manner as the dust containing spores of *B. popilliae* (Dutky *1941b*, *1942*, *1942a*). During 1939–51 over 7,000 pounds of this dust were produced at the Japanese Beetle Laboratory. Most of the spore dust was used in the cooperative colonization program in Maryland, but some of it was used to establish experimental colonies in New Jersey, New York, Pennsylvania, Delaware, Virginia, Connecticut, and Massachusetts. The pathogen became established at most of the sites where it was colonized, and it became dispersed over wide areas by natural agencies. It was not uncommon to find *B. lentimorbus* associated with *B. popilliae* in the field. Usually infection by *B. lentimorbus* was dominant among the grubs in the fall and early spring and infection by *B. popilliae* was dominant in the late spring (White and Dutky unpublished).

Bacillus euloomarahae Beard

When spores of *Bacillus euloomarahae* were injected into the blood, infection developed among grubs of *Anomala orientalis*, *Heteronychus sanctae-helenae*, *Popillia japonica*, *Sericesthis pruinosa*, and *Maladera castanea* (Beard *1956*). *Amphimallon majalis*

also became infected by injection of spores (Tashiro unpublished). However, when grubs of *P. japonica* and *A. majalis* were introduced into soil inoculated with the spores, none of them became infected (Tashiro unpublished).

Bacillus fribourgensis Wille

Spores of *Bacillus fribourgensis* injected into the blood of grubs of *Popillia japonica* and *Amphimallon majalis* had a low pathogenicity to them. When these grubs were introduced into soil inoculated with the spores, no infection developed among them (Tashiro unpublished).

Other Bacterial Diseases

The injection of a culture of *Bacillus alvei* Ches. and Chey., a bacterial infection associated with European foulbrood of the honey bee, killed *Popillia* grubs within a few days. However, when the culture of 11 strains of the organism was diluted before injection, the development of infection among the grubs was not consistent. It appeared that the lethal effect produced by the injection of the undiluted culture might be due to toxic materials developed in the culture medium during the growth of the organism rather than to the ability of the organism to multiply in the blood of the grubs. (Dutky unpublished).

Dutky (*1937*) isolated a red pigment-forming bacterium, similar in morphology and pigmentation to *Serratia marcescens* Bizio, from infected grubs in 1934. The injection of 275 to 2.8 million cells of this organism into the blood of grubs produced clots and killed the grubs within 3 days. The organism showed no reduced virulence after continuous culture on artificial media for more than 2 years. Grubs did not develop infection when introduced into soil inoculated with suspensions of the organism, although *S. marcescens* was causing a high mortality among grubs in storage for the winter. Grubs infected by this pathogen have been found from time to time in the field.

Rickettsia Disease

A fatal infection of the grubs caused by *Rickettsiella popilliae* (Dutky and Gooden) Philip was discovered at Nottingham, Pa., in 1940 (Dutky and Gooden *1952*). It has since been encountered in grubs from widely separated areas. At some localities large numbers of infected grubs were found repeatedly. Most of the recoveries were made from *Popillia* grubs, but the organism was found attacking grubs of *Phyllophaga anxia* LeC. and *P. ephilida* (Say). Possibly other species of Scarabaeidae may be susceptible to infection by this rickettsia. Affected grubs have a greenish-blue discoloration of the fat bodies, which led to the designation of blue disease.

Popillia grubs were infected by injecting them with suspensions of infected blood or with filtrates of these suspensions and also by introducing them into soil inoculated with the suspensions. The

attempts to isolate and culture this rickettsia on artificial media were not successful, but the organism was maintained by serial inoculation of grubs. Preliminary studies indicated that 86° F. is about the upper limit for its development. The threshold temperature has not been determined. Attempts to colonize the organism in the field were not successful.

SUMMARY

The Japanese beetle (*Popillia japonica* Newman) was discovered in southern New Jersey in 1916. Prior to that time it was known to occur only on the main islands of Japan, where it is not considered to be an important economic pest, probably because its natural enemies keep it under control. In New Jersey it found a generally favorable climate, large areas of turf for the development of the immature stages, almost 300 species of plants to satisfy its voracious appetite, and no important natural enemies.

The insect-feeding birds, small terrestrial mammals, and the few predaceous and parasitic insects indigenous to the Eastern United States were not able to cope with the twentyfold to thirtyfold reproductive potential of the beetle. It spread rapidly in its new environment and soon became a threat to American agriculture. The adults seriously damaged orchard crops, certain field crops, and ornamental trees and shrubs. The grubs destroyed large areas of turf and damaged the roots of some cultivated crops.

Foreign Predaceous and Parasitic Insects.—A search for effective predaceous and parasitic insects during 1920–33 in Japan, Korea, China, India, Formosa, and Australia disclosed that (1) adult beetles of the genus *Popillia* and related Scarabaeidae were parasitized by 7 species of Tachinidae and 1 species of Pyrgotidae; (2) the grubs of these species were parasitized by 2 species of Tachinidae, 2 species of Scoliidae, and 52 species of Tiphiidae; and (3) both stages were attacked by 1 of the Carabidae. The life history and habits of the more important parasites and a predator in the Far East were studied to evaluate their potential effectiveness in controlling the beetle in the United States.

The predator and the parasites that appeared most promising for control of the beetle were collected in their native habitats in the Far East, or reared there, and shipped to the United States. Methods had to be developed for rearing them and containers had to be devised for shipping them. The dipterous parasites with a long pupal period were sent in the pupal stage, but those with a short pupal period were forwarded as larvae within their living host. The hymenopterous parasites were shipped in the cocoon and adult stages. The coleopterous predator was sent in the adult stage. A total of 1,771,340 predators and parasites were shipped, including 1,185,963 Tachinidae, 483,593 Tiphiidae, 62,972 Scoliidae, 21,462 Pyrgotidae, 16,450 Carabidae, and 900 Ithonidae. Of these insects, 1,610,847 were shipped for control of *Popillia japonica*, 154,258 for control of *Maladera castanea* (Arrow), and 6,235 for control of *Anomala orientalis* Waterhouse. Since all the Tachinidae, Pyrgotidae, and some of the Scoliidae and Tiphiidae

were forwarded in their immature stages, methods had to be developed for holding them until the adults emerged and conditions were suitable for their liberation in the field.

The establishment of a foreign predator or parasite in a new environment is a slow tedious process extending over several years. Most species were not able to adapt themselves to their new environment. However, five parasitic species were known to have been established. *Hyperecteina aldrichi* Mesnil was colonized at 55 sites in 12 States and the District of Columbia. It was found at 59 percent of the sites surveyed in 1937 and at 43 percent of them in 1950. *Dexilla ventralis* Aldrich was released at 17 sites in six States and *Prosena siberita* (Fabricius) at 5 sites in two States, but each of these parasites was recovered at only 1 site. The Japanese strain of *Tiphia popilliavora* Rohwer was colonized at 716 sites in eight States and the Korean strain at 51 sites in eight States; the former was found at 51 percent of the sites surveyed in 1937 and at 47 percent of them in 1950 and the latter was recovered at 36 percent of the sites surveyed in 1950. *Tiphia vernalis* Rohwer, the most effective of these parasites, was colonized at 2,027 sites in 14 States and the District of Columbia. A survey of 351 sites in 1937 and 423 sites in 1950 showed that this parasite was well established at 64 and 63 percent of them, respectively.

The survival of these foreign parasitic insects in the United States is dependent to a large extent on having a suitable supply of food for the adult flies and wasps and an adequate population of the beetle. The adults of *Hyperecteina aldrichi, Dexilla ventralis,* and *Tiphia vernalis* feed on honeydew and nectar, whereas adults of *T. popilliavora* feed at the blossoms of wild carrot and *Prosena siberita* adults at various blossoms. *H. aldrichi, T. popilliavora,* and *T. vernalis* parasitize only *Popillia japonica* in this country; *D. ventralis* and *P. siberita* having a variety of hosts in the Far East might parasitize some other species of Scarabaeidae here.

Entomogenous Diseases.—The most important diseases of the Japanese beetle in the United States are the infections of the grubs caused by the parasitic nematode *Neoaplectana glaseri* Steiner and by the milky disease bacteria *Bacillus popilliae* Dutky and *B. lentimorbus* Dutky.

Grubs infected by the nematode were found in the spring of 1929 at Haddonfield, N.J. Although a search for the nematode was made in other parts of New Jersey and in Pennsylvania, it was not found at that time in any other area. Since the nematode attacks several species of white grubs, it is probable that its normal host was some other species of Scarabaeidae and that it had adapted itself to the Japanese beetle.

It was demonstrated that the nematode can be established in an area where it does not occur naturally, and when so established can cause a high mortality of the grubs. The most critical environmental factor is soil moisture. The nematode can not withstand desiccation. The nematode maintained itself for 14 years with a grub population of less than five per square foot; it survived for 1½ years in the absence of a host.

Methods were developed for rearing the nemas in large numbers

on artificial media. During 1940–46 it was colonized at 3½-mile intervals throughout New Jersey and at 100 sites in Maryland. No surveys were made to determine at how many of these sites it became established. Although *Neoaplectana glaseri* is not effective under so wide a range of conditions as is milky disease, in a suitable environment it is a worthwhile parasite of the beetle.

Bacillus popilliae, the most common of the milky disease complex within the area infested by the beetle, was investigated more extensively than *B. lentimorbus.* Thirteen species of Scarabaeidae were found infected by this pathogen in the field; 15 species became infected by feeding in soil inoculated with spores of the pathogen; 51 species became infected by injection of spores into the blood; 10 species did not become infected by feeding in inoculated soil; and 4 species did not become infected by injection of spores. There are many strains of this bacterium. A strain developed in grubs of one species may have a high or low virulence to grubs of other species. The incidence of infection among grubs is a graded response to the concentration of spores in the soil.

The vegetative stage of the bacterium is killed by exposure to sunlight, heat, or cold, but the spores are very resistant to adverse conditions and may remain viable for many years, ready to infect successive generations of grubs. In the absence of a practical method for the propagation of *Bacillus popilliae* on artificial media, a process was devised for producing the spores in the blood of the grubs and incorporating them in a dust for storage and distribution in the field. The spores were colonized extensively during 1939–53 in 14 States and the District of Columbia. It was estimated at the conclusion of the colonization that over 244,000 pounds of spore dust had been applied at over 160,000 sites.

Assays of the soil at 95 sites in six States and the District of Columbia during 1960–63 showed that *Bacillus popilliae* occurred at all the colonized turf sites and was well established at about one-third of them. It had spread naturally and had developed to a level where adequate control of the grubs could be expected in one-fifth of the uninoculated pastures within 1 or 2 miles of an inoculated site. It had spread naturally into uninoculated cultivated fields, but the populations of spores were low.

Status of Beetle in Older Infested Area.—In the 270 square miles in southern New Jersey and southeastern Pennsylvania occupied by the Japanese beetle in 1921, the annual beetle populations increased in magnitude and importance until by 1929 it was estimated that there were more than 500 million beetles per square mile. As the foreign parasitic insects became established and the incidence of disease among the beetle population increased, the density of the annual populations declined progressively until by 1945 the beetle occurred only at isolated sites throughout the area. It has persisted at many of these sites, but usually the populations are relatively small. This cycle of the rise and decline of beetle populations has been repeated with modifications as the beetle invaded new areas. Thus adequate biological control of the beetle has been attained in much of the area occupied by the insect in 1964.

LITERATURE CITED

ADAMS, J. A.
1944. JAPANESE BEETLE CONTROL BY PARASITIC INSECTS. Farm Res. 10: 1, 9.

———
1949. THE ORIENTAL BEETLE AS A TURF PEST ASSOCIATED WITH THE JAPANESE BEETLE IN NEW YORK. Jour. Econ. Ent. 42: 366–371.

——— and MATTHYSSE, J. G.
1949. THE JAPANESE BEETLE. Cornell Univ. Ext. Bul. 770, 14 pp.

——— and WHEELER, E. H.
1946. RATE OF DEVELOPMENT OF MILKY DISEASE IN JAPANESE BEETLE POPULATIONS. Jour. Econ. Ent. 39: 248–254.

AGEE, A.
1918. THIRD ANNUAL REPORT OF NEW JERSEY DEPARTMENT OF AGRICULTURE. N.J. Dept. Agr. Bul. 17, pp. 79, 145–147.

ALDRICH, J. M.
1923. TWO ASIATIC MUSCOID FLIES PARASITIC UPON SO-CALLED JAPANESE BEETLE. U.S. Natl. Mus. Proc. 63, pp. 1–4.

———
1928. FIVE NEW PARASITIC FLIES REARED FROM BEETLES IN CHINA AND INDIA. U.S. Natl. Mus. Proc. 74, pp. 1–7.

ALLEN, H. W.
1934. DESCRIPTIONS OF TWELVE NEW SPECIES OF TIPHIIDAE FROM THE UNITED STATES. Amer. Ent. Soc. Trans. 60: 295–316.

———
1935. NORTH AMERICAN WASPS OF THE GENUS NEOTIPHIA. Amer. Ent. Soc. Trans. 61: 53–65.

———
1961. TIPHIA (HYMENOPTERA) OF AMERICA NORTH OF MEXICO: KEY TO SPECIES, DESCRIPTIONS OF SIX NEW SPECIES AND NOTES ON SYNONYMY. Amer. Ent. Soc. Trans. 87: 1–20.

———
1962. TYPES OF TIPHIINAE IN THE BRITISH MUSEUM (NATURAL HISTORY). Amer. Ent. Soc. Trans. 88: 21–75.

———
1965. TIPHIINAE (HYMENOPTERA: TIPHIIDAE) OF WESTERN NORTH AMERICA. Acad. Nat. Sci. Phila. Proc. 117: 45–115.

———
1966. A REVISION OF THE TIPHIINAE (HYMENOPTERA: TIPHIIDAE) OF EASTERN NORTH AMERICA. Amer. Ent. Soc. Trans. 92: 231–356.

——— and BURRELL, R. W.
1934. METHODS OF OBTAINING EMERGENCE OF TIPHIA ADULTS FROM IMPORTED COCOONS FOR USE AGAINST THE JAPANESE BEETLE. Jour. Agr. Res. 49: 909–922.

——— and JAYNES, H. A.
1928. PROGRESS WITH PARASITES OF THE JAPANESE BEETLE DURING 1927. Jour. Econ. Ent. 21: 823–832.

——— and JAYNES, H. A.
1930. CONTRIBUTION TO THE TAXONOMY OF ASIATIC WASPS OF THE GENUS TIPHIA. U.S. Natl. Mus. Proc. 76, pp. 1–105.

——— and KROMBEIN, K. V.
1964. A REVISION OF NEOTIPHIA MALLOCH AND KROMBEINIA PATE (HYMENOPTERA: TIPHIIDAE). Amer. Ent. Soc. Trans. 89: 211–275.

BALOCK, J. W.
1934. THE STATUS OF TIPHIA VERNALIS ROHWER, AN IMPORTED PARASITE OF THE JAPANESE BEETLE, AT THE CLOSE OF 1933. Jour. Econ. Ent. 27: 491–496.

BARTLET, K. A., and LEFEBVRE, C. L.
1934. FIELD EXPERIMENTS WITH BEAUVERIA BASSIANA (BALS.) VUILL., A FUNGUS ATTACKING THE EUROPEAN CORN BORER. Jour. Econ. Ent. 27: 1147–1157.

BEARD, R. L.
1944. SUSCEPTIBILITY OF JAPANESE BEETLE LARVAE TO BACILLUS POPILLIAE. Jour. Econ. Ent. 37: 702–708.

———
1945. STUDIES ON THE MILKY DISEASE OF JAPANESE BEETLE LARVAE. Conn. Agr. Expt. Sta. Bul. 491, 505–582.
———
1946. COMPETITION BETWEEN TWO ENTOMOGENOUS BACTERIA. Science 103: 371–372.
———
1946a. CONNECTICUT STATE ENTOMOLOGISTS, FORTY-FIFTH REPORT, 1945. Conn. Agr. Expt. Sta. Bul. 501, pp. 66–71.
———
1956. TWO MILKY DISEASES OF AUSTRALIAN SCARABAEIDAE. Canad. Ent. 82: 640–647.
BELL, E. L.
1930. ENGLISH SPARROWS EATING THE JAPANESE BEETLE. Brooklyn Ent. Soc. Bul. 25: 8.
BRADLEY, W. G.
1952. THE EUROPEAN CORN BORER. U.S. Dept. Agr. Ybk. 1952: 614–621.
BROMLEY, S. W.
1945. ROBBER FLY AND JAPANESE BEETLE. Brooklyn Ent. Soc. Bul. 40: 44–47.
———
1946. WHEEL-BUG VS. JAPANESE BEETLE. Ent. News 57: 21.
BRUNSON, M. H.
1934. THE FLUCTUATION OF THE POPULATION OF TIPHIA POPILLIAVORA ROHWER IN THE FIELD AND ITS POSSIBLE CAUSES. Jour. Econ. Ent. 27: 514–518.
———
1937. INFLUENCE OF THE INSTARS OF THE HOST LARVAE ON SEX OF TIPHIA POPILLIAVORA ROH. Science 86: 197.
———
1938. INFLUENCE OF JAPANESE BEETLE INSTAR ON THE SEX AND POPULATION OF THE PARASITE TIPHIA POPILLIAVORA. Jour. Agr. Res. 57: 379–386.
BUCHHOLZ, A. B.
1942. REPORT OF BUREAU OF PLANT INDUSTRY. N.Y. (State) Dept. Agr. and Markets Ann. Rpt. for 1941, Leg. Doc. 1942 (83): 140–142.
———
1943. REPORT OF BUREAU OF PLANT INDUSTRY. N.Y. (State) Dept. Agr. and Markets Ann. Rpt. for 1942, Leg. Doc. 1943 (72): 132–133.
BURRELL, R. W.
1930. A HUMIDITY APPARATUS. Jour. Econ. Ent. 23: 994–997.
———
1931. DEXIA VENTRALIS ALDRICH, AN IMPORTED PARASITE OF THE JAPANESE BEETLE. Jour. Agr. Res. 43: 323–336.
CARTER, W.
1945. NOTES ON MILKY DISEASE OF JAPANESE BEETLE LARVAE. Hawaii. Acad. Sci. Proc., Ann. Meeting 16: 8–9.
CHADA, H. L., DITMAN, J. A., and DAIGH, F. C.
1942. PROGRESS DURING 1942 OF THE PROGRAM FOR THE COLONIZATION OF MILKY DISEASE OF JAPANESE BEETLE LARVAE IN DELAWARE. Peninsula Hort. Soc. [Del.] Trans. 32: 86–92.
CLAUSEN, C. P.
1927. HYPERALONIA OENOMAUS ROND., A PARASITE OF TIPHIA LARVAE. Ent. Soc. Amer. Ann. 21: 642–659.
———
1940. ENTOMOPHAGOUS INSECTS, 688 pp. McGraw-Hill Co., New York.
——— GARDNER, T. R., and SATO, K.
1932. BIOLOGY OF SOME JAPANESE AND CHOSENESE GRUB PARASITES (SCOLIIDAE). U.S. Dept. Agr. Tech. Bul. 308, 27 pp.
——— JAYNES, H. A., and GARDNER, T. R.
1933. FURTHER INVESTIGATIONS OF PARASITES OF POPILLIA JAPONICA IN THE FAR EAST. U.S. Dept. Agr. Tech. Bul. 366, 58 pp.
——— and KING, J. L.
1924. PRELIMINARY REPORT ON PARASITES OF POPILLIA JAPONICA. Jour. Econ. Ent. 17: 76–79.

CLAUSEN, C. P., KING, J. L., and TERANISHI, C.
1927. THE PARASITES OF POPILLIA JAPONICA IN JAPAN AND CHOSEN (KOREA) AND THEIR INTRODUCTION INTO THE UNITED STATES. U.S. Dept. Agr. Bul. 1429, 56 pp.

CORY, E. N.
1940. THE MILKY DISEASE VS. THE JAPANESE BEETLE. Sci. Monthly 50: 574–576.

——— and LANGFORD, G. S.
1944. THE JAPANESE BEETLE IN MARYLAND. Md. Univ. Bul. 88, 24 pp.

——— and LANGFORD, G. S.
1950. THE UTILIZATION OF BACILLUS POPILLIAE DUTKY AGAINST POPILLIA JAPONICA NEWM. 8th Internatl. Cong. Ent. Proc., pp. 883–886.

——— and LANGFORD, G. S.
1955. THE JAPANESE BEETLE RETARDATION PROGRAM IN MARYLAND. Md. Univ. [Agr.] Ext. Bul. 156, 20 pp.

DAVIS, J. J.
1920. THE GREEN JAPANESE BEETLE. N.J. Dept. Agr. Cir. 30, 33 pp.

———
1920a. THE GREEN JAPANESE BEETLE PROBLEM. Jour. Econ. Ent. 13: 185–194.

DICKERSON, E. L., and WEISS, H. B.
1918. POPILLIA JAPONICA NEWM., A RECENTLY INTRODUCED JAPANESE PEST. Canad. Ent. 50: 217–221.

DUMBLETON, L. J.
1945. BACTERIAL AND NEMATODE PARASITES OF SOIL INSECTS. New Zeal. Jour. Sci. and Technol. A27: 76–81.

DUTKY, S. R.
1937. INVESTIGATION OF DISEASES OF THE IMMATURE STAGES OF THE JAPANESE BEETLE. 113 pp. New Brunswick, N.J. (Thesis, Ph.D., Rutgers Univ.)

———
1940. TWO NEW SPORE-FORMING BACTERIA CAUSING MILKY DISEASE OF JAPANESE BEETLE LARVAE. Jour. Agr. Res. 61: 57–68.

———
1941. TESTING THE POSSIBLE VALUE OF MILKY DISEASES FOR CONTROL OF SOIL-INHABITING LARVAE. Jour. Econ. Ent. 34: 217–218.

———
1941a. SUSCEPTIBILITY OF CERTAIN SCARABAEID LARVAE TO INFECTION BY TYPE A MILKY DISEASE. Jour. Econ. Ent. 34: 215–216.

———
1941b. METHOD FOR THE CONTROL OF JAPANESE BEETLE. U.S. Patent 2,258,319.

———
1942. PROCESS FOR PROPAGATING BACTERIA. U.S. Patent 2,293,890.

———
1942a. METHOD FOR THE PREPARATION OF SPORE-DUST MIXTURES OF TYPE A MILKY DISEASE OF JAPANESE BEETLE LARVAE FOR FIELD INOCULATION. U.S. Bur. Ent. and Plant Quar. ET–192, 10 pp.

———
1947. PRELIMINARY OBSERVATIONS ON THE GROWTH REQUIREMENTS OF BACILLUS POPILLIAE DUTKY AND BACILLUS LENTIMORBUS DUTKY. Jour. Bact. 54: 267.

———
1957. REPORT ON WHITE GRUB CONTROL PROJECT IN CHILE. Chile, Dir. Nac. Agr., Agr. Téc. 17, pp. 92–105.

———
1959. INSECT MICROBIOLOGY. Adv. in Appl. Microbiol. 1: 175–200.

———
1963. THE MILKY DISEASES. *In* Insect Pathology, An Advanced Treatise. V. 2, pp. 75–115. Academic Press, New York.

——— and FEST, W. C.
1942. MICROINJECTOR. U.S. Patent 2,270,804.

——— and GOODEN, E. L.
1952. COXIELLA POPILLIAE, N. SP., A RICKETTSIA CAUSING BLUE DISEASE OF JAPANESE BEETLE LARVAE. Jour. Bact. 63: 743–750.

——— and WHITE, R. T.
1940. NOTE ON THE OCCURRENCE OF MICROSPORIDIAN INFECTION AMONG NATIVE SCARABAEID LARVAE. Jour. Parasitol. 26: 333.
EASTER, S. S.
1947. CONTROL OF THE JAPANESE BEETLE AT SECOND ARMY POSTS. Jour. Econ. Ent. 40: 632–634.
EDDY, B.
1943. DIVISION OF ENTOMOLOGY AND PLANT INDUSTRY. R.I. Dept. Agr. and Conserv. Ann. Rpt. 1943: 8.
FLEMING, W. E.
1958. CONTROLLING THE JAPANESE BEETLE. U.S. Dept. Agr. Farmers' Bul. 2004, 13 pp. (rev.)
———
1958a. BIOLOGICAL CONTROL OF THE JAPANESE BEETLE, ESPECIALLY WITH ENTOMOGENOUS DISEASES. 10th Internatl. Cong. Ent. Proc. 1956 (3), pp. 115–125.
———
1961. MILKY DISEASE FOR CONTROL OF JAPANESE BEETLE GRUBS. U.S. Dept. Agr. Leaflet 500, 6 pp.
———
1963. THE JAPANESE BEETLE IN THE UNITED STATES. U.S. Dept. Agr. Agr. Handb. 236, 30 pp.
——— and BAKER, F. E.
1932. HOT WATER AS AN INSECTICIDE FOR THE JAPANESE BEETLE IN SOIL AND ITS EFFECT ON THE ROOTS OF NURSERY PLANTS. U.S. Dept. Agr. Tech. Bul. 274, 42 pp.
FRENCH, G. T.
1941. THE MILKY DISEASE OF THE JAPANESE BEETLE, A NEW AND EFFECTIVE CONTROL REMEDY. Va. State Hort. Soc. Rpt. 46, pp. 55–60.
——— WILEY, C. R., FREUND, F. R., and MATHENY, W. H.
1949. THE JAPANESE BEETLE AND ITS CONTROL. 15 pp. Va. Dept. Agr. and Immigr., Richmond, Va.
FRIEND, R. B.
1940. CONNECTICUT STATE ENTOMOLOGIST, THIRTY-NINTH REPORT, 1939. Conn. Agr. Expt. Sta. Bul. 434, pp. 222–322.
———
1942. CONNECTICUT STATE ENTOMOLOGIST, FORTY-FIRST REPORT, 1941. Conn. Agr. Expt. Sta. Bul. 461, pp. 49–50.
GARDNER, T. R.
1934. COMPARATIVE OVIPOSITIONAL EFFICIENCY AND COLLECTION COSTS OF IMPORTED VERSUS ESTABLISHED TIPHIA VERNALIS ROHWER, A PARASITE OF THE JAPANESE BEETLE. Jour. Econ. Ent. 27: 497–499.
———
1938. INFLUENCE OF FEEDING HABITS OF TIPHIA VERNALIS ON THE PARASITIZATION OF THE JAPANESE BEETLE. Jour. Econ. Ent. 31: 204–207.
——— and PARKER, L. B.
1940. INVESTIGATIONS OF THE PARASITES OF POPILLIA JAPONICA AND RELATED SCARABAEIDAE IN THE FAR EAST FROM 1929 TO 1933, INCLUSIVE. U.S. Dept. Agr. Tech. Bul. 738, 36 pp.
GARMAN, P., BRIGHAM, W. T., SCHREAD, J. C., and SMITH, G. R.
1941. REPORT ON PARASITE WORK FOR 1940. Conn. Agr. Expt. Sta. Bul. 445, pp. 328–333.
——— BRIGHAM, W. T., SCHREAD, J. C., and SMITH, G. R.
1942. REPORT FOR PARASITE WORK FOR 1941. Conn. Agr. Expt. Sta. Bul. 461, pp. 487–490.
——— BRIGHAM, W. T., SCHREAD, J. C., and SMITH, G. R.
1943. REPORT ON PARASITE AND DISEASE WORK FOR 1942. Conn. Agr. Expt. Sta. Bul. 472, pp. 231–232.
GIRTH, H. B., MCCOY, E. E., and GLASER, R. W.
1940. FIELD EXPERIMENTS WITH A NEMATODE PARASITE OF THE JAPANESE BEETLE. N.J. Dept. Agr. Cir. 317, 21 pp.
GLASER, R. W.
1931. THE NEMATODE PARASITE OF THE JAPANESE BEETLE. N.J. Dept. Agr. Ann. Rpt. 16: 175–180.

GLASER, R. W.
1931a. THE CULTIVATION OF A NEMATODE PARASITE OF AN INSECT. Science 73: 614.

———
1932. STUDIES ON NEOAPLECTANA GLASERI, A NEMATODE PARASITE OF THE JAPANESE BEETLE (POPILLIA JAPONICA). N.J. Dept. Agr. Cir. 211, 34 pp.

———
1940. THE BACTERIA-FREE CULTURE OF A NEMATODE PARASITE. Soc. Expt. Biol. and Med. Proc. 43: 512–514.

——— and FARRELL, C. C.
1935. FIELD EXPERIMENTS WITH THE JAPANESE BEETLE AND ITS NEMATODE PARASITE. N.Y. Ent. Soc. Jour. 43: 345–371.

——— and FOX, H.
1930. A NEMATODE PARASITE OF THE JAPANESE BEETLE POPILLIA JAPONICA NEWM. Science 71: 16–17.

——— MCCOY, E. E., and GIRTH, H. B.
1940. THE BIOLOGY AND ECONOMIC IMPORTANCE OF A NEMATODE PARASITE OF INSECTS. Jour. Parasitol. 26: 479–495.

——— MCCOY, E. E., and GIRTH, H. B.
1942. THE BIOLOGY AND CULTURE OF NEOAPLECTANA CHRESIMA, A NEMATODE PARASITE IN INSECTS. Jour. Parasitol. 28: 123–126.

HADLEY, C. H.
1922. THE JAPANESE BEETLE. N.J. Dept. Agr. Cir. 46, 20 pp.

———
1938. PROGRESS OF JAPANESE BEETLE INVESTIGATIONS. N.Y. Ent. Soc. Jour. 46: 203–216.

———
1948. MILKY DISEASE FOR CONTROL OF JAPANESE BEETLE GRUBS. U.S. Bur. Ent. and Plant Quar. EC–4, 8 pp.

———
1948a. CONTROL OF THE JAPANESE BEETLE BY MEANS OF MILKY DISEASE. Brooklyn Inst. Arts and Sci., Bot. Gard. Rec., Plants and Gard. 4, pp. 188–190.

——— and HAWLEY, I. M.
1934. GENERAL INFORMATION ABOUT THE JAPANESE BEETLE IN THE UNITED STATES. U.S. Dept. Agr. Cir. 332, 23 pp.

HALLOCK, H. C.
1929. NORTH AMERICAN PREDACEOUS INSECTS ATTACKING JAPANESE BEETLE GRUBS. Ent. News 40: 76–78.

———
1929a. NOTES ON METHODS OF REARING SARCOPHAGINAE AND THE BIOLOGY OF SARCOPHAGA LATISTERNA PERK. Ent. Soc. Amer. Ann. 22: 246–250.

HAWLEY, I. M.
1944. NOTES ON THE BIOLOGY OF THE JAPANESE BEETLE. U.S. Bur. Ent. and Plant Quar. E–615, 18 pp.

———
1952. MILKY DISEASES OF BEETLES. U.S. Dept. Agr. Ybk. 1952: 394–400.

——— and WHITE, G. F.
1935. PRELIMINARY STUDIES OF THE DISEASES OF LARVAE OF THE JAPANESE BEETLE (POPILLIA JAPONICA NEWM.). N.Y. Ent. Soc. Jour. 43: 405–412.

HAYNES, W. C., ST. JULIAN, G., SKELTON, M. C., and others.
1961. PRESERVATION OF INFECTIOUS MILKY DISEASE BACTERIA BY LYOPHILIZATION. Jour. Insect Path. 3: 55–61.

HEADLEE, T. J.
1918. REPORT OF THE DEPARTMENT OF ENTOMOLOGY, 1916–17. N.J. Agr. Expt. Sta. Ann. Rpt. 1918: 440–441.

HOLLOWAY, J. K.
1931. TEMPERATURE AS A FACTOR IN THE ACTIVITY AND DEVELOPMENT OF THE CHINESE STRAIN OF TIPHIA POPILLIAVORA IN NEW JERSEY AND PENNSYLVANIA. N.Y. Ent. Soc. Jour. 39: 555–564.

HOWARD, L. O.
1918. REPORT OF THE ENTOMOLOGIST, 1917–18. 5 pp. U.S. Dept. Agr., Washington, D.C.

HURPIN, B.
1955. SUR UNE LAITEUSE DES LARVES DE MELOLONTHA MELOLONTHA L. Soc. de [Paris] Compt. Rend. Biol. 149: 1966–1967.

———
1957. SUR LA VIRILENCE DE BACILLUS POPILLIAE DUTKY PUR LES LARVES DU HANNETON CUMMUN MELOLONTHA MELOLONTHA. Soc. de Biol. [Paris] Compt. Rend. Biol. 151: 1833–1835.

———
1959. ETUDE DE DIVERS SOUCHES DE MALADIE LAITEUSE SUR LES LARVE DE MELOLONTHA MELOLONTHA L. ET SUR CELLES DE QUELQUES ESPECES VOISINES. Entomophaga 4, pp. 233–248.

JAYNES, H. A., and GARDNER, T. R.
1924. SELECTIVE PARASITISM BY TIPHIA SP. Jour. Econ. Ent. 17: 366–369.

KING, J. L.
1931. THE PRESENT STATUS OF THE ESTABLISHED PARASITES OF POPILLIA JAPONICA NEWMAN. Jour. Econ. Ent. 24: 453–462.

———
1937. METHODS OF REARING TIPHIIDAE AND SCOLIIDAE. *In* Culture Methods for Invertebrate Animals. 590 pp. Comstock Pub. Co., Ithaca, N.Y.

———
1937a. LIBERATIONS OF JAPANESE BEETLE PARASITES IN THE EASTERN STATES IN 1937. U.S. Dept. Agr. Insect Pest Survey Bul. 17, pp. 473–483.

———
1939. COLONIZATION OF JAPANESE BEETLE PARASITES IN THE EASTERN STATES IN 1938. U.S. Dept. Agr. Insect Pest Survey Bul. 19, Sup. to No. 1, pp. 25–28.

——— ALLEN, H. W., and HALLOCK, H. C.
1927. THE PRESENT STATUS OF WORK ON THE PARASITES OF POPILLIA JAPONICA NEWMAN. Jour. Econ. Ent. 20: 365–373.

——— and HALLOCK, H. C.
1925. A REPORT ON CERTAIN PARASITES OF POPILLIA JAPONICA NEWM. Jour. Econ. Ent. 18: 351–356.

——— and HOLLOWAY, J. K.
1930. THE ESTABLISHMENT AND COLONIZATION OF TIPHIA POPILLIAVORA, A PARASITE OF THE JAPANESE BEETLE. Jour. Econ. Ent. 23: 266–274.

——— and HOLLOWAY, J. K.
1930a. TIPHIA POPILLIAVORA ROHWER, A PARASITE OF THE JAPANESE BEETLE. U.S. Dept. Agr. Cir. 145, 11 pp.

——— and PARKER, L. B.
1950. THE SPRING TIPHIA, AN IMPORTED ENEMY OF THE JAPANESE BEETLE. U.S. Bur. Ent. and Plant Quar. E–799, 8 pp.

——— PARKER, L. B., and WILLARD, H. J.
1951. STATUS OF IMPORTED PARASITES OF THE JAPANESE BEETLE IN 1950. U.S. Dept. Agr. Insect Pest Survey Spec. Sup. 5, 18 pp.

KOWALCZYK, S. A.
1938. A REPORT ON THE INTERNAL PROTOZOA OF THE LARVAE OF THE JAPANESE BEETLE, POPILLIA JAPONICA NEWM. Amer. Micros. Soc. Trans. 57: 229–244.

LANGFORD, G. S., VINCENT, R. H., and CORY, E. N.
1942. THE JAPANESE BEETLE AS HOST AND DISSEMINATOR OF TYPE A MILKY DISEASE. Jour. Econ. Ent. 35: 165–169.

LEFEBVRE, C. L.
1931. PRELIMINARY OBSERVATIONS ON TWO SPECIES OF BEAUVERIA ATTACKING THE CORN BORER PYRAUSTA NUBILALIS (HBN.). Phytopathology 27: 1115–1128.

LIGHT, J. H.
1943. REPORT PENNSYLVANIA DEPARTMENT OF AGRICULTURE FOR 1939–43. [n.p.] Harrisburg, Pa.

MCCOY, E. E., and CARVER, C. W.
1941. A METHOD OF OBTAINING SPORES OF THE FUNGUS BEAUVERIA BASSIANA (BALS.) VUILL. IN QUANTITY. N.Y. Ent. Soc. Jour. 49: 205–210.

McCoy, E. E., and Girth, H. B.
1938. THE CULTURE OF NEOAPLECTANA GLASERI ON VEAL PULP. N.J. Dept. Agr. Cir. 285, 12 pp.
——— Girth, H. B., and Glaser, R. W.
1938. NOTES ON A GIANT FORM OF THE NEMATODE NEOAPLECTANA GLASERI. Jour. Parasitol. 24: 471–472.
——— and Glaser, R. W.
1936. NEMATODE CULTURE FOR JAPANESE BEETLE CONTROL. N.J. Dept. Agr. Cir. 265, 10 pp.
Montgomery, R. W.
1943. REPORT OF THE DIVISION OF ENTOMOLOGY AND PATHOLOGY. Queensland Bur. Sugar Expt. Stas. Ann. Rpt. 43: 18–19.
Moul, E. T.
1945. NOTES ON ARILUS CRISTATUS (LINNAEUS) IN YORK COUNTY, PENNSYLVANIA AND ON ITS PREY. Ent. News 56: 57–59.
Parker, L. B.
1934. NOTES ON THE LIFE HISTORY AND BIOLOGY OF CENTETER UNICOLOR ALDRICH. Jour. Econ. Ent. 27: 486–491.
———
1935. THREE NEW SPECIES OF TIPHIA FROM EASTERN ASIA. N.Y. Ent. Soc. Jour. 43: 395–404.
———
1937. SEVEN NEW SPECIES OF ASIATIC TIPHIA. N.Y. Ent. Soc. Jour. 45: 269–290.
Polivka, J. B.
1950. DISTRIBUTION AND CONTROL OF THE JAPANESE BEETLE IN OHIO. Ohio Agr. Expt. Sta. Res. Cir. 4, 16 pp.
———
1956. EFFECTIVENESS OF MILKY DISEASE IN CONTROLLING JAPANESE BEETLE IN OHIO. Jour. Econ. Ent. 49: 4–6.
———
1960. MILKY DISEASE CAUSES MORTALITY OF JAPANESE BEETLE GRUBS. Ohio Farm and Home Res. 45: 58–59.
Rex, E. G.
1940. A PROMISING PATHOGEN OF ADULT JAPANESE BEETLES. N.Y. Ent. Soc. Jour. 48: 401–403.
Rice, P. L.
1943. FINAL REPORT ON THE PROGRAM FOR THE COLONIZATION OF THE CAUSAL AGENT OF THE MILKY DISEASE OF JAPANESE BEETLE LARVAE IN DELAWARE. Peninsula Hort. Soc. [Del.] Trans. 33: 121–124.
Roberts, R.
1930. SEVEN NEW NAMES IN THE GENUS TIPHIA. Canad. Ent. 62: 189–190.
Rohwer, S. A.
1924. DESCRIPTION OF THREE NEW SPECIES OF TIPHIA PARASITIC ON POPILLIA JAPONICA. Wash. Ent. Soc. Proc. 26: 87–92.
———
1927. A NEW TIPHIA FROM KOREA. Wash. Ent. Soc. Proc. 29: 19.
Schaerffenberg, B.
1957. BEAUVERIA BASSIANA (VUILL.) LINK ALSO PARASIT DES KARTOFFELKAFERS (LEPTINOTARSA DECIMLINEATA SAY). Anz. f. Schädlingsk. 30: 69–74.
Schread, J. C.
1944. REPORT ON DISEASE WORK IN 1943. Conn. Agr. Expt. Sta. Bul. 481, pp. 264–267.
———
1945. REPORT ON DISEASE WORK IN 1944. Conn. Agr. Expt. Sta. Bul. 488, pp. 331–339.
———
1946. REPORT ON DISEASE WORK IN 1945. Conn. Agr. Expt. Sta. Bul. 501, pp. 64–65.
———
1947. REPORT ON DISEASE WORK IN 1946. Conn. Agr. Expt. Sta. Bul. 512, pp. 49–50.

SCHWARDT, H. H., LINCOLN, G. C., and KERR, T. W.
1943. ENTOMOLOGY AND LIMOLOGY. N.Y. (Cornell) Agr. Expt. Sta. Ann. Rpt. 55 (1942): 121–132.
SIM, R. J.
1934. SMALL MAMMALS AS PREDATORS ON JAPANESE BEETLE GRUBS. Jour. Econ. Ent. 27: 482–483.
SMITH, E. H.
1941. JAPANESE BEETLE CONTROL WITH NATURAL ENEMIES. Farm Res. 7: 2, 10.
——— and DANIEL, D. M.
1942. THE STATUS OF BIOLOGICAL CONTROL OF THE JAPANESE BEETLE IN NEW YORK STATE. N.Y. (Geneva) Agr. Expt. Sta. Bul. 898, pp. 6–9.
SMITH, L. B.
1928. THE JAPANESE BEETLE, PRESENT STATUS AND CONTROL. Acad. Nat. Sci. Phila. Ybk. 1928: 5–15.
———
1930. THE JAPANESE BEETLE IN 1929. Jour. Econ. Ent. 23: 495–501.
——— and HADLEY, C. H.
1926. THE JAPANESE BEETLE. U.S. Dept. Agr. Cir. 363, 67 pp.
STEARNS, L. A., FASSIG, W. W., and BEACHER, J. H.
1941. COLONIZATION OF MILKY DISEASE OF JAPANESE BEETLE LARVAE IN DELAWARE. Peninsula Hort. Soc. [Del.] Trans. 31: 46–51.
STEINER, G.
1929. NEOAPLECTANA GLASERI, N. G., N. SP. (OXYURIDAE), A NEW NEMIC PARASITE OF THE JAPANESE BEETLE (POPILLIA JAPONICA NEWM.). Wash. Acad. Sci. Jour. 19: 436–440.
STEINHAUS, E. A.
1947. INSECT MICROBIOLOGY. 763 pp. Comstock Pub. Co., Ithaca, N.Y.
STEINKRAUS, K. H.
1957. STUDIES ON THE MILKY DISEASE ORGANISMS. I. Parasitic Growth and Sporulation of *Bacillus popilliae*. Jour. Bact. 74: 621–624.
———
1957a. STUDIES ON THE MILKY DISEASE ORGANISMS. II. Saprophytic Growth of *Bacillus popilliae*. Jour. Bact. 74: 625–632.
——— and PROVVIDENTI, M. L.
1958. STUDIES OF THE MILKY DISEASE ORGANISMS. III. Variability Among Strains of *Bacillus popilliae* Sporulating on Artificial Media. Jour. Bact. 75: 38–42.
——— and TASHIRO, H.
1955. PRODUCTION OF MILKY DISEASE SPORES (BACILLUS POPILLIAE DUTKY AND BACILLUS LENTIMORBUS DUTKY) ON ARTIFICIAL MEDIA. Science 121: 873–874.
STOLL, N. R.
1953. CONTINUED INFECTIVITY FOR JAPANESE BEETLE GRUB OF NEOAPLECTANA GLASERI (NEMATODA) AFTER SEVEN YEARS OF AXENIC CULTURE. Thapar Commemoration V. 1953, pp. 259–268. Lucknow, India.
———
1959. CONDITIONS FAVORING THE AXENIC CULTURE OF NEOAPLECTANA GLASERI, A NEMATODE PARASITE OF CERTAIN INSECT GRUBS. N.Y. Acad. Sci. Ann. 77, pp. 126–136.
TASHIRO, H.
1957. SUSCEPTIBILITY OF EUROPEAN CHAFER AND JAPANESE BEETLE LARVAE TO DIFFERENT STRAINS OF MILKY DISEASE ORGANISMS. Jour. Econ. Ent. 50: 350–352.
——— and WHITE, R. T.
1954. MILKY DISEASE OF EUROPEAN CHAFER LARVAE. Jour. Econ. Ent. 47: 1087–1092.
TILLARD, R. J.
1922. THE LIFE HISTORY OF THE AUSTRALIAN MOTH LACEWING ITHONE FUSCA NEWM. Bul. Ent. Res. 13: 205–224.
TIMONIN, M. I.
1939. PATHOGENICITY OF BEAUVERIA BASSIANA (BALS.) VUILL. ON COLORADO POTATO BEETLE LARVAE. Canad. Jour. Res. 17 D: 103–107.

WEISS, H. B.
1918. THE CONTROL OF IMPORTED PESTS RECENTLY FOUND IN NEW JERSEY. Jour. Econ. Ent. 11: 122–125.

———
1918a. NEW INSECT AND PLANT DISEASE PESTS IN NEW JERSEY. N.J. Dept. Agr. Cir. 14, 12 pp.

WHEELER, E. H.
1943. EFFECT OF MILKY DISEASE ON 1942–43 JAP BEETLE GRUBS. Farm Res. 9: 8.

———
1943a. EXPERIMENTS WITH MILKY DISEASE FOR NATURAL CONTROL OF JAPANESE BEETLE IN NEW YORK. N.Y. (Geneva) Agr. Expt. Sta. Bul. 703, pp. 14–17.

——— and ADAMS, J. A.
1945. PROGRESS IN JAP BEETLE CONTROL BY MILKY DISEASE. Farm Res. 11: 14–15.

WHITE, R. T.
1940. THE RELATION OF ANTS TO THE JAPANESE BEETLE AND ITS ESTABLISHED PARASITES. N.Y. Ent. Soc. Jour. 48: 85–99.

———
1940a. SURVIVAL OF TYPE A MILKY DISEASE OF JAPANESE BEETLE UNDER ADVERSE FIELD CONDITIONS. Jour. Econ. Ent. 33: 303–306.

———
1941. DEVELOPMENT OF MILKY DISEASE ON JAPANESE BEETLE LARVAE UNDER FIELD CONDITIONS. Jour. Econ. Ent. 34: 213–215.

———
1943. EFFECT OF MILKY DISEASE ON TIPHIA PARASITES OF JAPANESE BEETLE LARVAE. N.Y. Ent. Soc. Jour. 51: 213–218.

———
1946. EFFECT OF THE SUN'S RAYS UPON THE VIABILITY OF SPORES OF BACILLUS POPILLIAE DUTKY, THE ORGANISM CAUSING MILKY DISEASE OF JAPANESE BEETLE LARVAE. U.S. Bur. Ent. and Plant Quar. E–703, 4 pp.

———
1947. MILKY DISEASES INFECTING CYCLOCEPHALA LARVAE IN THE FIELD. Jour. Econ. Ent. 40: 912–914.

———
1948. APPLICATION OF MILKY DISEASE SPORE DUST WITH A COMMERCIAL FERTILIZER. Jour. Econ. Ent. 41: 113–114.

——— and DUTKY, S. R.
1940. THE EFFECT OF THE INTRODUCTION OF MILKY DISEASES ON POPULATIONS OF JAPANESE BEETLE LARVAE. Jour. Econ. Ent. 33: 306–309.

——— and DUTKY, S. R.
1942. COOPERATIVE DISTRIBUTION OF ORGANISMS CAUSING MILKY DISEASE OF JAPANESE BEETLE GRUBS. Jour. Econ. Ent. 35: 679–682.

——— and MCCABE, P. J.
1943. COLONIZATION OF THE ORGANISMS CAUSING MILKY DISEASE OF JAPANESE BEETLE LARVAE. U.S. Bur. Ent. and Plant Quar. E–605, 7 pp.

——— and MCCABE, P. J.
1946. COLONIZATION OF THE ORGANISM CAUSING MILKY DISEASE OF JAPANESE BEETLE LARVAE, 1939–1945. U.S. Bur. Ent. and Plant Quar. E–704, 8 pp.

——— and MCCABE, P. J.
1950. THE EFFECT OF MILKY DISEASE ON JAPANESE BEETLE POPULATIONS OVER A TEN-YEAR PERIOD. U.S. Bur. Ent. and Plant Quar. E–801, 3 pp.

——— and MCCABE, P. J.
1951. COLONIZATION OF THE ORGANISM CAUSING MILKY DISEASE OF JAPANESE BEETLE LARVAE, 1939–1949. U.S. Bur. Ent. and Plant Quar. E–816, 10 pp.

WILLE, H.
1956. BACILLUS FRIBOURGENSUS, N. SP., ERREGER EINER "MILKY DISEASE" IM ENGERLING VON MELOLONTHA MELOLONTHA L. Schweiz. Ent. Gesell. Mitt. 29, pp. 271–282.

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