START

57

GREENBUGS: DETERMINING BIOTYPES, CULTURING, AND SCREENING FOR PLANT RESISTANCE

With Notes on Rearing Parasitoids

Technical Bulletin No. 1556

Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

CONTENTS

ILLUSTRATIONS

TABLES

GREENBUGS: DETERMINING BIOTYPES, CULTURING, AND SCREENING FOR PLANT RESISTANCE
With Notes on Rearing Parasitoids

By K. J. Starks and R. L. Burton[1]

ABSTRACT

Characteristics for separating the four biotypes (A, B, C, and D) of the greenbug, *Schizaphis graminum* (Rondani), are discussed, including morphological differences and response to resistant varieties and insecticides. Techniques for culturing greenbugs on caged plants in the greenhouse are described. In addition, methods are suggested for screening for plant resistance both in the greenhouse and in the field. Sources of plant resistance to the greenbug are listed. Culturing techniques for both primary and secondary parasitoids are covered. Difficulties that may be encountered are discussed for all phases of rearing and screening.

KEYWORDS: biotypes, *Charips*, greenbug, *Lysiphlebus testaceipes*, plant pest resistance, primary parasitoids, *Schizaphis graminum*, secondary parasitoids.

INTRODUCTION

The greenbug, *Schizaphis graminum* (Rondani),[2] is a major pest of wheat, barley, oats, rye, and sorghum in the Midwest. This insect injects a toxicant during feeding, and a relatively small number of greenbugs can cause more damage than a much larger number of some other species of aphids. The pest also has a high parthenogenetic reproductive rate. Direct damage and indirect control expeditures have cost grain sorghum producers about $12 million per year from 1968 to 1976. Dahms et al. estimated that in outbreak years losses exceed 50 million bushels of small grains (4).[3] Furthermore, the greenbug, along with other species of aphids, transmits the causal agents of plant diseases such as barley yellow dwarf and maize dwarf mosaic.

There are several species of natural enemies of the greenbug. Some of these increase to large numbers, but usually the increases during current cultural practices are too late to prevent economic losses. For this reason, insecticides have been heavily relied upon for greenbug control. Organophosphorus chemicals afforded reliable control at relatively small dosages for about 20 years, but in 1974 tolerance to these commonly used insecticides became pronounced. An alternative method of control, host-plant resistance, has received increased emphasis. Sources of resistant germplasm have now been located in all the crop species, and some plant resistance to the greenbug has been released by State agricultural experiment stations and the Agricultural Research Service (ARS).

This publication is intended for research workers in public agencies and industry who are rearing the greenbug or starting programs on plant resistance. A discussion of all the known biotypes of the greenbug is not now available in one article. Rearing and screening for plant resistance have been discussed in journal articles but not at length, and difficulties are seldom mentioned. Procedures for rearing parasitoids may be helpful for researchers in biological control.

ECONOMICALLY IMPORTANT BIOTYPES OF THE GREENBUG

The greenbug has been collected from at least 78 species of *Gramineae* (Poaceae) and other plant families (*3*). With such a comparatively wide host range, and with reproduction rapid and mainly, if not entirely, by parthenogenesis in the United States (*8*), several biotypes might be expected. An acre of grain sorghum can harbor several million individuals, and so the chance for genetic variation is great. However, at present most entomologists in the Great Plains separate the greenbug into only four biotypes of importance (A, B, C, and D) on field crops.

Characteristics for separating the four major biotypes are shown in figure 1 and are based partly on designations made by Wood et al. (*18*). Some morphological differences among the biotypes listed by these authors are difficult to determine, and these have been omitted as distinguishing characteristics. Other morphological characteristics that are distinguishable with about ×5 magnification can be helpful. Usually, biotypes A and B appear a darker green than C and D, but this distinction may be unreliable because greenbugs become darker as they age and because food sources can influence insect color. The black tips on the cornicles of biotypes A and B are usually prominent throughout the life, but there are gradations that make some individuals difficult to separate. Morphological characteristics are generally not as reliable as physiological characteristics based on fecundity and survival on host plants, especially in regard to temperature differences and to tolerance to specific insecticides.

Biotype A was probably preceded by others, but no attempt was made to separate biotypes until after Dickinson selection 28–A (DS 28–A) wheat was found to be resistant. Subsequently, a biotype designated as B overcame

FIGURE 1.—Differences in the economic biotypes of the greenbug.

the resistance of DS 28-A. Biotype B had mainly replaced A in small fields in Oklahoma by 1966, even though the two biotypes have similar ecological and reproductive patterns. Biotype C caused a severe outbreak of greenbugs on grain sorghum in 1968, and since that time has largely replaced B on small grains in much of the Great Plains. This new biotype was better able to withstand summer temperatures, and male alates were prevalent during certain times of the year (9). Biotype C also had a higher reproductive rate than previous biotypes (table 1). Plant entries resistant to C were also resistant to A and B until CI 1579 and CI 1580 oats indicated susceptibility to B. Some plant selections resistant to all known biotypes are listed in table 2.

Biotype D probably gives the same reaction on plants as biotype C, although a more thorough study is needed. Biotype D, however, has as much as a thirtyfold resistance to some organophosphorus insecticides (10, 17). A population of biotype D may resort back to insecticidal susceptibility near that of other biotypes, but the resistance will again become pronounced once there is repeated subjection to certain insecticides. Biotype D was first reported on sorghum in West Texas in the summer of 1974, but it was probably present

TABLE 1.—*Interactions of three biotypes of the greenbug and some host plants*

Host plant	Number of nymphs per adult for biotype—			Damage rating for biotype[1]—		
	A	B	C	A	B	C
Barley:						
'Rogers'	82	74	116	5.8	5.8	5.9
'Will'	58	47	51	1.8	1.7	1.6
Sorghum:						
RS-610	23	25	64	...	1.2	5.3
PI 264453	0	1	19	...	1.0	2.2
'Deer'	0	4	64	...	1.6	5.1

[1] Ratings ranged from 1, no visible damage, to 6, dead or dying plant.

on wheat in New Mexico prior to this. In 1975, it was reported in Texas, Oklahoma, Kansas, Nebraska, and South Dakota.

No doubt other biotypes based on reactions to plants are already present in other countries, and new ones can appear in the United States. Correspondence with an Argentine scientist indicates that DS 28-A wheat still maintains field resistance, although the greenbug is now a serious pest on sorghum in Argentina.[4] In Eastern Europe, greenbug eggs

[4] Personal communication from H. O. Arriaga, Universidad Nacional de La Plata, La Plata, Argentina.

TABLE 2.—*Some plants resistant to the three known biotypes of the greenbug and commercial cultivars developed from these sources*

Resistant plant source	Commercial cultivars in use
Sorghum:	
PI 221613 (IS 809)	Will be used in several hybrids.
KS-30	Do.
SA 7536-1 (Shallu)	Do.
PI 264453	Used in forage-type sorghums.
PI 308976	Grassy and not used at present.
PI 229828	Do.
PI 220248	Do.
PI 302178	Do.
PI 302231	Do.
PI 226096	Do.
Barley:	
CI 7580 (Kerney)	'Will', 'Nebar', 'Kambi'.
CI 5144 (Omugi)	'Kerr'.
'Ludwig'	'Era'.
'Dicktoo'	None.
Rye: Insave FA	'Okema'.
Triticale: Insave FA	'Gaucho'.
Oats: PI 186270	None.
Wheat: 'Amigo'	Several varieties are being developed.

hatch readily, whereas none have been demonstrated to be viable in the United States. Alate greenbugs are known to be distributed for long distances by air currents (7), and so biotypes can be widely distributed. In the United States, agricultural scientists need to be especially watchful for greenbug damage to corn, since this important crop and sorghum are often planted in the same areas. Also, any greenbug population that can kill 'Will' barley, 'Gaucho' triticale, or IS-809 sorghum would be considered a new biotype.

CULTURING THE GREENBUG

The greenbug can be cultured on an artificial diet or on growing plants. The artificial diet is a modified pea aphid diet, with dissolved and suspended ingredients upon which greenbugs feed through a sachet membrane (2). This diet has a limited use for such research as nutritional studies, but it is impractical for rearing large numbers of greenbugs through several generations, since it is costly and time consuming.

We grow culture plants in the greenhouse in 8-in plastic pots containing a 3:1:1 mixture of soil, sand, and peat, although this composition is not critical. A small amount of complete fertilizer should be included in the mixture. Sterilization of the soil mixture should be done if plant diseases become a problem, but sterilization can induce plant-growth problems. Barley is the preferred host of the greenbug, and this small grain can be expected to give the most rapid buildup of populations. However, barley can be quickly killed by greenbug damage, and it does not grow well at ambient temperatures much above 80° F. For these reasons, grain sorghum can be used as a substitute culture plant, or a mixture of grain sorghum and feed barley may be chosen. About 30 seeds treated for soil-borne diseases but not treated with an insecticide can be distributed in each pot. Seeds of resistant selections should not be used for cultures, since fecundity will be reduced appreciably; in fact, food sources can precondition greenbugs for two generations. Some of the resistant selections are listed in table 2. Resistant sorghum hybrids are available commercially, and these have a designation of resistance on the label or bag.

PN-5226
FIGURE 2.—Pots and cages for culturing greenbugs.

Cylindrical plastic cages are placed over culture plants to exclude extraneous insects and to confine the greenbugs (fig. 2). We make the cages from transparent nitrocellulose film, 0.02 inches thick and polished on both sides.[3] Although nitrocellulose film is inflammable, it is still preferred since some other types of plastics are toxic to greenbugs (1).

The size of the cage depends upon the dimensions of the sheet of plastic film. We have used a 14- by 14-inch piece of film. Two ventilation holes about 3 inches in diameter are cut about 3 inches from the edges of corners diagonally across from each other. The piece of plastic is then rolled into a cylinder, and the edges are overlapped at least one-half of an inch and secured with a cellulose nitrate base adhesive. The edges at the seam may need to be clamped for a few minutes until the glue sets.

The ventilation holes and the top of the cage are covered with glue-on cloth cut about one-half of an inch larger than the diameter of the opening. The cloth should have a weave sufficiently large to allow aeration but small enough to block the passage of insects. Cages may be reused and can be washed with a mild soap. They darken with age and become brittle, especially if stored in direct sunlight under high temperature. The bottom may curl, but a thin strip can be cut off. Cages can be made larger or smaller, depending on needs.

The bottom of the cage is pressed into the 1 inch of sand placed on top of seed. About 2 weeks after planting, new cultures may be infested by putting two or three plants from previous cultures into the cages containing the new plantings. In about 2 or 3 additional weeks, the culture should have a maximum number of greenbugs.

Temperature and humidity are dictated more by the culture plant needs than by greenbug requirements. Greenbugs, especially biotype C, can reproduce from 60 to 90 F, although about 72 F is best, since at this temperature 100 nymphs per female can be produced over a 20-day period.

There is a period, mainly in February and March in Oklahoma and Texas, when alate (winged) adults can predominate in cultures. Although alate females give birth to nymphs, they do so at a reduced rate and often congregate in the top of cages—an action that is perhaps related to migration. In addition to alate females, 15 to 20 percent of the winged individuals can be males. Males are small, and the tarsi and the apices of the tibiae are dark. There is a conspicuous C-shaped ventral at the tip of the abdomen. These males commonly mate with apterous (wingless) oviparous (egg-laying) females that can represent up to one-half of the total population in the spring. The oviparae have swollen and dark hind tibiae with many sensoria. Eggs are laid readily on culture plants and the cloth of cages. They darken with age, but none has been observed to hatch (8). The production of sexuals and alates is influenced by temperature and probably other factors (9). Ordinarily, the population will resort back to apterous oviparous females in the spring, and rearing returns to being relatively simple.

It is rare to find a greenbug in culture that has died from an infectious pathogen. However, parasitoids and predators can cause serious problems in cultures. The main parasitoid in the Great Plains is *Lysiphlebus testaceipes* (Cresson), small braconid. The females oviposit in all instars of the greenbug, and straw-colored mummies are formed. Greenbug reproduction is greatly reduced because of parasitization and annoyance from the wasps (6). Another native parasitoid, *Aphelinus nigritus* (Howard), less frequently invades cultures
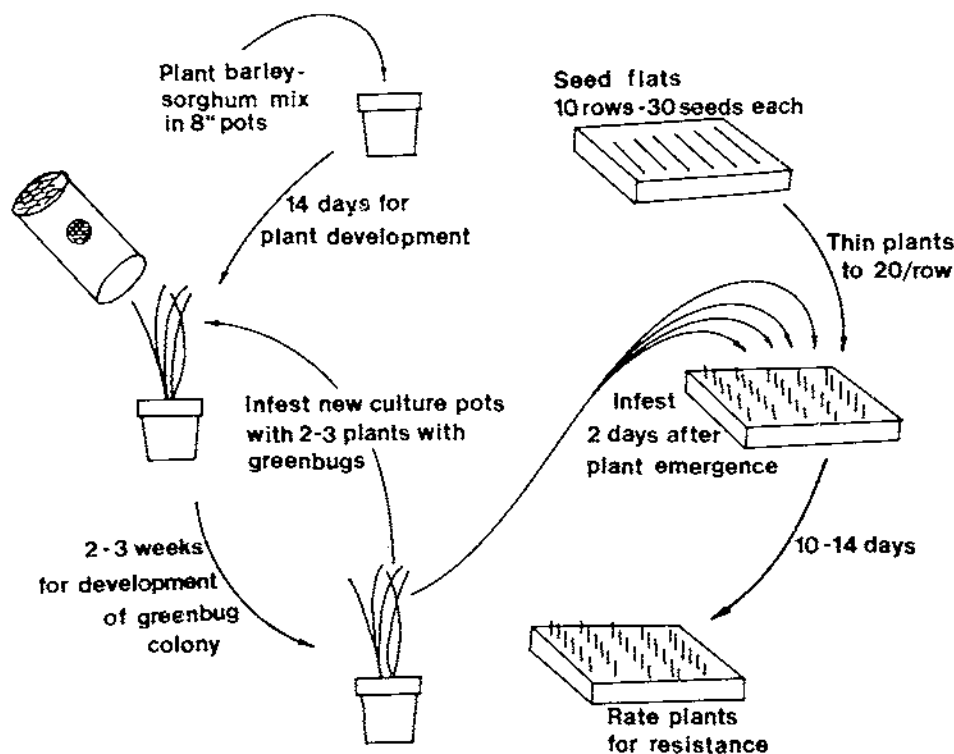
FIGURE 3.—Screening techniques for plant resistance.

and forms a black mummy. The best way to cope with parasitoids is to start with "clean" cultures and discard promptly any pots with evidence of parasitoids. The cages help to reduce parasitization and interference from other undesirable pests, but sometimes oviposition occurs through the mesh of the ventilation cloth. Mites can also pass through the mesh of the cloth, but these are seldom a problem unless plants are kept unnecessarily long.

Watering plants too frequently may promote mildews and some other diseases that can kill plants. Plants should not be watered until there is the first indication of wilting.

GREENHOUSE SCREENING FOR PLANT RESISTANCE

The greenbug is comparatively simple to manage in the greenhouse in a plant-resistance program (fig. 3). Greenbugs, unlike some insects, do not need to be caged over plants. Environmental factors such as light, temperature, and relative humidity can vary widely without deleterious effects on the greenbug. More consideration should be given to the growth needs of the plant than to those of the greenbug.

For our resistance screening, we use greenhouse flats filled to about 1 inch of the top with a unsterilized 3:1:1 mixture of soil, sand, and peat moss. Ten equally spaced rows about 1 inch deep are made in each flat by pressing a planting board on the top of the soil (fig. 4).

About 30 seeds are planted in each row and covered with sand up to the top of the flat. Before planting, seeds are treated with a fungicide but no insecticidal seed treatment is used. Flats are uniformally watered when necessary. Usually, a susceptible entry of the same species is randomly placed in each flat, and a known resistant entry is included once or more in each test. A test can contain any number of flats, depending upon the supply of greenbugs in the cultures and available spaces in the greenhouse. We prefer to use about 20 flats per test and have a series of tests spaced at weekly intervals, allowing about 180 entries (excluding susceptible checks) to be evaluated in each test. This number can be doubled by dividing the rows crosswise with a thin partition. (Of course, the number of seeds per entry should be reduced in half.) After emergence, plants can be thinned to 20 per row, but the number of plants will not influence results to any extent. Plants are infested with greenbugs of all ages and mixed biotypes about 2 days after emergence by either 1) brushing or shaking aphids from culture plants fairly uniformly over flats or 2) placing uprooted infested culture plants between rows and allowing greenbugs to crawl to the test plants (fig. 5). Two days after infestation, we examine flats, and those without adequate greenbugs receive additional infestations. About two greenbugs per

FIGURE 4.—Planting board and flat ready for seeding.
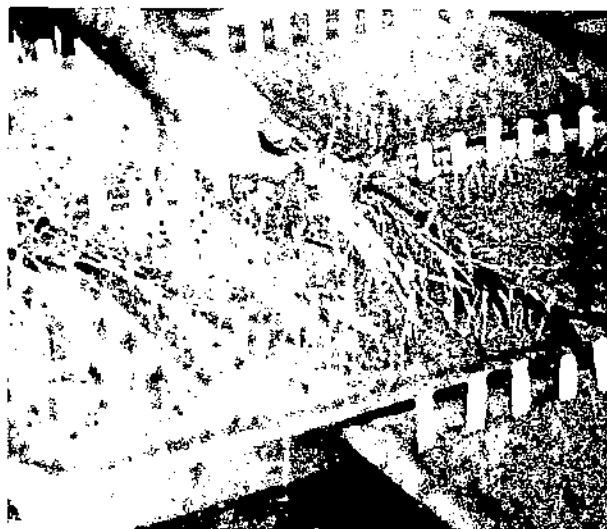
FIGURE 5.—Infestation of flats with greenbug-infested plants.

6

small grain plant and four per sorghum seedling are considered adequate at this time. Uniformity of the infestation can be encouraged by gently sprinkling the flats with water.

About 10 to 14 days after infestation, depending on growth conditions, plants are rated for resistance. Instead of using a predetermined period, we get more uniform results by rating each flat when the plants in the susceptible row are dying. We use a visual rating system of 1 for no injury to 6 for dead or dying plants. A scale divisible by 3 allows grouping into resistant, intermediate, and susceptible categories. At the start, a person may wish to make a photograph of the injury groups and use the picture as a reference for rating. Experience should soon make the reference unnecessary. For screening collections, an overall rating of the rows can be made; for segregating material, individual plants can be rated. If an additional criterion is considered necessary, compare average plant heights for each row taken at the time of infestation and again when the susceptible checks start to die.

Counts of greenbugs in flat tests at the time of rating mean little, since the aphids will move readily from dying plants to growing ones. Such migration is why greenhouse screening in flats is considered a severe test of resistance. In fact, we have yet to find levels of resistance in the greenhouse that would not prevail in the field. Also, resistance in seedlings has been shown to last until plants are mature (14).

Each screening test lasts for about a month. Soil mixtures can be reused if plant diseases are not a problem. Plants can be transplanted to large containers, fertilized, and taken to maturity.

Both damage ratings and plant height differences in flat tests measure confounding effects of antibiosis, nonpreference, and tolerance, but the last is emphasized. If resistance mechanisms are to be separated, then more detailed, better controlled tests are needed. Procedures for this are given by Schuster and Starks (11). In these tests, plants are caged with small plastic cylinders constructed like those used for rearing. A modification of the caging of the entire plant is the use of small snap-on plastic boxes on leaves (fig. 6). These can be used either in greenhouse or field tests
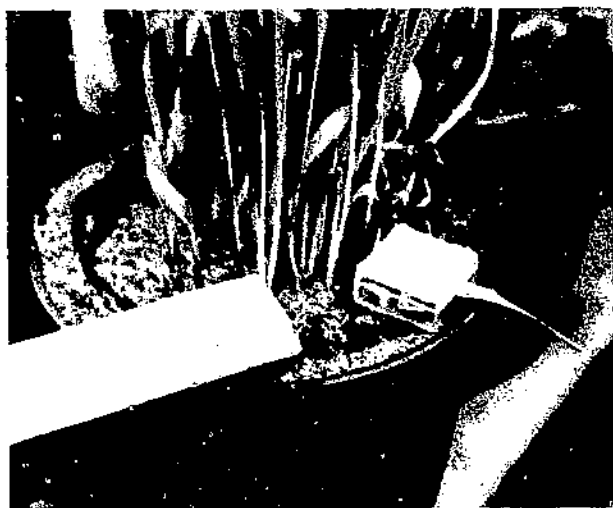
PN-5229

FIGURE 6.—Small snap-on plastic cage.

(15). Harvey and Hackerott used rooted seedlings in vials containing tapwater (5). A glass cylinder joined to each vial by a plastic fitting confined the greenbugs to the plants for detailed tests. Screening durations each year depend largely on greenhouse temperatures. We do most of our screening from November to April, but the sorghum screening period could be increased. Greenbugs may reproduce poorly during February and March, and these low populations may not cause enough damage to the plants to allow an adequate test. Also, the winged forms tend to leave the plants during this time. These problems usually do not persist beyond March.

Most greenhouses are not sufficiently tight to exclude unwanted invertebrates. Spiders can invade flats but cause limited problems. Marauding ants in southern locations can be disruptive. Plant pests such as other species of aphids, thrips, and mites can infest plants, but usually individual greenbug tests are finished before these pests reproduce sufficiently to interfere with results. By far the biggest problem is likely to be with L. testaceipes. Reproduction of this native parasitoid is rampant in screening tests where there is an abundance of the host. Prompt discarding of concluded tests will help to reduce problems. Even so, it usually is necessary to fumigate once or more during the year. Before fumigation, cultures should be removed, and the precautions printed on the fumigant label should be closely followed.

7

Although fumigants are valuable for reducing unwanted pests, other insecticides should not be used in greenhouses intended for greenbug-resistance research. Some insecticides present in greenhouses can have a long residual effect, making plant-resistance data unreliable. Even frequent use of short residual materials can result in problems. Mites and some other greenhouse pests may develop tolerance, making control extremely difficult.

The use of a mixture of greenbug biotypes is advisable, or else the resistance selection may be too narrow. Advanced plant entries should be evaluated with field biotypes from the area where released material is likely to be grown.

Experience should overcome the problem of heavily infested areas in flats (hotspots). Usually, repeated light applications of greenbugs give uniform infestations, whereas a single heavy application can cause hotspots.

Escapes (inadequately infested plants) are the menace of any plant-resistance program. If numerous, escapes can greatly increase the number of entries in progeny tests and delay progress. It is important to get all plants infested. On the other hand, heavy infestations can prevent the discovery of low or even moderate resistance levels that might be adequate for plant protection in the field. Since it is impractical to infest plants individually with greenbugs in screening tests, we suggest light infestations in unreplicated tests be used to eliminate most of the entries. Then, tests with two or three replicates and heavier infestations can be used to further reduce the number of entries. When plant-entry reactions are nearly the same, and further reductions are necessary, these should be done on an agronomic basis. Thus, in the screening process it is advisable to keep as wide a plant germplasm base as possible.

FIELD SCREENING
FOR PLANT RESISTANCE

So far, plant resistance to the greenbug located in greenhouse screening has held up under field conditions. Resistance has not broken down because of either high or low plant-growth temperatures. Instead, temperature extremes tend to enhance the detrimental effects of resistant plants on the greenbug (19). Also, maturation of the plant adversely affects the greenbug, and resistant plants tend to be less suitable hosts sooner than susceptible plants (14).

Resistance in the field may be complemented by weather. For example, hard rains, especially if accompanied by strong winds, can dislodge greenbugs from plants. Since plant resistance tends to make greenbugs restless, there may be an increased proportion of insects that are knocked off the plant and fail to reestablish.

The little research that has been done on the interaction of plant resistance and natural enemies indicates that the two control factors are compatible toward reducing greenbug populations and plant damage (13). Beneficial species such as L. testaceipes can increase to high numbers in the field, but usually their population buildup lags too far behind that of the greenbug to prevent economic damage. Plant resistance can hold the greenbug population in check or allow the plant to withstand high pest populations until native enemies become established.

For the above reasons, intermediate levels of resistance may look more promising under field conditions than in the greenhouse. All measurable levels of resistance should be later evaluated in the field. Space limitations and other factors will dictate the amount of material that can be taken to the field.

Plot size need not be large for nonsegregating plant material. (We usually do not try to select segregates in the field.) Also, replication is not necessary if the only objective is screening. For advanced material, plot size and replication can be similar to designs used for yield trials. It is often advisable to have plantings at more than one location to increase the chances of reaching adequate greenbug infestations.

Usually, sorghum planted early does not escape greenbug infestations. Greenbug-resistance tests taken to yield may have less interference from midges if planted about the same time as other sorghum in the area. On the other hand, sorghum planted late in an area may receive increased greenbug damage, since it takes fewer greenbugs to damage small sorghum plants. Only 10 to 15 greenbugs per

seedling can cause serious damage, whereas 1,500 greenbugs per plant may be necessary to cause yield losses to larger plants.

A greenbug count as a criterion of resistance may not be reliable, especially with low population buildup, since the variation from plant to plant can be relatively large. A high buildup with an average of several hundred greenbugs per plant makes detailed counts impractical. Estimates in groups of 10 or even 50 have to be made. Even though greenbug counts are variable, they should still be made, for they furnish valuable information on the magnitude of the infestation and the distribution over a given period. Greenbug counts are also an indication of antibiosis or nonpreference. Remember that populations can decline rapidly on plants severely injured or unhealthy for other reasons. We usually make weekly greenbug counts on 10 entire plants in each 20 or 30 ft of a row.

Leaf-damage ratings are fairly easy to make and offer a good measurement of tolerance. A scale similar to the one suggested earlier for greenhouse ratings may be used. Teetes et al. (16) used the following categories: "1—no red spotting on leaves; 2—red spotting on leaves; 3—portion of a leaf killed by greenbugs; 4—1 leaf killed; 5—2 leaves killed; 6—4 leaves killed; 7—6 leaves killed; 8—8 leaves killed; and 9—dead plants." This system would be suitable only for plants with more than eight leaves developed. Regardless of the scale, known susceptible and resistant entries should be repeated about every 10th row for comparison. We make weekly ratings, and often more than one person will separately rate the same material.

A count of dead leaves can be misleading, since sorghum bottom leaves normally die early or are removed by cultivation. A live-leaf count should be made downward from the flag leaf. The sorghum types on which live leaves are counted need to be similar to those of the standards used for comparison.

Yield is the final evaluation of a resistance program. Standard agronomy yield methods can be used. In addition to threshed grain weight, a head count, moisture percentages, and grams per 1,000 kernels can furnish useful information. Often greenbug injury will cause the main stalk to die, and tillers will be produced. The small heads may mature late or unevenly. Greenbug injury can also cause shrunken seed, especially if the infestation progresses to the panicle.

An alternative to natural infestations of greenbugs in the field is the use of cages for small tests. The cages can be relatively large (10 ft^2 or more) and enclose entire plants, or they can be small plastic cages (1 to 4 in^2) attached to a portion of a leaf. Large cages need to be constructed from wire screen with a small mesh so that parasitoids will be excluded. In large cages, greenbug populations after artificial infestation usually increase rapidly and damage can be accentuated. However, yield data may be meaningless because of unnatural plant growth conditions. Small plastic cages on leaves need cloth-covered ventilation holes on at least one side. Five to ten adult greenbugs can be put in each small cage, and damage ratings of the enclosed areas and progeny counts can be made. Artificial infestation in the field without cages has not been successful. Natural infestations must be relied upon. Many areas where small grains and sorghum are produced may not have dependable natural infestations of greenbugs. For this reason, field tests may have to be repeated several times before reliable results can be obtained.

Commonly, plant-resistance evaluations have not been possible because of insecticidal contamination of plots in the field. The insecticide may drift from a nearby field, especially during aerial application. Uneven contamination may reduce greenbugs in some spots but not in others, giving erroneous data on plant resistance. Insecticides should not be used on or near plant-resistance evaluation trials.

Plant diseases may cause leaf injury that confuses greenbug-damage ratings and confounds yield reductions. It may be necessary to introduce greenbug resistance and disease resistance separately into a program. For example, IS 809 sorghum is highly resistant to the greenbug but is highly susceptible to a maize dwarf mosaic virus (MDMV) that is transmitted by the greenbug and other aphids. There are available sources of MDMV resistance, and this should be incorporated into any material that is being developed with IS 809.

CULTURING PRIMARY AND SECONDARY PARASITOIDS

A prerequisite for rearing aphid parasitoids is the successful rearing of a host, such as the greenbug. The cages described earlier can be used for parasitoids. All parasitoids in the following techniques are handled as adults by using aspirators made from small bottles and flexible tubing with pasteur capillary pipettes as collecting tips. Collection is made from upper portions of the cages, since the adults are positive phototropic. Infestation of new pots only require placing the open bottle inside the new cages and allowing the adults to escape. Parasitoids should be fed a 10 percent sugar or honey solution impregnated onto cotton, since this procedure will increase larval production. Mating usually occurs soon after adult emergence.

Primary parasitoids. — We culture a native braconid parasitoid, *Lysiphlebus testaceipes* (Cresson), of the greenbug in the greenhouse. This primary parasitoid is found commonly throughout the Great Plains, sometimes in large numbers when aphid populations are high. It plays an important role in natural control of aphids, particularly the greenbug, its preferred host (*6*).

Figure 7 shows a schematic for culturing *L. testaceipes.* Fourteen-day-old sorghum or barley plants are infested with about 100 adult greenbugs. At the same time, approximately 40 unsexed parasitoids are added to the cage. After 12 to 14 days, the first generation of *L. testaceipes* can be harvested. Some original greenbugs and their offspring escape parasitization by initial infestation and are parasitized by emerging adults before collection, creating
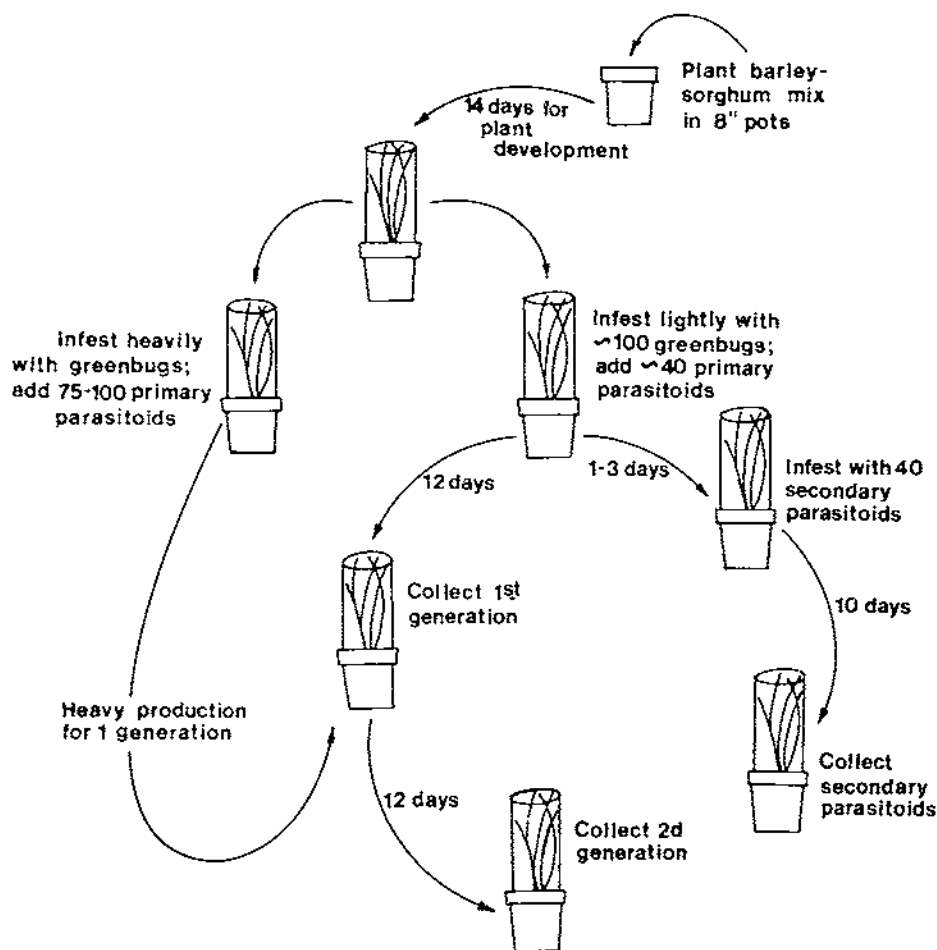

FIGURE 7.—Culturing techniques for primary and secondary parasitoids.

a second generation another 12 to 14 days later. While this two-generation technique has a decided advantage in maintaining a continuous colony, it does produce insects more slowly. For maximum production over a shorter period, an alternate technique is used. The pot is heavily infested with greenbugs, and about twice the number of parasitoids (75 to 100) are added. Plants can survive the greenbug attack just long enough to allow sufficient development of the parasitoids, thus producing only one generation. If larger quantities are required, a technique for rearing in flats was described by Starks et al. (12).

Secondary parasitoids. — By means of the above cultures of primary parasitoids, the secondary parasitoid, *Charips* sp., can be cultured (fig. 7). One to three days after infesting plants with greenbugs and adding 40 primary parasitoids (fig. 3), about 40 unsexed secondary parasitoids are added to the cage. Emergence occurs about 10 days later, depending on temperature.

Possible difficulties. — One of the more serious problems in culturing parasitoids and secondary parasitoids is the accidental mixing of colonies. Rearing greenbugs can be a problem if colonies are contaminated with *L. testaceipes*, and, likewise, *L. testaceipes* rearing can be a problem if contaminated with secondary parasitoids. The best solution is separate rearing areas for each entity, that is, plants, greenbugs, primary parasitoids, and secondary parasitoids. Proper sanitation of equipment such as washing cages can prevent cross-contamination. Proper caging of pots can prevent much of the contamination but not all. Two other preventative suggestions include the use of only *L. testaceipes* adults (not mummies) for infestation and the use of separate aspirators for each insect.

The short adult life (2 to 3 days) of *L. testaceipes* should be kept in mind, and processing of this stage expedited as soon as possible after emergence to fully realize the potential of the insects. *Charips* sp. lives 5 to 7 days and survives handling better than *L. testaceipes*.

An unmated *L. testaceipes* will lay eggs, and the resulting offspring will be all males. A predominance of males may mean that mating was not adequate.

LITERATURE CITED

(1) Chada, H. L. 1959. Toxicity of cellulose acetate and vinyl plastic cages to barley plants and greenbugs. J. Econ. Entomol. 52: 276–279.

(2) Cress, D. C., and Chada, H. L. 1971. Development of a synthetic diet for the greenbug. *Schizaphis graminum*. 1. Greenbug development on two synthetic diets. Ann. Entomol. Soc. Am. 64: 1237–1240.

(3) Dahms, R. G., Connin, R. V., and Guthrie, W. D. 1954. Grasses as hosts of the greenbug. J. Econ. Entomol. 40: 1151–1152.

(4) ———, Johnson, T. H., Schlehuber, A. M., and Wood, E. A., Jr. 1955. Reaction of small grain varieties and hybrids to greenbug attack. Okla. Agric. Exp. Stn. Tech. Bull. T–55, 61 pp.

(5) Harvey, T. L., and Hackerott, H. L. 1969. Plant resistance to a greenbug biotype injurious to sorghum. J. Econ. Entomol. 62: 1271–1274.

(6) Hight, S. C., Eikenbary, R. D., Miller, R. J., and Starks, K. J. 1972. The greenbug and *Lysiphlebus testaceipes*. Environ. Entomol. 1: 205–209.

(7) Jensen, R. E., and Wallin, J. R. 1965. Weather and aphids: A review. U.S. Dep. Commer., Environ. Sci. Serv. Admin. Tech. Note 5–AGMET–1, 19 pp.

(8) Mayo, Z. B., Jr., and Starks, K. J. 1972. Sexuality of the greenbug, *Schizaphis graminum*, in Oklahoma. Ann. Entomol. Soc. Am. 65: 671–675.

(9) ———, and Starks, K. J. 1974. Temperature influences on alary polymorphism in *Schizaphis graminum*. Ann. Entomol. Soc. Am. 67: 421–423.

(10) Peters, D. C., Wood, E. A., Jr., and Starks, K. J. 1975. Insecticide resistance in selections of the greenbug. J. Econ. Entomol. 68: 339–340.

(11) Schuster, D. J., and Starks, K. J. 1973. Greenbugs: Components of host-plant resistance in sorghum. J. Econ. Entomol. 66: 1131–1134.

(12) Starks, K. J., Burton, R. L., Teetes, G. L., and Wood, E. A., Jr. 1976. Parasitoid release for greenbug control on sorghum. U.S. Dep. Agric., Agric. Res. Serv. [Rep.] ARS–S–91, 12 pp.

(13) ———, Muniappan, R., and Eikenbary, R. D. 1972. Interaction of plant resistance and parasitism against the greenbug on barley and sorghum. Ann. Entomol. Soc. Am. 65: 650–655.

(14) ———, and Wood, E. A., Jr. 1974. Greenbugs: Damage to growth stages of susceptible and resistant sorghum. J. Econ. Entomol. 67: 456–457.

(15) Teetes, G. L., and Johnson, J. W. 1973. Damage assessment to the greenbug in grain sorghum. J. Econ. Entomol. 66: 1181–1185.

(16) ———, Johnson, J. W., and Rosenow, D. T. 1975. Response of improved resistant sorghum hy-

brids to natural and artificial greenbug populations. J. Econ. Entomol. 68: 546–548.

(17) ———, Schaefer, C. A., Gipson, J. R., McIntyre, R. C., and Latham, E. E. 1975. Greenbug resistance to organophosphorus insecticides on the Texas High Plains. J. Econ. Entomol. 68: 214–216.

(18) Wood, E. A., Jr., Chada, H. L., and Saxena, P. N. 1969. Reaction of small grains and grain sorghum to three greenbug biotypes. Okla. State Univ. Agric. Res. Prog. Rep. P-618, 7 pp.

(19) ———, and Starks, K. J. 1972. Effect of temperature and host plant interaction on the biology of three biotypes of the greenbug. Environ. Entomol. 1: 230–234.

END