SCREENING ALFALFA SEEDLINGS FOR RESISTANCE TO THE TARNISHED PLANT BUG, LYGUS LINEOLARIS (PALISOT DE BEAUVOIS)

by

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TABLE OF CONTENTS

	PAGE
INTRODUCTION	1
REVIEW OF LITERATURE	4
MATERIALS AND METHODS	14
Rearing	14
Overwintering	15
Methods of Screening Plants	16
Seedling Damage Experiments	19
RESULTS AND DISCUSSION	25
SUMMARY AND CONCLUSIONS	55
LITERATURE CITED	59

Introduction

Three species of the genus Lygus Hahn (Hemiptera: Miridae) have been reported as causing major damage to alfalfa, (Medicago sativa Linnaeus) especially to seed production. The tarnished plant bug, L. lineolaris (formerly known as L. pratensis and L. oblineatus), is primarily an eastern species although occurring as far west as California. This is the most prevalent species of Lygus near Manhattan, Kansas. L. hesperus Knight and L. elisus Van Duzee are primarily western species, but also occur in small numbers in the Midwest. For historical information and distribution of Lygus spp. see Crosby and Leonard (1914), Knight (1917), Keck (1928), Shull (1933b), Stitt (1940), Knight (1941), and Hughes (1943).

According to studies made by Carlson (1940), Hughes (1943), and Jeppson and MacLeod (1946), the type of damage to alfalfa caused by these three Lygus species is very similar. Although Stitt (1944) reported some differences among species concerning the amount of damage to alfalfa seed production per individual insect, the differences between L. hesperus and L. lineolaris were not significant. Significant differences were obtained only in the amount of brown seed caused by L. hesperus and L. elisus. All three species reduced pod-set significantly and caused highly significant increases in percentage of brown (nonviable) seed. There have been no known studies concerning differences between the three species in reducing the vegetative growth of alfalfa. Also, it was found that some authors made no distinction as to which species was being reported on, referring to "lygus bugs" or Lygus spp.

It was decided, therefore, to include information concerning western species of Lygus, as much quantitative research data have resulted from these studies. If a paper concerned particular species it shall be so stated. In other cases, lygus bugs or Lygus spp. will be used.

Although the damage caused by Lygus spp. to alfalfa is generally well known, there have been few published studies concerning alfalfas resistant to these insects. Perhaps the reason for this is that plant damage caused by Lygus spp. is generally not of a spectacular nature and is therefore not as easily noticed as that caused by other insects. Also, differences in resistance to Lygus spp. among varieties or plants may not be as large as those found in studies concerning plant resistance to other insects.

Much of the search for alfalfas resistant to Lygus spp. has involved mature plants studied in the field. This seems to be a logical approach due to the severe damage to seed production caused by these insects. However, Lygus spp. can also markedly reduce the vegetative growth of alfalfa.

The primary objectives of this study were to develop methods for rapidly screening large numbers of alfalfa plants for resistance to Lygus spp., especially L. lineolaris. In an effort to accomplish this, alfalfa seedlings were used in all experiments. If a reliable method of screening seedlings for resistance to these insects is developed, the work of locating resistant plants will take less time, facilities, and expense.

Other objectives included:

1. What are the effects of given Lygus populations on different seedling growth stages?
2. What are the effects of Lygus on seedling growth?

3. Do adults or nymphs cause heavier seedling damage?
4. Can varietal differences in resistance be detected in seedling growth stages?
5. What are the effects of temperature on plant damage?

The above categories are general ones, and individual experiments sometimes had more than one objective.

The definition of resistance used in this study was that of Painter (1951): "the relative amount of heritable qualities posessed by the plant which influence the ultimate degree of damage done by the insect." Painter also pointed out that resistance is generally relative and is "definable only in terms of other and usually more susceptible varieties."

Review of Literature

Biology and life cycle

The tarnished plant bug undergoes incomplete metamorphosis with the nymphal period consisting of five instars. Eggs are whitish to gray in color, about 1 mm long and .25 mm wide, flask-shaped, and with the anterior end slightly curved and compressed toward the apex (Crosby and Leonard, 1914). Stitt (1940) reported data concerning the location of Lygus spp. eggs deposited on alfalfa plants. Of those eggs observed, 44.9% were in flower buds, 24.7% in stem internodes, and 14.5% in and near stem nodes. No eggs were found in leaves or leaf petioles.

First and second instar nymphs are yellowish green and are sometimes mistaken for aphids. Tarnished plant bug nymphs can be distinguished from aphids by the absence of cornicles, the dark scent gland on the dorsal side of the abdomen, and their very rapid movements. Distinct wing pads appear in fourth and fifth instar nymphs. Adults vary somewhat in their coloration and size, but they are generally brownish, mottled with various shades of yellowish and reddish brown and are about one-fourth inch in length (Smith and Franklin, 1961).

Ridgway and Gyrisco (1960a) conducted experiments dealing with the effect of temperature on length of the life cycle. The mean incubation period of eggs ranged from 42.08 days at 15 C to 6.04 days at 35 C. Nymphs hatching at 35 C did not survive. The mean nymphal period ranged from 31.47 days at 20 C to 14.91 days at 30 C; the fifth instar averaged the longest at all temperatures. The above data generally agree with that reported by Smith and Franklin (1961), although these authors included no temperature data. Beards

and Leigh (1960) reported that L. hesperus eggs hatched in 6 to 8 days, with nymphal development taking approximately 16 days at 78-80 F. High bug mortality occurred when laboratory temperatures exceeded 95 F.

In laboratory studies on the life history of L. hesperus, Leigh (1963) found that adult males lived an average of 35.2 days and females an average of 43.4 days when reared on bean sections at 80 $\pm$ 2 F. Landes and strong (1965) reported that L. hesperus adult males survived an average of 46.4 days when reared on fresh bean juice ingested through a Parafilm membrane.

Curtis and McCoy (1964) and Taksdal (1963) found that oviposition preferences of female tarnished plant bugs generally did not correlate with feeding preferences of first instar nymphs.

Shelford (1951) found that size of tarnished plant bug populations showed correlations with rainfall, temperature, and amount of sunlight. Smith and Michelbacher (1946) reported that the population increase of Lygus spp. was variable, depending on temperature and moisture conditions. Hot and/or dry conditions caused a rapid life cycle due to the insects' faster development. The life cycle was longest, and Lygus were more numerous, when moisture was plentiful.

Haseman (1918) stated that in Missouri 20-25 days were required to complete the life cycle in summer; this allowed four or five generations per year. Ridgway and Gyrisco (1960b) reported at least two and probably three annual generations in New York. Hixon (1955) reported that data indicated a generation of Lygus spp. for each alfalfa hay crop in Nebraska, with five generations per year possible in an undisturbed area. In Kansas there are at least three generations per year with time for a fourth, but a fourth generation has not been positively identified. Smith and Franklin (1961) found the

third generation maturing in late August and early September; this allowed time for a fourth generation. These authors stated that, in Kansas, tarnished plant bugs were more numerous in alfalfa fields in the fall than many other insects.

Crosby and Leonard (1914) found males and females overwintering in about equal abundance in New York. Forbes (1884) stated that older nymphs also overwintered under leaves of mullein plants (Verbascum sp. Linnaeus). Hughes (1943) reported that only adults were known to overwinter in Minnesota, as did Haseman (1918) concerning Missouri. Nymphs have not been found overwintering in Kansas, but adults have been taken from under and about alfalfa plants every month during the winter. Overwintering individuals were darker in color than those found during the summer (Smith and Franklin, 1961). These authors also reported finding overwintering eggs of the tarnished plant bug and rapid plant bug, Adelphocorus rapidus Say, in alfalfa stems. The eggs failed to hatch when brought into the greenhouse.

These insects have been found overwintering in many different hibernating sites, but they are generally found underneath some sort of leaf or lumber debris. Keck (1928) found tarnished plant bug adults hibernating in bunch grass, under leaf litter, around old buildings, in alfalfa stubble, and in sudan grass. Overwintering females were taken and placed on alfalfa in the greenhouse. Fertile eggs were deposited on the plants within a few days.

Stitt (1949) stated that practically all Lygus spp. infestations in the main alfalfa seed crop came from surrounding cultivated or irrigated land. Stitt also noted that a change in cropping practices caused a change in the percentage of each Lygus species in some areas of southern Arizona and California. Formerly 90% of the lygus bugs were L. hesperus, but with a rise

in sugar beet acreage, over 50% of the bugs in adjacent alfalfa fields were L. lineolaris. Smith and Michelbacher (1946) reported difficulty in determining population trends in any particular field because of migrations from other wild and cultivated hosts and uneven cutting of neighboring alfalfa fields. Hixon (1955) stated that alfalfa fields larger than 30 acres had larger tarnished plant bug populations per sample unit than smaller fields.

Other data on the biology and life cycle of Lygus spp. have been gathered by Sorenson (1932), Shull (1933a), Knowlton and Sorenson (1943), Hughes (1943), Salt (1945), and Strong and Landes (1965).

Lygus spp. damage to alfalfa

Keck (1928) stated that plants infested with male tarnished plant bugs were killed as quickly as those infested with females. Shull et al. (1934), in experiments dealing with the relation of L. hesperus and L. elisus to flower fall, found that this type of injury was caused both by males and females. In five test cages containing only males, no seed pods formed.

Stitt (1940) stated that both adults and nymphs fed on the young ovary, causing loss of seed pods. Anderson et al. (1952), in giving instructions for sampling the Lygus spp. population to determine insecticide application time and dosage, stated that nymphs should count double (one nymph equals two insects) in determining numbers present. This indicates that Lygus nymphs cause at least as much damage to alfalfa as adults.

Anderson et al. (1952) stated that Lygus spp. are the most important insect pests of alfalfa seed and may destroy the entire crop. In Kansas, plant bugs, of which Lygus spp. are the most numerous, probably cause the most severe damage to the alfalfa seed crop (Sorensen et al., 1958). Franklin

(1951) reported the tarnished plant bug to be one of the most abundant injurious insect in alfalfa fields near Manhattan, Kansas, and cited their feeding habits as causing damage far out of proportion to their numbers.

Stitt (1940) placed Lygus spp. effect on seed production into two classes: 1) injury to flower buds and flowers by feeding and egg laying, causing "blasted buds" and "flower fall"; 2) feeding on the immature seed pods before they are mature. In fields observed, there was an average loss of about 17% in seed production. Carlson (1946) reported that an uncontrolled infestation of Lygus spp. reduced seed production about as effectively as the absence of pollinating insects. This author also stated that the consistently lower percentage of tripped flowers in lygus-infested plots suggested a possible reluctance of pollinating insects to visit lygus-infested alfalfa. Sorenson (1944) cited insect pests, of which Lygus spp. were the most injurious, as one of the main causes for the decline of alfalfa seed yields in the West from 1941-1945. Klostermeyer (1962) stated that in Washington one lygus bug caused about as much damage as 40 pea aphids, Acyrthosiphon pisum (Harris).

Hughes (1943) reported 100% blasted buds after 240 hours exposure to Lygus spp. at a 1:3 insect to raceme ratio, and 40% blasted buds after 48 hours at a 1:2 ratio. This compared with 14% blasted buds on check plants. There was an average of 63.50% flower fall after 24 hours exposure at a 1:10 insect to flower ratio, compared with 35% flower fall in control cages. Sorenson (1932) reported 92.40% flower fall after plants were exposed to Lygus spp. for 10 days at a 1:30 insect to flower ratio. Carlson (1940) stated that bleached and discolored buds gave an early indication of Lygus spp. damage to alfalfa in the full-bloom stage. Rosetting, characterized by the development of bud racemes near the tips of main stems and branching

into disk- or knoblike clusters, was a later effect. Other data concerning Lygus spp. in relation to seed production have been gathered by Sorenson (1936), Carlson (1945), Bolton and Peck (1946), and others.

Shull et al. (1934), working primarily with L. hesperus and L. elisus, found that hay yields were reduced 17.5% compared with uninfested plants. The conclusion was that sufficient numbers of Lygus would considerably reduce hay yields. Sorenson (1939) noted an 8 to 35% reduction in stem lengths after confining L. hesperus and L. elisus on alfalfa plants. Carlson (1940) measured stem lengths of plants grown in the field under a natural infestation (primarily L. hesperus and L. elisus) and observed an average difference of 6.8 inches in 22 days of growth between infested plants and plants protected by dusting 5-7 times a week. Also observed was an increase in the number of stems on infested plants (40 stems) compared with uninfested plants (24 stems). Air-dried forage of continuously infested plants weighed significantly more than that from plants uninfested from prebud stage through harvest.

MacLeod and Jeppson (1942), working with the same two species as Carlson, found that, in general, both green and dry weights of plants were reduced in direct proportion to the number of insects feeding on them. In a later paper (Jeppson and MacLeod, 1946), these authors reported that the daily growth rate of alfalfa was consistently reduced by 6, 8, or 16 Lygus per plant, compared with uninfested checks. Two or four insects per plant reduced daily growth increment 30-60% if plants were infested within 4 days after cutting. These authors also stated that the possible reason for Carlson's (1940) data reporting infested plants to weigh more was an inhibition of the plants' reproductive growth by the insects which resulted in the continuation of

vegetative growth over a longer period. Stitt (1948) found that plant height was reduced 49 to 56% by 10 or 25 Lygus spp. feeding on plants from the time they were 2-4 inches tall (after cutting) until the next hay crop would normally be cut.

Phytotoxicity and histological studies of Lygus spp. injury

King and Cook (1932) classified Lygus spp. as cotton pests and found damage by individual bugs quite variable. This was cited as evidence indicating the presence of an irritant or phytotoxin in the saliva. Carter (1939) reviewed much of the literature dealing with phytotoxicity of Lygus spp. and other mirids. Hughes (1943) reported that injury to buds, flowers, and young seed pods was thought to be mainly phytotoxic. In histological studies, injury to flowers was found to be localized, with injury surrounding feeding punctures spreading to other parts of the injured flower. This injury did not affect the raceme as a whole, however, since a single injured flower fell off within a few days. Jeppson and MacLeod (1946) found that large areas of cell disintegration near terminal and lateral bud primordia followed feeding by L. hesperus and L. elisus. There was only a small area of disintegration surrounding the path made by the mouthparts, but a large area around the point of feeding. A lateral bud usually substituted for the damaged terminal meristem which enabled stem growth to continue; however when alfalfa in the reproductive growth stages was damaged by feeding, there was no evidence of such a substitution. This was offered as a possible explanation for the consistent injury by Lygus spp. to seed production.

Flemion et al. (1954) stated that the stylets of L. lineolaris: 1) were moved rapidly when in plant tissue; 2) were frequently plunged into and

withdrawn from tissue; and 3) travelled in random directions. The stylets travelled both intra- and intercellularly. Many cells in the feeding area were affected, either by being collapsed or in various stages of disorganization.

Control of Lygus spp.

The tarnished plant bug is a widespread insect pest that has been recorded feeding on more than 50 economic plants as well as weeds and grasses (Knight, 1941). Taksdal (1963) stated that L. lineolaris has a host range including 120 plant species representing 30 plant families; this wide host range coupled with their very active nature often make effective control of these insects difficult. Sorenson (1944) cited population density, a wide range of host plants, high reproductive capacity, and difficulty of control as the major reasons for their injurious nature to alfalfa. Smith and Michelbacher (1946) stated three reasons for the difficulty of controlling lygus bugs with insecticides: 1) large scale migrations of the insects from field to field; 2) development of large populations early in the season, the result in part of a wide range of hosts; and 3) the long period that buds, blossoms, and seeds must be protected. Andres et al. (1955) found that lygus bugs increased their tolerance to DDT 3-4 times during the growth of one alfalfa seed crop. Ridgway and Gyrisco (1961), Bacon et al. (1960), Scholl and Medler (1947), and others reported data concerning insecticidal control of Lygus spp.

Stitt (1949) stated that controlling the weed hosts of Lygus spp. would probably reduce populations that attack alfalfa.

Biological control of Lygus spp. in the United States is apparently not very successful. Stitt (1940) reported no parasite of adults, nymphs, or

eggs of Lygus spp. Some predators, such as Geocoris spp. (Hemiptera: Lygaeidae), Nabis ferus (Linnaeus) (Hemiptera: Nabidae), Formica peripilosa (Wheeler) (Hymenoptera: Formicidae), and two species of spiders were reported to have fed on nymphs. F. peripilosa was the only predator found that noticeably reduced nymphal numbers in any area. Smith and Franklin (1961) observed two species of spiders, a small black ant, and a tree cricket feeding on nymphs. Knowlton (1944) reported the green collops, Collops hirtellus (Coleoptera: Malachiidae), feeding on lygus bugs. McGregor (1927) reported some species of birds, spiders, and reduviids as destroying considerable numbers of lygus bugs. Wene and Sheets (1962) stated that predators present in Arizona cotton fields were not very effective against lygus bugs.

Crosby and Leonard (1914) reported a mymarid parasite as destroying small numbers of tarnished plant bug eggs. Muesebeck (1936) reported rearing two species of parasitic wasps from Lygus spp.

Resistance of alfalfa to Lygus spp.

Aamodt and Carlson (1938) found some alfalfa varieties able to flower in spite of lygus bug injury. The insects were present in about equal numbers on all varieties tested; the difference in injury apparently lying in the ability of some varieties, especially Grimm, to recover. Some strains more resistant than the average were found within Grimm. Malcom (1953) reported significant differences in Lygus spp. populations on alfalfa varieties tested in Washington. Ranger and Buffalo had the highest populations whereas Ladak and Turkestan had the lowest. Nielson and Schonhorst (1965) sampled Lygus spp. populations on many alfalfa varieties in Arizona and found significant differences. Zia, Rhizoma, and Stoneville polycross no. 1 were the most promising varieties. These authors stated that alfalfa varieties of different

growth classes should be evaluated separately, because alfalfa varieties "of different growth characteristics under simultaneous observations may tend to show corresponding differences in the lygus bug population, . . . thus masking any real evidence of resistance that may be present."

Taksdal (1963) suggested that there was a possibility that L. lineolaris evolved races, if sufficiently isolated from other plant communities, according to the composition of the plant community where the insects lived. His data indicated that adult L. lineolaris populations collected from different plant families preferred to feed and rest on plants representing the family from which they were originally collected. If these plant communities consisted of cultivated crops, a population may develop an increased ability to survive and multiply on the crop and cause increased damage, thus decreasing the permanence of any resistance that may be developed.

Use of seedlings in resistance screening

Screening alfalfa seedlings for resistance to insect pests is a relatively recent technique. This method was successful in the development of Cody alfalfa, which is resistant to the spotted alfalfa aphid, Therioaphis maculata (Buckton), (Harvey et al., 1960). Alfalfa seedlings have also been screened for resistance to the pea aphid, Acyrthosiphon pisum (Harris), (Hackerott et al., 1963).

Materials and Methods

Rearing

The primary objectives of the rearing methods used in this study were to develop techniques of maintaining insect populations large enough for resistance screening. No attempt was made to gather life history data when using the different methods.

Rearing was attempted following the methods of Beards and Leigh (1960) for L. hesperus. Fresh green bean pods (Phaseolus vulgaris L.) served as food and oviposition sites. Quart mason jars, circular clear plastic containers 2 3/4 inches high and 3 3/4 inches in diameter, and shell vials 95 mm high and 25 mm in diameter were used as containers for the insects. Mason jars were topped with fine mesh screen, and the plastic containers and shell vials were topped with Saran Wrap. Bean pods were replaced every other day, and if found to contain eggs, placed in a jar to await egg hatch.

Alfalfa seedlings combined with fresh bean pods were used in rearing trials. A few fresh bean pods were placed in a flat of alfalfa seedlings, allowing the insects to feed and oviposit on both. Bean pods were replaced every other day. Alfalfa seedlings combined with carrot roots, celery stalks, and frozen green bean pods also were used following the same method. Frozen beans were removed from the freezer and placed in a refrigerator to thaw overnight before being introduced into cages containing insects. When included with alfalfa, frozen beans were replaced daily; the other food sources were replaced about every three days. Some preliminary rearing experiments using frozen beans alone were attempted.

Sprouted alfalfa seeds also were used as a possible food source in rearing. Seeds were allowed to sprout on moist blotters in clear circular plastic containers topped with Saran Wrap. A certain number of nymphs was then introduced into the containers. Nymphs were transferred to another container with newly-sprouted seeds about every 7 days.

Rearing was attempted both in the greenhouse, where temperatures fluctuated widely, and in two Percival E-57 plant growth chambers. Various temperature ranges were used in all experiments in the plant growth chambers, with a 10 F difference between day and night temperatures. Rearing temperatures were generally 80 F (day) and 70 F (night).

Overwintering

An overwintering study was conducted during the winter of 1965-66. The objectives of this study were to determine good overwintering sites for these insects, and if overwintering Lygus could be collected, placed in one area, and gathered when needed for resistance screening.

One part of the study consisted of placing five cages in the field and providing different overwintering sites (alfalfa hay, leaf litter, uncut alfalfa, etc.). All cages were bottomless, but cardboard squares matching cage dimensions were placed over the ground in two cages.

Four square cages, 24 inches on each side and 20 inches high, were placed in a somewhat protected area behind the south wall of a building. A fifth cage, 60 inches by 35 inches and 16 inches high, was placed in an uncut area of an alfalfa field. All cages had wood frames covered with fine mesh screen. Cage bases were placed in 1 to 2 inch deep trenches matching cage dimensions in an attempt to minimize escapes by bugs.

Adult tarnished plant bugs were collected in November and various numbers introduced into the cages. The insects were removed approximately 120 days later and recovery data recorded.

Another part of the study was conducted in a refrigerated room in the Department of Entomology. Various numbers of field-collected adult insects were caged either on mullein or alfalfa plants taken from the field and planted in pots. The number of males and females was determined before being placed in the cages. The temperature in the room was 45 $\pm$ 3 F, with light provided 12 hours per day by two 40-watt fluorescent lamps. After approximately 90 days the insects were removed and survival data recorded.

Methods of screening plants for resistance

Three different cage types were used in various experiments:

1) Large (11 inches by 20 inches) wood flats with 8 to 16 alfalfa varieties grown in rows were used. Plants were enclosed by rectangular glass cages 7 inches high and slightly smaller than the dimensions of the flat. The cages were topped with Lumite inserted in a wood frame that fitted over the top of the glass. Two to four replications were planted in each flat, depending on the number of varieties used and the planting arrangement.

Various methods were employed in an attempt to obtain even distribution of insects on the plants within these cages. In some cases black cloth was placed over tops of the cages to prevent the insects from flying toward the overhead lights, thus gathering at the cage tops. In one instance green construction paper was attached to the glass in an attempt to lessen the insects' tendency to congregate around the cage sides. Cages with lower tops also were used in an effort to keep more insects on the plants below.

2) Round plastic pots 4 inches and 12 inches in diameter were used, and, depending on the size of pot used, one to four varieties were grown in each. Plants were not grown in rows but were planted in different quarters of the pots. Glass battery jars 8 inches in diameter and 12 inches high with Lumite tops served as cages on large pots, with lantern globes used on small pots. Embroidery hoops with Lumite inserted were used as cage tops on lantern globes. Screen strips approximately 1 1/2 inches wide were attached to bottoms of both battery jars and lantern globes to aid air circulation inside the cages.

Groups of one or two varieties were planted in small pots. If two varieties were planted, each variety occupied one-half of the pot. Four varieties were planted in the large pots; each variety occupied one-fourth of the area within a pot.

3) Square aluminum pans (9 inches on each side and 2 inches deep) were used, in which eight rows of plants spaced 1 inch apart were grown. One to three varieties were grown in each pan. Plants were enclosed by square glass cages 5 inches high and covered with Lumite screen inserted in a wood frame that fitted over the top of the glass.

Experiments were conducted in two types of plant growth chambers. Much of the early work in this study was done in a growth chamber with constant temperature and continuous illumination. Illumination was provided by overhead 40-watt fluorescent lamps. Although not measured, light intensity varied considerably throughout the chamber. Later experiments were conducted in two Percival E-57 plant growth chambers in which temperature, light, and day length could be regulated.

The insect to plant ratios discussed below refer to the number of Lygus per plant at the beginning of an experiment. No attempt was made to maintain a constant insect population after the start of an experiment and no distinction was made between sexes of Lygus used.

In some cases, when large numbers remained alive after termination of an experiment, the insects were removed from cages and used in another experiment or in rearing. To accomplish this without raising the cage top and allowing many insects to escape, a 1-inch hole was cut in the cage top, and the rest of the top and sides of the cage were covered with black cloth. Two holes were cut in a clear plastic box measuring 3 inches by 2 inches by 1 1/4 inches. One hole was cut in the bottom of the box and was slightly larger than the hole in the cage top. Another 3/8-inch hole was cut at one end of the box and a short piece of plastic tubing inserted. Two cellulose nitrate centrifuge tubes were taped head to head, with a hole matching the diameter of the plastic tubing cut at the end of one tube. A piece of fresh green bean pod was placed inside the centrifuge tubes and the free end of the plastic tubing inserted into the hole. This apparatus was placed on top of the cage with the large hole in the plastic box over the hole in the cage top. A light was placed above the cage to attract the insects to the hole. Centrifuge tubes containing Lygus were removed daily and replaced by new tubes containing fresh bean pods. Insects were removed from the tubes with an aspirator and transferred to rearing cages or another screening experiment. Remaining adults and nymphs were removed by allowing them to gather on fresh bean pods placed in the cage. Beans were removed daily and placed in rearing containers.

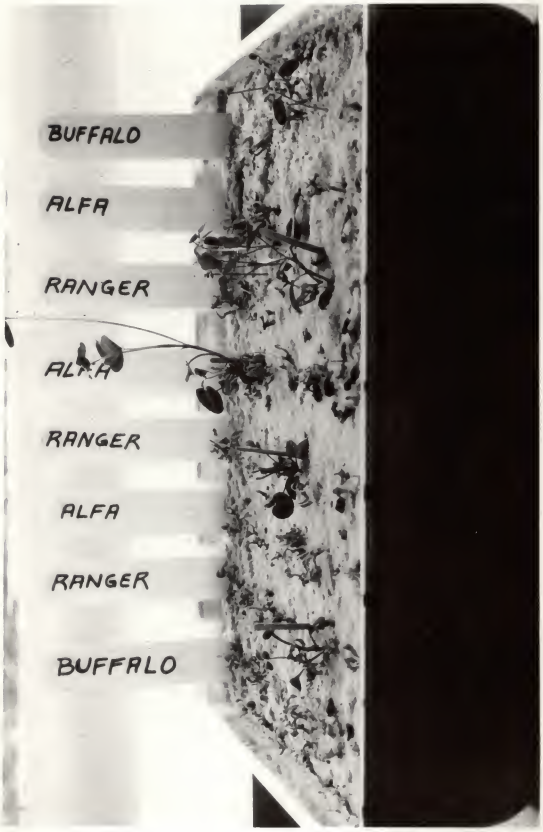
Soil was sterilized in an autoclave before plantings were made. Seedlings in one of the three early growth stages (cotyledon, unifoliolate, trifoliolate) were used in all experiments. Plant damage was measured by percent plant mortality, plant growth reduction, and number and percent of unifoliolate leaves upright. Not all of these damage measurements were made during the same experiment. In all experiments, surviving plants, especially those surrounded by dead or dying plants, were saved and grown to obtain seed (Fig. 1).

Varieties screened for resistance were originally selected to represent a wide variety of germ plasm. A few of the most resistant and most susceptible varieties (on the basis of preliminary experiments) were then selected for further testing in order to evaluate the screening techniques used.

Effect of given Lygus populations on different seedling growth stages

In one experiment, two alfalfa varieties (Sirsa #9 and Lahontan), replicated four times, in the cotyledon, unifoliolate and trifoliolate growth stages were subjected to an insect to plant ratio of 1:2 for 10 days at 75 F and continuous illumination. The varieties were randomized within each replication and the growth stages randomized within each variety. Planting dates were spaced in time so that all three growth stages could be tested in the same flat with the same insect population. The 25 plants per row were infested when the last planting was in the cotyledon stage. A second flat with uninfested plants served as a check. A border row flanked the tested varieties at each end of the flat. Flats were rotated one-half turn daily to decrease the possible effect of light intensity differences within the growth chamber. The insects were removed after 10 days and a count made of

Figure 1. An example of an experiment where surviving plants were saved and grown to obtain seed. Plants marked with toothpicks were surrounded by dead or dying plants.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plants apparently dead or dying. A final plant mortality count was made 6 days later to determine any after-effects of the insects' feeding or oviposition.

An experiment was conducted to determine the effect of different insect populations on seedlings in the same growth stage. Lahontan seedlings were grown in 4-inch plastic pots, each pot containing 25 unifoliolate seedlings and serving as one replication. The temperature was 75 F with continuous illumination. Insect to plant ratios of 1:2, 1:3, and 1:4 were used, with four replications for each ratio tested. The insects were left on the plants for one week, removed, and plant mortality data recorded.

Effect of Lygus on seedling growth

As there were few deaths among plants in the trifoliolate stage in the above experiment, 50 plants of each variety were measured and compared with 50 of each variety from the check flat. Plants were measured from the base of the cotyledon leaves to the base of the tallest trifoliolate leaf.

Another experiment using three varieties (Sirsa #9, Alfa, Grimm) was conducted in a Percival growth chamber at a temperature range of 75 F (day) and 65 F (night) and a day length of 12 hours. The three varieties were planted twice in the same order in each pan (e.g. Grimm, Sirsa #9, Alfa, Grimm, Sirsa #9, Alfa), with 25 unifoliolate seedlings per row. A border row flanked the tested varieties on both sides. Plants in another pan served as a check. Plants were infested for 10 days at a 1:2 insect to plant ratio with adult Lygus removed from an overwintering experiment. Plants were measured from the base of the cotyledon leaves to the base of the unifoliolate leaf and compared with uninfested check plants.

Damage to seedlings by adults and nymphs

An experiment comparing damage done to the same varieties by adults and late instar nymphs was conducted in a Percival growth chamber at a temperature range of 75 F (day) and 65 F (night) and with a day length of 12 hours. Alternate rows of Alfalfa and Sirsa #9 seedlings, each containing 25 unifoliolate seedlings, were grown in aluminum pans. There were three rows of each variety per pan and a border row flanking the tested varieties on both sides. Plants in one pan were infested with late instar nymphs at a 1:4 insect to plant ratio, and plants in a second pan were infested with the same number of adults. A third pan with uninfested plants served as a check. Plant damage was measured by counting the number of upright unifoliolate leaves after certain periods of infestation and measuring plant height after 7 days of infestation.

Experiments to determine if varietal differences in resistance could be detected

Various methods were used in this phase of the study. In one experiment, four varieties with 30 unifoliolate seedlings per variety grown in 12 inch plastic pots, replicated four times, were subjected to a 1:2 insect to plant ratio for 7 days at 75 F and continuous illumination. The location of the varieties was randomized within each pot. The insects were removed from the plants after 7 days and plant mortality data recorded. Pots were rotated one-half turn daily to standardize light intensity differences.

A large varietal test was attempted using 16 alfalfa varieties, replicated four times, and planted in a large wood flat. The varieties were randomized within each replication. The 25 unifoliolate seedlings per row were subjected to a 1:3 insect to plant ratio for 7 days at 75 F and

continuous illumination. The flat was rotated one-half turn daily to standardize light intensity differences. The insects were removed from the plants after 7 days and plant mortality data recorded. All subsequent experiments were conducted with varieties selected on the basis of this experiment.

Effects of temperature on plant damage

These experiments were conducted in two Percival E-57 plant growth chambers at two temperature ranges. There was a 10 F difference between day and night temperatures in all experiments. Plants were grown in square aluminum pans at the same temperature and moved to their respective chambers shortly before experiments began. In one experiment, two varieties (Sirsa #9 and Alfa), each planted in three consecutive rows, were tested at two temperature ranges: 70-60 F and 80-70 F. In another experiment, two varieties (Ranger and Alfa) were planted in alternate rows and tested at temperature ranges of 85-75 F, and 75-65 F. Day length was 12 hours in both experiments. Plant damage was measured by recording the number and per cent of upright unifoliolate leaves after certain periods of infestation.

A final experiment was conducted with Sirsa #9 and Alfa seedlings planted in alternate rows. The temperature ranges were 80-70 F and 75-65 F and a day length of 14 hours. Plant damage was measured by recording the number of apparently dead or dying plants at the end of the experimental period.

There were 25 seedlings per row in the above experiments, and all plants were infested in the early unifoliolate stage with a 1:2 insect to plant ratio.

Results and Discussion

Rearing

While none of the rearing methods attempted in this study produced sufficient numbers of adult tarnished plant bugs for extensive resistance screening, the few insects produced were used in some experiments. The use of fresh green bean pods alone and combined with alfalfa seedlings were the most successful of the methods attempted. Eggs and large numbers of nymphs were obtained readily when the above methods were used; however obtaining large numbers of adults proved difficult. Many nymphs would develop until the fourth or fifth instar, where a high mortality occurred, leaving few nymphs alive to develop into adults. Rearing was more successful in the growth chambers than in the greenhouse, where high temperatures often caused high Lygus mortality.

The above results did not compare with those reported by Beards and Leigh (1960) for L. hesperus. These authors stated that insect mortality was relatively low unless laboratory temperatures exceeded 95 F. It is probable that rearing was not done on a large enough scale in the present study to yield the large numbers of insects required for resistance screening. This is being investigated further at the present time. Relative humidity was not measured, but it may have been too low during a critical part of the life cycle.

As the supply of fresh bean pods was undependable during the winter months, other food sources were used in rearing experiments. The insects fed on carrot roots, celery stalks, and frozen green bean pods that were allowed to thaw overnight before being introduced into cages containing

insects. Eggs were found deposited only on carrot roots, but no nymphs were observed hatching from these eggs. When nymphs were fed only frozen bean pods, they generally survived 7-10 days but did not molt. Most nymphs in a flat of alfalfa seedlings combined with frozen bean pods were dead a few days after removing all bean pods.

The use of sprouted alfalfa seeds as a rearing method was attempted on a small scale. In small preliminary experiments with this method, approximately 50% of the first instar nymphs tested developed into adults. This method is being investigated further, both in rearing and as a possible resistance screening method.

Overwintering studies

Results of the field study showed that high insect mortality occurred in all cages with all overwintering sites and materials tested, but more Lygus were recovered from the field cage (uncut alfalfa) under more "natural" conditions (Table 1). Much fungus formed in the cage with alfalfa hay as an overwintering site, which may have had some bearing on the low number of insects recovered from this cage. All cages except the one that contained uncut alfalfa were located in a poorly-drained area behind a building, and the damp conditions which resulted also may have affected the number of live insects recovered. Another possibility is the possible escape of the insects, since none of the cages used had bottoms.

It may or may not be significant to note that approximately 90% of the insects recovered alive were females. The sex ratio of the insects actually placed in the field cages was not determined; however population samples taken at that time indicated the sex ratio to be approximately 1:1.

Table 1. Recovery of adult Lygus in different field overwintering sites, from November 20, 1965, to March 20, 1966.

Overwintering site	No. insects placed in cage	Insects recovered alive	
		No.	Per cent
Leaf litter, grass, no cage bottom	140	20	14
Leaf litter, cardboard over ground	140	12	8
Leaf litter, cardboard over ground	140	13	9
Alfalfa hay	140	2	1
Uncut alfalfa	300	70	23
Totals	860	117*	14

* Over 90% of recovered insects were females.

In a similar overwintering study with L. lineolaris in Kansas (Keck, 1928), 33.7% of the recovered insects were dead after being caged on alfalfa clumps from November to January.

Survival of Lygus in the refrigerated room was also low, but higher than in the field cages (Table 2). More females than males survived under these conditions, and more insects survived on alfalfa than mullein.

After being removed from the field cages and refrigerated room, most of the surviving insects were used in a resistance screening experiment (Table 6), which is discussed below.

Results from different cages used in screening studies

Use of the large wood flats in resistance screening caused many difficulties. One difficulty was the tendency of adult Lygus to "fight their confinement" by gathering near the sides and flying to the tops of the cages (Fig. 2). This resulted in severe damage to plants near the sides of the cages and little damage to plants in the center of the cages. Covering cage tops with black cloth prevented many insects from flying to the tops, but more insects gathered around the sides. The black cloth also retarded plant growth in the center of the cages. The use of cages with lower tops also was unsuccessful in keeping more insects down on the plants. Attaching green construction paper to cage sides did not prevent the insects from gathering near the sides.

Rotating the flats and pots one-half turn at least daily was as effective a method as any used in obtaining more even distribution of Lygus on the plants. The use of the smaller pans and round pots also helped in that the ratio of border area to total area within the cages was more favorable;

Table 2. Survival of adult Lygus on potted plants of two species in a refrigerated room, December 21, 1965, to March 19, 1966.

Plant	Origianl no. males in cage	Per cent survived	Original no. females in cage	Per cent survived	Total no. insects in cage	Per cent survived
Alfalfa	12	33	13	69	25	52
Alfalfa	11	27	7	28	18	28
Mullein	13	8	12	25	25	16
Mullein	13	0	12	25	25	12
Totals	49	16	44	39	93	27

Figure 2. Insects gathered at the top of a cage during a resistance screening experiment.

that is, if most insects stray only a given distance from the edge regardless of cage size, using cages with less area between edges insures a more even distribution of insects on the plants.

The effect of given insect populations on different seedling growth stages

Results of the seedling-growth-stage experiment are recorded in Table 3. This experiment was conducted only to determine the effect of the same insect population on seedlings in different growth stages. The two alfalfa varieties tested were selected on the basis of their growth characteristics, so that one variety could serve as a check on the other concerning the amount of plant damage caused in each growth stage. Plants were infested for 10 days at a 1:2 insect to plant ratio.

There was significantly higher mortality among plants infested in the cotyledon stage than among plants in the unifoliolate and trifoliolate stages. Mortality differences between plants in the unifoliolate and trifoliolate stages were not significant at the .05 level. Mortality differences between Sirsa #9 and Lahontan were not significant at each growth stage in this experiment.

A plant mortality count made 6 days after removal of the insects showed that plants in the cotyledon stage had increased mortality in all but one case, where plant mortality remained the same. Plant mortality differences between the 10th and 16th day were variable in the other growth stages.

Lygus reduction of seedling growth

In the same experiment, measurements made on plants infested in the trifoliolate stage show growth of both varieties reduced over 50% during the 10-day experimental period. Sirsa #9 showed slightly less growth reduction than Lahontan (Table 4).

Table 3. Per cent plant mortality in two alfalfa varieties in different seedling growth stages infested for 10 days at a 1:2 insect to plant ratio and 75 F, July 1965.

Variety and growth stage	Days after infestation* 10 Replication 1	2	3	4	Mean	16 Replication 1	2	3	4	Mean
Sirsa #9										
Cotyledon	27	54	77	56	52	40	68	81	68	63
Unifoliolate	16	7	28	15	16	20	7	28	15	17
Trifoliolate	0	12	24	0	10	0	17	24	0	10
Lahontan										
Cotyledon	48	56	54	64	56	52	56	58	84	63
Unifoliolate	24	12	25	22	20	28	4	20	18	18
Trifoliolate	8	25	20	16	14	7	16	27	12	16

* All insects removed from plants after 10 days

L.S.D. .05 = 12.2
.01 = 17.1

Analysis of variance

Source of variation	df	Sum of squares	Mean square	Observed F	Required .05	F .01
Main plots	7	1231.8				
Replications	3	776.8	258.9	1.74	9.28	29.46
Varieties	1	8.2	8.2	.05	10.13	34.12
Error(a)	3	446.8	148.9			
Sub-plots	23	15256.5				
Growth stages	2	12469.7	6234.8	49.82**	3.88	6.93
Var. x growth stages	2	53.1	26.6	2.12	3.88	6.93
Error(b)	12	1501.9	125.2			

Adult Lygus removed from overwintering sites were used in another experiment to determine the effect of the insects on seedling growth as well as if insects removed from overwintering sites could be used to screen plants for resistance (Table 5). Over 90% of the insects used in this experiment were females. Plants were infested in the unifoliolate stage at a 1:2 Lygus to plant ratio and a temperature range of 75-65 F. During the 10-day experiment, growth was reduced one-fourth to one-third in the three varieties tested. Sirsa #9 showed less growth reduction than either Alfa or Grimm.

The insects removed from overwintering sites did not appear to cause as much plant damage as those collected in the field during the summer months, even when plants were infested at a higher insect to plant ratio than in a previous experiment (Table 4).

Damage to seedlings by adults and nymphs

Results of an experiment comparing damage done to the same two varieties by adults and late-instar nymphs are shown in Table 6. Plants were infested for 7 days at a temperature range of 75-65 F and a 1:4 insect to plant ratio. The insect to plant ratio in this experiment was low due to the limited number of late-instar nymphs available. Nymphs reduced growth more than adults in both varieties during the length of the experiment. In a different damage measurement recorded during this experiment, there were fewer upright unifoliolate leaves on plants infested with nymphs in both varieties compared with plants infested with adults.

All nymphs were dead at the end of the 7-day experimental period, making it questionable whether the nymphs lived long enough to cause a maximum

Table 4. Growth reduction in two alfalfa varieties after 10 days of infestation in the trifoliolate stage by adult Lygus at 75 F and a 1:3 insect to plant ratio, July, 1965.

Variety	Mean height*(mm) of infested plants	Mean height*(mm) of uninfested plants	Per cent growth reduction
Sirsa #9	60	126	52
Lahontan	57	134	56

* Plants measured from the base of the cotyledon leaves to the base of the tallest trifoliolate leaf

Table 5. Growth reduction in three alfalfa varieties after 10 days of infestation in the unifoliolate stage by adult Lygus at a 1:2 insect to plant ratio and a temperature range of 75-65 F. Of the insects used, 90% were females removed from overwintering cages, March, 1966.

Variety	Mean height*(mm) of uninfested plants	Mean height*(mm) of infested plants	Per cent growth reduction
Sirsa #9	58	43	26
Alfa	48	32	33
Grimm	51	34	33

* Plants measured from the base of the cotyledon leaves to the base of the tallest trifoliolate leaf

Table 6. Growth reduction in two alfalfa varieties after 7 days of infestation in the unifoliolate stage by Lygus adults and nymphs at a temperature range of 75-65 F and a 1:4 insect to plant ratio, February, 1966.

Variety	Mean height*(mm) of uninfested plants	Mean height* (mm) of plants infested with:			
		Nymphs	Per cent reduction	Adults	Per cent reduction
Sirsa #9	47	27	42	31	34
Alfa	40	22	45	27	32

* Plants measured from the base of the cotyledon leaves to the base of the unifoliolate leaf.

amount of damage. Nymphs did not appear to survive for long periods when alfalfa seedlings were the only food sources available.

These results agreed with those reported by Leigh (1963) in a paper dealing with the life history of L. hesperus. In that study, few nymphs developed into adults when alfalfa terminals without flower buds or seed pods were used as food sources.

Differences in growth reduction between Sirsa #9 and Alfa were slight whether infested with adults or nymphs. Sirsa #9 showed slightly less growth reduction than Alfa when infested with nymphs and slightly more growth reduction when infested with adults. It is possible that the insect to plant ratio was too low to reveal any varietal differences that may actually have been present.

Effect of different Lygus populations on seedlings in the same growth stage

Damage caused by different adult Lygus populations to alfalfa seedlings in the same growth stage is recorded in Table 7. Plants were infested in the unifoliolate stage for 7 days at 75 F. A significant decrease in plant mortality occurred when the insect to plant ratio was decreased from 1:2 to 1:3. Although there was slightly higher plant mortality at the 1:4 insect to plant ratio than at 1:3, this difference was not significant at the .05 level.

Experiments to detect varietal resistance differences

Varieties chosen for this phase of the study were selected to obtain a relatively wide range of germ plasm. Due to the limited data reported in the literature concerning the screening of alfalfa varieties for resistance to Lygus spp., the varieties actually tested were chosen almost at random from those available.

Table 7. Per cent plant mortality of Lahontan alfalfa seedlings after 7 days of infestation in the unifoliolate stage at 75 F by different adult Lygus populations, July, 1965.

Insect to plant ratio	Replication 1	2	3	4	Mean per cent mortality
1:2	36	20	20	36	30
1:3	0	4	12	8	6
1:4	12	4	4	20	10

L.S.D. .05 = 11.54
.01 = 17.48

Analysis of variance

Source of variation	df	Sum of squares	Mean square	Observed F	Required .05	F .01
Populations	2	1098.67	549.34	12.36**	5.14	10.92
Replications	3	245.34	81.78	1.84	4.76	9.78
Error	6	266.66	44.44			
Total	11					

** Signigicant at .01

In one preliminary experiment, plant mortality data were recorded for four alfalfa varieties after 7 days of infestation by adult Lygus at 75 F and a 1:2 insect to plant ratio (Table 8). Plants were infested in the unifoliolate stage. There were originally four replications in this experiment, but one replication was eliminated from the table because most of the insects escaped from the cage. There was much variation in plant mortality from replication to replication in this experiment, and differences between the varieties tested were not significant at the .05 level.

Another experiment of this type was attempted using 16 varieties replicated four times and planted in the same flat (Fig. 3 and Table 9). With this planting arrangement all replications were subjected to the same insect population. All plants were infested in the unifoliolate stage for 10 days at 75 F and a 1:3 insect to plant ratio.

Significant differences in plant survival were obtained among the varieties tested and also among replications (Table 9). Differences between replications can probably be explained on the basis of the planting arrangement, light intensity differences within the growth chamber, and habits of the insects. Replications one and two, with the lowest mean plant survival, were located on the side of the flat toward the strongest light. Replication four, located farthest away from the strong light but near the cage side where the insects had a tendency to gather (see discussion of cages), had lower plant survival than replication three, which was located both away from strong light and the side of the cage. The flat was rotated one-half turn after plants had been infested for 2 days; however most of the heavy damage to replications one and two had already occurred and many of the insects were dead (see below).

Table 8. Per cent plant mortality in four alfalfa varieties after 7 days of infestation in the unifoliolate stage by adult Lygus at 75 F and a 1:2 insect to plant ratio, July, 1965.

Variety	Replication 1	2	3	Mean per cent mortality**
Du Puits	62	76	47	61
Lahontan	67	57	47	57
Cody	37	77	30	48
Sirsa #9	23	60	33	39

** Differences not significant at .05 level.

Figure 3. Mean per cent plant survival shown by 16 alfalfa varieties infested in the unifoliolate stage for 10 days at 75 F and a 1:3 insect to plant ratio. August, 1965.

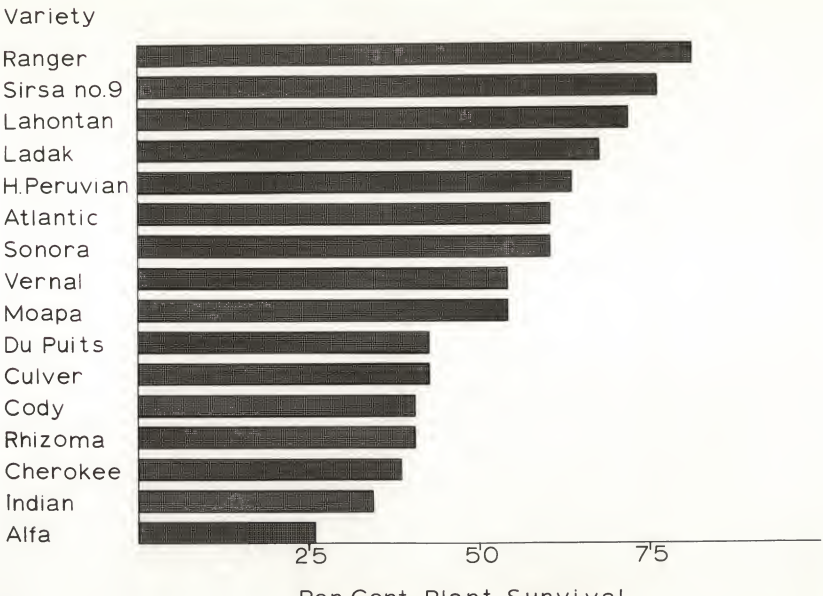

Variety
Ranger
Sirsa no.9
Lahontan
Ladak
H.Peruvian
Atlantic
Sonora
Vernal
Moapa
Du Puits
Culver
Cody
Rhizoma
Cherokee
Indian
Alfa
2'5
5'0
7'5
Per Cent Plant Survival

Table 9. Per cent survival shown by 16 alfalfa varieties infested in the unifoliolate stage with adult Lygus for 10 days at 75 F and a 1:3 insect to plant ratio, August, 1965.

Variety	Replication 1	2	3	4	Mean
Ranger	61	75	92	92	80
Sirsa #9	50	93	88	75	76
Lahontan	28	92	92	76	72
Ladak	25	82	83	84	68
H. Peruvian	24	72	79	76	63
Atlantic	38	71	80	50	60
Sonora	18	72	88	60	60
Vernal	64	8	77	68	54
Moapa	11	64	84	57	54
DuPuits	20	22	60	67	42
Culver	46	44	38	50	42
Cody	20	29	70	39	40
Rhizoma	20	10	70	58	40
Cherokee	0	6	68	76	38
Indian	29	6	68	35	34
Alfa	13	20	35	36	26

L.S.D. .05 = 25.31
.01 = 33.81

Analysis of variance

Source of variation	df	Sum of squares	Mean square	Observed F	Required .05	F .01
Varieties	15	15724.75	1048.32	3.32**	1.90	2.47
Replications	3	17476.63	5825.54	18.44**	2.82	4.25
Error	45	14218.37	315.96			
Total	63					

** Significant at .01

As a result of this experiment, two varieties, Sirsa #9 and Alfa, were selected for further testing in order to determine if consistent differences in plant damage could be obtained. Sirsa #9 had one of the highest mean survival percentages and Alfa had the lowest mean survival percentage in this experiment.

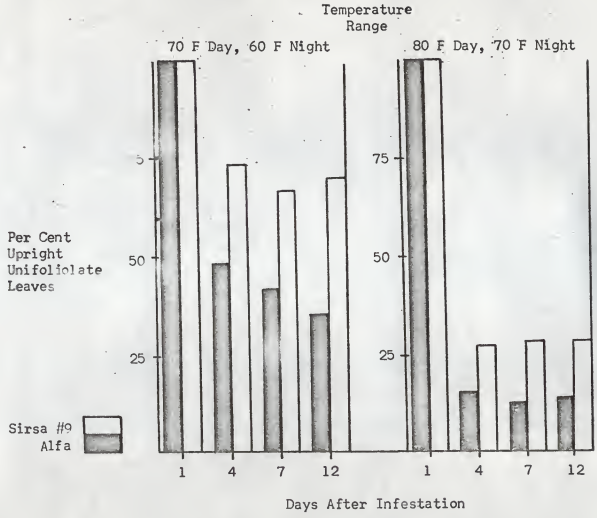
In the experiments with the varieties selected, plants were grown in small aluminum pans in an attempt to eliminate the uneven distribution of insects on the plants that occurred in previous experiments. The experiments also were conducted at two temperature ranges in an attempt to determine the effect of different temperatures on the amount of plant damage.

In one experiment conducted at two temperature ranges, Sirsa #9 and Alfa seedlings were infested in the unifoliolate stage for 12 days at a 1:2 Lygus to plant ratio (Figs. 4 and 5). In this experiment, plant damage was measured by recording the number of upright unifoliolate leaves in each variety at intervals after the plants were infested. Sirsa #9 had more upright unifoliolate leaves than Alfa at each temperature range and each time data were recorded. The slight increase in per cent of upright unifoliolate leaves shown by Sirsa #9 at both temperature ranges after 12 days was apparently recovery from damage by some plants. Most of the insects were dead after 10 days. Alfa showed recovery only at the 80-70 F temperature range.

More plant damage occurred at 80-70 F than at 70-60 F. However, plant damage differences between the two varieties were greater at 70-60 F. Most plant damage occurred within the first few days after infestation at both temperature ranges with minor changes the following days.

Figure 4. An experiment conducted at two temperature ranges. Plants on the left infested at 70-60 F, plants on the right infested at 80-70 F. Alfa on the left and Sirsa #9 on the right in both cages (three rows of each).

Figure 5. Per cent of unifoliolate leaves upright in two alfalfa varieties infested with adult Lygus at two temperature ranges and a 1:2 insect to plant ratio, December, 1965.


Temperature Range
70 F Day, 60 F Night
80 F Day, 70 F Night
Per Cent Upright Unifoliolate Leaves
75
50
25
75
50
25
Sirsa #9
Alfa
1
4
7
12
1
4
7
12
Days After Infestation

In an experiment without Sirsa #9, Ranger and Alfa were infested in the unifoliolate stage for 8 days at a 1:2 insect to plant ratio and temperature ranges of 85-75 F and 75-65 F (Fig. 6). Plant damage was measured by recording the number of upright unifoliolate leaves in each variety at intervals after plants were infested. Damage differences between the two varieties were slight at each temperature range. However, Ranger, which had more upright unifoliolate leaves than Alfa at 75-65 F, had slightly fewer than Alfa at 85-75 F. More plant damage occurred at 75-65 F. Most plant damage again occurred within the first few days after infestation.

Results of another experiment with Sirsa #9 and Alfa are shown in Table 10. Plants were infested in the unifoliolate stage for 6 days at a 1:2 Lygus to plant ratio. Temperature ranges in this experiment were 80-70 F and 75-65 F, with six replications at each temperature range. Plant damage was measured by recording the number of apparently dead or dying plants in each variety.

Significantly differences were obtained between means of both varieties at each temperature range. Differences between the two varieties in the number of dead or dying plants were relatively consistent from replication to replication, with the exception of replication three at the 75-65 F temperature range. In this replication, Sirsa #9 had two more dead plants than Alfa.

As occurred in a previous experiment (see above), more plant damage was recorded at the 75-65 F temperature range than at the 80-70 F range. Out of 300 plants at each temperature range, 145 plants were dead or dying after 6 days at 75-65 F, while 132 plants were dead or dying at 80-70 F.

Figure 6. Per cent of unifoliolate leaves upright in two alfalfa varieties infested with adult Lygus at two temperature ranges and a 1:2 insect to plant ratio, January, 1966.

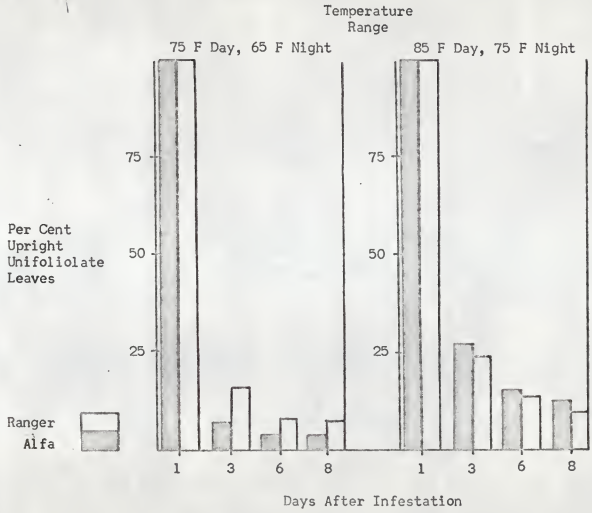

Temperature
Range
75 F Day, 65 F Night
85 F Day, 75 F Night
Per Cent
Upright
Unifoliolate
Leaves
75
50
25
75
50
25
Ranger
Alfa
1
3
6
8
1
3
6
8
Days After Infestation

Table 10. Number of dead or dying plants in two alfalfa varieties out of an original 25 seedlings per variety per replication after 6 days of infestation in the unifoliolate stage by adult Lygus at two temperature ranges and a 1:2 insect to plant ratio, June, 1966.

Temperature range	Variety	Replication 1	2	3	4	5	6	Mean
80 F day, 70 F night	Alfa	13	12	14	15	12	14	13.33
	Sirsa #9	8	11	10	10	6	7	8.67
75 F day, 65 F night	Alfa	15	18	14	9	13	14	13.83
	Sirsa #9	8	13	16	7	8	10	10.33

L.S.D. .05 = 2.00
.01 = 2.77

Analysis of variance

Source of variation	df	Sum of squares	Mean square	Observed F	Required .05	F .01
Replications	5	51.71	10.34	1.96	2.90	4.56
Treatments	(3)	(109.13)	36.38	6.90**	3.29	5.42
Temp.	1	7.04	7.04	1.34	4.54	8.68
Varieties	1	100.04	100.04	18.98**	4.54	8.68
T x V	1	2.05	2.05	.39	4.54	8.68
Error	15	79.12				
Total	23					

** Significant at .01 level

It is difficult to compare results obtained in these experiments with those found in the literature. Most of the previous studies concerning Lygus spp. resistance in alfalfa varieties have involved recording insect populations on mature plants in the field. In the present study, seedlings were used exclusively and no population data were recorded. Nielson and Schonhorst (1965), after recording Lygus spp. populations on alfalfa varieties in Arizona, suggested that alfalfa varieties of different growth characteristics should be evaluated separately, because Lygus spp. populations would possibly be highest on those varieties in the bud or bloom stage at the time population samples were taken.

Whether resistance in seedling growth stages will be carried over into reproductive stages of plant growth has not yet been determined. Further studies will be undertaken to determine this.

Summary and Conclusions

The primary objectives of this study were to develop methods of screening large numbers of plants for resistance to Lygus spp., especially L. lineolaris, by using alfalfa seedlings. Various combinations of cage size, temperature, seedling growth stage, and insect populations were studied.

An attempt was made to develop efficient culture methods for rearing Lygus populations large enough for resistance screening. Rearing experiments were conducted using fresh green bean pods, green bean pods combined with alfalfa seedlings, carrot roots, celery stalks, frozen green bean pods, and sprouted alfalfa seeds. Rearing sufficient insects for extensive resistance screening was not successful with any of the food sources used.

Overwintering experiments were conducted in the field and in a refrigerated room. In these experiments, various numbers of adult insects were caged over different overwintering sites for 90 to 120 days, removed and recovery data recorded. There was high insect mortality in both field and refrigerated room overwintering cages. Surviving insects were used in a seedling growth reduction experiment.

Various cage types and planting arrangements were used in seedling screening experiments. Plants were grown in large (11 inches by 20 inches) wood flats, in round plastic pots, 4 to 12 inches in diameter, and in aluminum pans (9 inches square). The insects were most evenly distributed on the plants when plants were grown in round pots or aluminum pans.

Experiments were conducted in two different types of plant growth chambers. Earlier experiments were conducted in a growth chamber with constant temperature and continuous illumination. Later experiments were

conducted in two Percival E-57 plant growth chambers where light, temperature, and day length could be regulated.

The insects were confined on the plants from 6 to 12 days at various insect to plant ratios. Plant damage was measured by per cent plant mortality, growth reduction during the period of the experiment, and per cent of unifoliolate leaves upright. Not all of these damage measurements were made during each experiment. Surviving plants, especially those surrounded by dead or dying plants, were saved and grown to obtain seed.

Differences in plant mortality were not significant at the .05 level in a preliminary resistance screening experiment using four varieties. However, significant differences were obtained among 16 varieties in a larger experiment. There was much variation in plant survival from replication to replication, possibly due to differences in light intensity within the growth chamber used, the planting arrangement, and habits of the insects. On the basis of this experiment, varieties were chosen for further testing to determine whether or not consistent damage differences could be obtained. These experiments were conducted at two temperature ranges. More plant damage occurred at the 75-65 F temperature range than at other temperature ranges used.

In a growth stage experiment, a higher percentage of seedlings in the cotyledon stage were killed than in the unifoliolate or trifoliolate stages.

Seedling growth was reduced from 26 to 56% during experimental periods, depending on the variety and the insect to plant ratio used. Insects removed from overwintering sites reduced plant growth less than insects collected in the field during the summer months. Late-instar nymphs reduced

plant growth more than adults in both varieties tested, but most nymphs were dead a few days after being caged on the plants.

A 1:2 insect to plant ratio caused significantly higher mean per cent plant mortality than the 1:3 or 1:4 insect to plant ratios. Differences in mean per cent plant mortality between the 1:3 and 1:4 insect to plant ratios were not significant at the .05 level.

Conclusions

1) Lygus spp. were capable of killing young alfalfa plants.
2) Rearing of Lygus populations large enough for resistance screening is possible if done on a larger scale than that attempted in this study. Fresh green bean pods combined with alfalfa seedlings appeared to be better food sources than bean pods alone.
3) Placing Lygus in overwintering cages for later use in resistance experiments was not practical with the methods used. Insects removed from overwintering sites did not cause as much plant damage as summer field-collected insects, and insect mortality was high with the overwintering sites used.
4) Aluminum pans, 9 inches square, appeared to be the best plant containers used in seedling screening experiments, because of the more even distribution of insects on the plants.
5) It was possible to detect varietal resistance differences in seedling growth stages with the methods used in this study. How large the differences are and the best ways to measure them are still open to question. The unifoliolate stage appeared to be the best growth stage for detecting differences. Plant mortality was a satisfactory

damage measurement when this growth stage was used. With older plants, however, some other damage measurement (differential growth reduction, bud-blasting, flower-fall, etc.) may have to be used.

6) There appeared to be an optimum temperature when most plant damage occurred. In this study more plant damage occurred when the daytime temperature was 75 F than with other temperatures used.

7) Based on information obtained in this study, the best differentiations between possible resistant and susceptible plants were obtained when plants were infested in the unifoliolate stage at a daytime temperature of 75 F and a 1:2 insect to plant ratio.

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SCREENING ALFALFA SEEDLINGS FOR RESISTANCE TO THE TARNISHED PLANT BUG, LYGUS LINEOLARIS (PALISOT DE BEAUVOIS)

by

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ABSTRACT

The primary objectives were to develop methods of screening large numbers of alfalfa plants (Medicago sativa Linnaeus) for resistance to Lygus spp. (Hemiptera: Miridae), especially L. lineolaris (Palisot de Beauvois), by using seedlings. Various combinations of cage size, temperature, seedling growth stage, and Lygus populations were studied.

A higher percentage of plants in the cotyledon stage were killed than plants in the unifoliolate or trifoliolate stages. A 1:2 insect to plant ratio caused a significantly higher plant mortality percentage than did 1:3 or 1:4 insect to plant ratios. The best differentiations between possible resistant and susceptible plants were obtained when plants were grown in 9 inch square aluminum pans and infested in the unifoliolate stage at a daytime temperature of 75 F and a 1:2 Lygus to plant ratio.

It was possible to detect varietial resistance differences in seedling growth stages whether plant damage was measured by per cent plant mortality, growth reduction, or per cent of upright unifoliolate leaves. The differences between varieties tested were not always statistically significant, but were generally consistent.

Rearing of laboratory populations of L. lineolaris great enough for large scale resistance screening was unsuccessful, but the few insects produced were used in some experiments.