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The Effect of Threecornered Alfalfa Hopper Populations on Alfalfa Growth and the Development of Host Plant Resistance Screening Techniques.

Daniel John Moellenbeck

Louisiana State University and Agricultural & Mechanical College

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The effect of threecornered alfalfa hopper populations on alfalfa growth and the development of host plant resistance screening techniques

Moellenbeck, Daniel John, Ph.D.
The Louisiana State University and Agricultural and Mechanical Col., 1992
THE EFFECT OF THREECORNERED ALFALFA HOPPER POPULATIONS 
ON ALFALFA GROWTH AND THE DEVELOPMENT OF HOST 
PLANT RESISTANCE SCREENING TECHNIQUES 

A Dissertation 
Submitted to the Graduate Faculty of the 
Louisiana State University and 
Agricultural and Mechanical College 
in partial fulfillment of the 
requirements for the degree of 
Doctor of Philosophy 

in 

The Department of Entomology 

by 
Daniel J. Moellenbeck 
B. S., Iowa State University, 1986 
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ABSTRACT

This dissertation research was designed with three main objectives: to evaluate the effects of threecornered alfalfa hopper, *Spissistilus festinus* (Say), feeding on alfalfa, *Medicago sativa* L., yield, regrowth, root carbohydrate composition, and disease incidence; to screen alfalfa cultivars for resistance to the threecornered alfalfa hopper and develop effective screening techniques; and to determine the mechanism(s) of this resistance.

Greenhouse studies determined the effects of threecornered alfalfa hopper infestations on alfalfa growth and their relationship with *Fusarium* crown-rot. Significant interaction effects between insect population and the presence of *Fusarium* were found for number of harvestable stems and acid detergent fiber concentration. The main effect of inoculation did not affect any of the plant parameters studied. Insect populations increased plant chlorosis and accelerated plant maturity. Insect populations also reduced plant height, root carbohydrate concentration, stem regrowth, and forage quality. Threecornered alfalfa hoppers also caused an increase in *Fusarium* crown-rot severity.

Choice and no-choice tests were conducted under greenhouse conditions to determine the presence of alfalfa antibiosis or antixenosis to the threecornered alfalfa hopper. Adult threecornered alfalfa hoppers were released
into cages containing six alfalfa cultivars. Fewer threecornered alfalfa hoppers and girdles were found on cultivars 'Cimarron VR' and 'Zia'. In the no-choice test, one newly hatched nymph was placed on each plant and development was monitored daily. Nymphal duration was longest when nymphs developed on 'Florida 77' and shortest on 'Zia'. Adult weights were reduced when nymphs developed on 'Dona Ana' and 'Cimarron'.

Screening techniques were developed to evaluate alfalfa resistance under field conditions. Resistance was characterized by adult populations, girdle damage, and stand persistence. 'Cimarron VR', 'Dona Ana', and 'GA Plains' exhibited resistance to the threecornered alfalfa hopper by having less girdle damage and greater stand persistence. 'Ladak' showed the highest level of susceptibility. Adult population monitoring was not an effective screening criterion. In addition, multiple girdle counts must be taken to accurately categorize alfalfa cultivars.
INTRODUCTION

Alfalfa, *Medicago sativa* L., is the most important forage crop species in the United States and Canada (Barnes et al. 1988). Recent emphasis on low-input sustainable agriculture will further increase the importance of alfalfa as a rotation crop. Alfalfa is an effective source of nitrogen fixation and an excellent source of protein yield per hectare. In addition, it is important in improving soil tilth (Barnes et al. 1988) and is a soil-conserving perennial crop (Stuteville & Erwin 1990). Cotton yields can be increased up to 20 percent following alfalfa compared to continuous cotton (Francis & Clegg 1990). Currently, less than 8,000 ha of alfalfa is grown annually in Louisiana (Wilson & Quisenberry 1987). The benefits of alfalfa as a rotation crop, and its potential profitability as an agronomic crop in Louisiana ($181.70 per ha per yr, Quisenberry et al. 1987) warrant further study to develop production strategies suitable for Louisiana growing conditions.

The threecornered alfalfa hopper, *Spissistilus festinus* (Say), is an important late summer pest of alfalfa in Louisiana (Wilson & Quisenberry 1987). Threecornered alfalfa hopper populations have been found to reduce both dry matter alfalfa yield and alfalfa quality (Wilson & Quisenberry 1987). In addition, damage caused by the threecornered alfalfa hopper has long been linked with reduced alfalfa stand persistence (Graham
Stand persistence is thought to be a major factor limiting alfalfa production in Louisiana (Wilson & Quisenberry 1987).

Control of the threecornered alfalfa hopper is generally achieved through the use of insecticide applications. Currently, only one insecticide (carbaryl) is labelled for threecornered alfalfa hopper control on alfalfa (Anonymous 1991). Insecticide applications are generally aimed toward adult populations, even though nymphs are the most damaging life stage (Moore & Mueller 1976). Nymphal populations are difficult to control with insecticides because they are generally found near the base of plants, and are protected from spraying by the upper canopy (Miner 1959). In addition, more persistent insecticides cannot be used because alfalfa may be harvested monthly during Louisiana summers. Because populations are difficult to control with insecticides, host plant resistance is a viable alternative control strategy.

Previous researchers have screened a large number of alfalfa cultivars for resistance to threecornered alfalfa hoppers, and several have been recommended as selection sources for increased resistance (Kulash & Hanson 1949; Nielson & Schonhorst 1965; Randolph & Meisch 1970). Currently, no alfalfa cultivars are classified as being resistant to the threecornered alfalfa hopper (Manglitz & Ratcliff 1988). To facilitate the development of
resistant cultivars suitable for commercial use in Louisiana and the southern United States, it is necessary to determine the effects of the threecornered alfalfa hopper on alfalfa. According to Painter (1951), "studies of injury are required because separate genes for resistance may be found to each of the different kinds that may be present." A better understanding of threecornered alfalfa hopper damage to the alfalfa plant will allow researchers to identify and develop resistant alfalfa cultivars more easily.

The development of screening techniques is also necessary to correctly categorize resistance. Standard screening techniques for threecornered alfalfa hopper resistance have not been developed. Methods of determining resistance under field and greenhouse conditions need to be developed to effectively screen alfalfa germplasm for insect resistance.

Objectives

1. To evaluate the effect of threecornered alfalfa hopper feeding on alfalfa yield, forage quality, stem regrowth, root carbohydrate composition, and disease severity.

2. To screen alfalfa cultivars and somaclones for resistance to the threecornered alfalfa hopper and develop effective field and greenhouse screening techniques.

3. To determine the mechanism(s) of alfalfa resistance to the threecornered alfalfa hopper.
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The threecornered alfalfa hopper, *Spissistilus festinus* (Say) is present throughout most of the soybean production areas of the United States and has gained pest status on soybean in much of the Gulf States area (Sparks & Boethel 1987). Concurrent with the rapid expansion of acreage planted to soybean, the populations of the threecornered alfalfa hopper have increased dramatically in alfalfa fields so that injurious populations now occur annually (Newsom et al. 1983). The threecornered alfalfa hopper has long been recognized as a pest of alfalfa (Osborn 1911; Graham 1938) and has become the major mid-to late-season pest of alfalfa production in Louisiana (Quisenberry et al. 1987). The threecornered alfalfa hopper has been shown to reduce both alfalfa yield and forage quality (Wilson & Quisenberry 1987) and also has been linked with poor stand persistence (Duggar 1929). In order to better understand the biology and importance of this important pest, a review of previous work on this insect is necessary. This chapter will discuss the life cycle, feeding habits, and general biology of the threecornered alfalfa hopper. In addition, plant response to damage caused by this insect, and threecornered alfalfa hopper control strategies will be discussed.

**Life Cycle.** The threecornered alfalfa hopper is widely distributed because of its ability to utilize a wide variety of host plants (Sparks 1984; Mueller & Dumas...
In addition to soybean and alfalfa, the threecornered alfalfa hopper can also damage many other field crops, including peanut (Todd et al. 1979), cotton (Fletcher 1937), potato (Swezey 1937), and a variety of clovers (Moore & Mueller 1976). It is also a pest of several horticultural crops such as tomato, where it was first reported as a crop pest (Oemler 1888), watermelon (Watson 1919), cowpeas (Graham & Ellisor 1938), and many species of beans (Wildermuth 1915). The threecornered alfalfa hopper also can damage a variety of tree species including black locust (Snyder 1935), almond (Cockerell 1899), apple (Hawley 1923), and hickory (Watson 1919). In addition, the threecornered alfalfa hopper utilizes many weed species including cocklebur (Wildermuth 1915), goldenrod (Watson 1919), and prickly seda (Moore & Mueller 1976). In general, legumes are the preferred host (Mueller & Dumas 1987).

In Louisiana, the threecornered alfalfa hopper overwinters as an adult in a state of reproductive diapause using pine, Pinus spp., as a maintenance host (Wildermuth 1915; Newsom et al. 1983). Other plants associated with overwintering adults include alfalfa, Sporobolus airoides (Torr.), Rumex spp., and Andropogon spp. with feeding only occurring on alfalfa (Newsom et al. 1983). In the southern United States, the threecornered alfalfa hopper completes four or possibly five generations each year (Graham & Ellisor 1940). A single generation
occurs on spring legumes, and the adults of this generation move into soybean (Sparks & Boethel 1987). In soybean, the second and third generations are produced. As these crops begin to mature, the population moves either into alfalfa and other perennial legumes or into pine stands for overwintering (Mitchell & Newsom 1984b). A fourth, and possibly a fifth, generation can be produced on perennial legumes (Graham & Ellisor 1940). During the fourth and fifth generations, the threecornered alfalfa hopper population peaks and causes the most damage to alfalfa (Chamberlain 1956; Wilson & Quisenberry 1987).

Nymphal development consists of five instars. A useful key to the instars was provided by Jordan (1952). Nymphal stage duration depends on both temperature and host plant species (Spurgeon & Mack 1990). Several studies have reported development time for this insect on several hosts and temperature conditions (Meisch & Randolph 1965; Moore & Mueller 1976; Mitchell & Newsom 1984b; Spurgeon & Mack 1990). Spurgeon & Mack (1990) studied nymphal duration on soybean under several different temperature regimes. They found the nymphal stage lasted from 18 to 50 d at 32.2 and 18.3 °C, respectively.

Oviposition. The female threecornered alfalfa hopper oviposits directly under the epidermis of its host plant (Graham 1938). At oviposition, the female makes a long slit in the plant tissue with her ovipositor and then
places egg clusters into the slit (Graham 1938). One to 17 eggs are laid in a cluster depending on the host plant and site of oviposition (Mitchell & Newsom 1984b; Daigle et al. 1988). Hyperplasia of epidermal cells results in gall formation at the ovipositional site and can result in death of the stem (Wildermuth 1915). On soybean, oviposition can occur shortly after plant emergence, with an increase in egg laying throughout the growing season (Daigle et al. 1988). Oviposition peaks late in the soybean plant's development coinciding with the start of the third generation.

Meisch & Randolph (1965) found that oviposition can result in the shredding of stem tissue that, along with feeding, can greatly damage the plant. Oviposition in the terminal can also distort the apical meristematic tissue (Rice & Drees 1985). Rice & Drees (1985) listed ovipositional trends exhibited by the threecornered alfalfa hopper on soybean plants. First, all portions of the plant can be utilized for oviposition, including root tissue below the soil line. Second, both nodes and terminals are preferred oviposition sites with the internodes being used infrequently. Finally, as plants develop into more advanced vegetative stages, fewer eggs are deposited in the lower nodes as compared to the upper nodes. This upward shift in the preferred oviposition site is a result of the lower stem tissues becoming woodier and less favorable (Daigle et al. 1988).
Feeding Habits. Each nymphal stage and the adult threecornered alfalfa hopper are phytophagous and can damage its host plant. Wildermuth (1915) reported that the threecornered alfalfa hopper displays two types of feeding patterns: random feeding and girdling. The first and second instars are not capable of girdling and do not cause serious damage (Moore & Mueller 1976). The third through fifth instars and adults are capable of girdling (Rice & Drees 1985), and cause the greatest amount of damage and economic loss. The fourth instar is considered to be the most injurious and is capable of girdling a stem in less than 24 h (Moore & Mueller 1976). Fifth instars seem to be less injurious than the fourth, but more so than earlier instars (Johnson & Mueller 1989).

The type of damage most often associated with the threecornered alfalfa hopper is stem girdling and the subsequent plant response. Threecornered alfalfa hoppers girdle a plant stem by puncturing continuously around the stem forming a ring of feeding scars (Jordan 1952). In alfalfa and early season soybeans, girdling occurs just above the soil level (Eddy 1936, Rice & Drees 1985). As the insect works around the stem, there is an initial constriction and necrosis at the girdle site (Rice & Drees 1985). The first visible symptom of girdle damage is a depression encircling the stem (Brown & Gibson 1922). The girdle site becomes more pronounced over time. Eventually, the constriction becomes clearly visible, and
the stem above the border of the girdle expands beyond the diameter of the ungirdled area (Brown & Gibson 1922).

The threecornered alfalfa hopper primarily feeds in phloem tissue by puncturing the stem with its piercing-sucking mouthparts (Mitchell & Newsom 1984a). Smith (1933) and King & Cook (1932) reported that insoluble sheaths left by feeding threecornered alfalfa hoppers remain in vascular tissues and disrupt translocation. Mitchell & Newsom (1984a) reported that the feeding punctures result in a severe disruption of the normal arrangement of vascular tissue directly beneath the external necrotic, girdled region, greatly reducing the amount of translocation in the damaged stem. In addition, the disruption of vascular arrangement results in the eventual loss of structure in all stem tissues external to the xylem, causing the depression in the girdled region (Mitchell & Newsom 1984a). Later in the growing season, girdle sites in soybean shift from the lower main stem to branches, petioles, and the upper main stem (Mitchell & Newsom 1984a), and thus, the insect avoids girdling woodier stem tissue.

The second type of feeding exhibited by this insect is random feeding (Wildermuth 1915). Random feeding punctures on the stem can be identified by the presence of small spots (Johnson & Mueller 1989). In this type of feeding, punctures are not restricted to a ring around the stem, and translocation in the vascular tissue is not
blocked. Random feeding can cause a reduction in soybean yield that is termed late season damage (Sparks 1984). Sparks & Newsom (1984) found that feeding shifts from the main stem to the pod region, especially the pedicels and peduncles, during pod fill. Although populations of nymphs and adults can be very high during this time, girdling seldom occurs at these sites (Sparks & Newsom 1984). Feeding near the pod drains the nutrient supply to the seeds (Mitchell & Newsom 1984a), and causes a loss in yield due to a reduction in the number of pods produced per plant and the number of seeds produced per pod (Sparks & Newsom 1984). This type of damage has only been studied in soybean, but probably is important in other seed and fruit crops.

The threecornered alfalfa hopper is capable of random feeding for food intake yet expends energy to girdle a plant. A girdle greatly reduces the translocation rate in the stem. This reduction in translocation has been proven to be very beneficial to the threecornered alfalfa hopper. The interruption of the flow of nutrients in the phloem results in a nutrient sink being formed above the girdle (Hicks et al. 1984). After a girdle is completed, nymphs become very sedentary and feed above the girdle, exploiting the nutrient sink created (Mitchell & Newsom 1984a).

Random feeding and girdling are complementary. Although food intake does occur during the formation of
the girdle, girdling functions primarily to enhance the nutritional quality of further feeding above the girdle (Mitchell & Newsom 1984a). More than one nymph may be found feeding above a completed girdle; however, the girdling does not appear to be a combined effort of more than one insect (Mitchell & Newsom 1984a). Rice & Drees (1985) reported that girdling and oviposition by a female hopper on the same plant creates a readily available nutrient sink that may increase the progeny's chance of survival. As discussed earlier, girdles are not formed by nymphs or adults feeding near soybean pods. Mitchell & Newsom (1984a) theorized that the pod is a nutrient sink and hoppers exploit the concentrated phloem supply in the pod without expending energy to girdle petioles.

Effect of Feeding Damage. As a result of girdling and the accompanying reduced rate of translocation, the affected stems either exhibit a purple color (Graham 1936) or become chlorotic (Brown & Gibson 1922). Reduced growth and yellowing of girdled plants is often thought to be caused by drought, inadequate fertilization, or disease (Kulash & Hanson 1949), resulting in the importance of the damage caused by this insect being overlooked. Alfalfa plants girdled by this insect grow at a much slower rate than non-girdled plants, causing a delay in plant maturity. It has been shown in Louisiana that this delay in maturity could amount to the loss of one-third of a harvest period (Wilson & Quisenberry 1987).
The most detrimental aspect of the girdle is the reduction in plant translocation. Radiotracer studies have demonstrated a high concentration of radiolabeled carbon above girdles, indicating extensive interference with translocation (Hicks et al. 1984). The blockage of translocation caused by the girdle prevents the movement of carbohydrates from the upper parts of the plant to the roots (Jordan 1952) or seed (Mitchell & Newsom 1984a). In alfalfa, the reduction in carbohydrate movement into the roots can greatly affect the plant's ability to overwinter (Jordan 1952). Alfalfa plants use stored root carbohydrates for regrowth after a harvest or in the spring. The reduction in carbohydrate translocation to the roots results in less available energy for regrowth. Eventually, plant mortality occurs because they do not have the necessary energy for regrowth. This greatly reduces the alfalfa plant stand and may reduce the profitability of alfalfa production by making it necessary to reseed (Graham 1938).

Leguminous plants rely on a symbiotic relationship with Rhizobium bacteria for their nitrogen needs. Hicks et al. (1984) reported significant reductions in the amount of nitrogen fixed when soybean plants were exposed to intense, prolonged feeding by the threecornered alfalfa hopper. They found that decreased nitrogen fixation could be attributed to a reduced number of nodules and a reduced nodule dry weight. Hutchinson (1979) stated the reduction
in nitrogen fixation was attributed to a decrease in the amount of photosynthate translocated from the leaves to the root nodules. As less photosynthate reaches the roots, there is a reduction in the nutrient supply available to Rhizobium (Mitchell & Newsom 1984a). Higher densities of the threecornered alfalfa hopper and longer periods of feeding cause further decreases in nitrogen fixation ability (Hicks et al. 1984). A major reduction in nitrogen fixation can greatly affect crop yield.

Girdle damage also reduces the structural strength of the stem (Jordan 1952). Girdles on the lower part of the main stem may cause the plant to break over and lodge, reducing yield and increasing harvest difficulties (Mueller 1980). Girdle damage occurring before plants are 20 cm tall causes the highest amount of soybean plant lodging (Bailey et al. 1970). Lodging caused by girdle damage generally occurs subsequent to the insect damage, often when wind, rain, or cultivation practices further cause the plant to lodge (Herzog et al. 1975). The greatest yield loss occurs when soybean plants heavily loaded with pods break at the girdle site due to the weight of the seed (Bailey 1975). Plant lodging in forage crops also can reduce harvestable yields. In addition, lodging, in combination with decreased translocation, reduces nitrogen fixation to even a greater extent (Hutchinson 1979).

Overall, damage caused by the threecornered alfalfa
hopper has a great effect on plant growth and thus, the quantity and quality of harvested seed and forage. Sparks & Newsom (1984) reported a significant soybean yield reduction with high late-season populations of the threecornered alfalfa hopper. In alfalfa, Quisenberry et al. (1987) found a 20% yield reduction could be expected when the threecornered alfalfa hopper population reached two adults per sweep. In addition, Wilson & Quisenberry (1987) found that girdling can significantly reduce alfalfa forage quality. The concentration of detergent fiber in alfalfa forage, an antiquality component, increased significantly, while crude protein concentration decreased in plots infested with the threecornered alfalfa hopper. Therefore, forage growers are faced with a yield reduction combined with a reduction in forage quality. Perhaps most important, plants stressed by the threecornered alfalfa hopper are thought to be more susceptible to plant disease, weed pressure, and other environmental conditions (Quisenberry et al. 1987).

Plant Disease Interaction. Although the threecornered alfalfa hopper has not been found to be a vector of plant disease (Bagga & Laster 1968, Weimer 1937, Blanton 1937, Smith 1924), it has been shown that girdling damage can increase plant disease. Herzog et al. (1975) found a positive relationship between girdle damage and incidence of infection by Sclerotium rolfsii Saccardo, the causal agent of Sclerotial blight on soybean. The girdle
site was thought to provide an infection site for the pathogen. Russin et al. (1986) studied the relationship between girdle damage and the incidence of stem canker on soybean. They found that the presence of girdles significantly increased canker lengths. In addition, yields from individual plants illustrated an apparent additive relationship between girdles and stem canker severity. It is thought that the girdle site provides a path for disease organisms to enter the plant, and girdling increases susceptibility to disease by reducing plant vigor.

Plant Response. Although damage by this insect can be severe, soybeans are able to compensate for girdle damage. Undamaged soybean plants compensate for girdled plants by producing more pods and beans. According to Mueller & Jones (1983), over 65% of the plants must be girdled early in the season before yields are reduced. Sparks (1984) found that although plant densities are lowered by threecornered alfalfa hopper girdling, even heavily damaged stands exceed the necessary 9.8 plants/row-meter necessary for maximum yield. In alfalfa, this ability to compensate for lost plants has not been studied.

Physiologically, plants may be able to overcome girdle damage. Often a gall-like callus forms above the girdle (Jordan 1952). This callus can increase in size until it completely covers the girdled area, a process
called "scabbing over" (Mueller 1976). Scabbing of the wound in soybean allows the plant to grow normally, with little reduction of soybean yields (Mueller 1976). It is thought that scabbing protects the girdle from disease and detrimental environmental factors. It is not known if translocation can resume after scabbing is complete. The strength of the girdle site is still reduced after scabbing, and the stem is still susceptible to breakage and lodging (Mueller 1976).

The reduction in translocation caused by a single girdle may be temporary in soybean petioles (Spurgeon & Mueller 1991). A study by Spurgeon & Mueller (1991) followed the translocation of glyphosate in girdled soybean plants. Translocation rates in petioles 7 and 14 d after girdling were similar to rates in ungirdled petioles. The investigators hypothesized that a proliferation of secondary phloem in the vicinity of the girdle resulted in the increased rate of translocation at older girdle sites. The ability of a plant to overcome girdle damage in this manner needs further study; however, this may be an important trait in developing plants with resistance to this insect.

Soybean stems may compensate for girdle damage by developing adventitious roots immediately above the girdle site (Herzog et al. 1975). These roots can increase in length until they reach soil level, and then may act as brace roots for the weakened plant (Herzog et al. 1975).
Johnson et al. (1988) observed that these root growths exhibited tissues organized as observed in true roots. When adventitious roots grow into the soil, they assume normal root development and appearance by producing lateral roots and root hairs. The anomalous growth observed could be induced by chemical substances present in threecornered alfalfa hopper saliva, such as plant hormone analogues, or may be caused by the physical wounding (Johnson et al. 1988). It is not yet known whether root growth is beneficial to the plant by creating a route around the translocation blockage or detrimental due to the rerouting of carbohydrates from the main tap root to the adventitious roots. More research needs to be done to determine if scabbing over and adventitious root growth are beneficial or detrimental to the damaged crop plant. If these two reactions are found to be beneficial, they could become important traits to use in developing cultivars resistant to threecornered alfalfa hopper damage.

Control. Control of the threecornered alfalfa hopper in soybean is generally achieved through the use of insecticide applications. Several insecticides including fenvalerate, acephate, and esfenvalerate are recommended for use against the threecornered alfalfa hopper on soybean (Anonymous 1991a). In alfalfa, only carbaryl (Sevin XLR) is labelled for threecornered alfalfa hopper control (Anonymous 1991b). Both carbaryl and
cypermethrin, however, have been shown to give adequate residual control (Isenhour 1985). Insecticidal control of threecornered alfalfa hopper populations on alfalfa is generally aimed toward adult populations even though late-instar nymphs are the most damaging stages (Moore & Mueller 1976). Nymphal populations are difficult to control with insecticides because they are found near the base of plants and are protected from spraying by the upper foliage canopy (Miner 1959). In addition, persistent insecticides cannot be used on alfalfa because it is harvested almost monthly during the growing season.

Graham & Ellisor (1938) suggested early harvest as a cultural control method against the threecornered alfalfa hopper. They reported that, in the spring, 97% of threecornered alfalfa hopper eggs are deposited more than two inches above the soil surface. Thus, an early harvest before the initial hatching of the eggs could reduce subsequent nymphal populations. This method may reduce initial spring populations; however, most of the late season adults migrate into alfalfa from soybean fields (Mitchell & Newsom 1984b). These more damaging populations would not be affected by this control strategy.

Biological control of the threecornered alfalfa hopper also has been studied. Several species of arthropods have been identified as being natural enemies of the threecornered alfalfa hopper. A strepsiteran
parasitoid, *Membracixenos jordani* Pierce, parasitizes the adult stage (Jordan 1953; Pierce 1952). Although this parasitoid does not cause the death of its host, no female threecornered alfalfa hopper parasitized by *M. jordani* has ever been found to contain eggs (Jordan 1953). The effectiveness of this parasitoid in reducing threecornered alfalfa hopper populations has not been studied. Jordan (1953) found during a 6 week period that 3 to 30% of collected adults were parasitized by *M. jordani*. Subsequent samples the following year, however, showed a very low parasitism rate.

Four egg parasitoids of the threecornered alfalfa hopper, *Gonatocerus ornatus* Gahan, *Polynema imitatrix* Gahan, *Paracentrobia periditrix* (Gahan), and *Anaphes ovijentatus* (Crosby and Leonard), have been identified (Gahan 1918; Stoner & Surber 1969). However, Daigle et al. (1988) and Rice & Drees (1985) found that parasitism of threecornered alfalfa hopper eggs is generally low. In addition, Jackson & Graham (1983) reported that *A. ovijentatus* only occasionally parasitizes the threecornered alfalfa hopper. Predacious arthropods, such as spiders and ants, also may play a small role in threecornered alfalfa hopper population suppression.

Insect resistant alfalfa cultivars have played an important role in the management of several insect species, including the spotted alfalfa aphid, *Theroioaphis maculata* (Buckton), and the pea aphid, *Acyrthosiphon pisum*
Harris, (Sorenson et al. 1988). No alfalfa cultivars are currently classified as being resistant to the threecornered alfalfa hopper (Manglitz & Ratcliff 1988). A large number of alfalfa cultivars have been screened for resistance to this insect. Kulash & Hanson (1949) screened 76 alfalfa cultivars, but reported none as being resistant. Nielson & Schonhorst (1965) screened 40 cultivars, and although none showed strong indications of resistance, 'Chilean 21-5', 'Kansas 67-1108', and 'Sirsa #9' were recommended as resistant germplasm sources. Randolph & Meisch (1970) screened 25 cultivars both in greenhouse and field studies. 'Zia', 'Socheville', 'Ladak', and 'Hairy Peruvian' showed higher levels of resistance to the threecornered alfalfa hopper in the greenhouse; however, none of the cultivars showed resistance under field conditions.

This review of the literature on the threecornered alfalfa hopper shows the importance of this insect to alfalfa production. The significant reduction in alfalfa yield, forage quality, and the decline in stand persistence associated with populations of the threecornered alfalfa hopper demonstrate the need for an effective control strategy. Because of the lack of effective insecticidal, cultural, and biological control strategies, host plant resistance need further investigation as an alternative method of control.
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CHAPTER I

Effects of Nymphal Populations of Threecornered Alfalfa Hopper (Homoptera: Membracidae) on 'Florida 77' Alfalfa Plants

This chapter is modified from manuscript # J90-390 submitted to and published by the Journal of Economic Entomology 84: 1889-1893 (1991).
The threecornered alfalfa hopper, *Spissistilus festinus* (Say), is recognized as an important late-summer pest of alfalfa, *Medicago sativa* L., in Louisiana (Farlow et al. 1981, Wilson & Quisenberry 1987). Feeding by the threecornered alfalfa hopper often results in a girdle that completely encircles the plant stem (Wildermuth 1915). The third through fifth nymphal instars of the threecornered alfalfa hopper are the most injurious developmental stages and cause significantly more girdling of soybean, *Glycine max* (L.) Merr., stems than adults or earlier instars (Moore & Mueller 1976). First and second instars have not been shown to girdle a stem, but rather injure the host plant by random feeding punctures (Moore & Mueller 1976).

Threecornered alfalfa hopper girdles on soybean stems interfere with the movement of translocates in the phloem, resulting in an increase of carbohydrates above the girdle (Hicks et al. 1984). Carbohydrate movement to root tissue may be reduced in alfalfa plants damaged by stem girdling (Jordan 1952). Because alfalfa plants rely on energy stored as root carbohydrates for regrowth after each harvest, a reduction in translocation to the roots could reduce stem regrowth. Lack of regrowth may reduce stand density, making it necessary to reseed the alfalfa stand annually (Graham 1938). Wilson & Quisenberry (1987) found that, in a field study, threecornered alfalfa hopper damage caused a 20% reduction in alfalfa dry-matter yield
during the sixth cutting period. In addition, populations of the three-cornered alfalfa hopper significantly reduced forage quality by reducing crude protein and increasing acid detergent fiber concentrations. Our greenhouse study was undertaken to determine the effects of three-cornered alfalfa hopper feeding on alfalfa growth, root carbohydrate content, and alfalfa yield and quality.

Materials and Methods

The experiment was conducted on 'Florida 77' alfalfa planted 21 November 1988. One plant per pot (4 liters) was maintained in a soil mixture consisting of Sunshine Growers Mix No. 1 (Fisons Western, Downers Grove, Ill.), vermiculite, and peat moss in a 3:1:1 ratio. The soil mixture was steam-sterilized for 12 h at 100°C before planting. All seeds were inoculated with Pelinoc (Nitrogen Company, Milwaukee, Wis.) to ensure the presence of nitrogen-fixing Rhizobium. The plants were grown under a photoperiod of 16:8 (L:D) and temperature was maintained at 27 ± 5°C. During the test period, all plants were watered three times weekly, once with tap water only and twice with a complete nutrient solution (Sheehy et al. 1980). This experiment consisted of infestation levels of zero, three, and six nymphs per plant with nine replicates. The study was continued through three harvest periods. Each treatment within a replicate consisted of six plants, two of which were destructively sampled at each harvest.
Plants were sprayed for the control of twospotted spider mite (*Tetranychus urticae* Koch) with methamidophos (Monitor 4 emulsifiable concentrate [EC] at 1.39 kg/1.89 liters; Mobay Corporation, Kansas City, Mo.) on 21 January and 27 February. Whitefly populations were controlled with carbofuran (Furadan 4 flowable [F] at 12.5ml/1.89 liters; FMC Corporation, Philadelphia, Pa.) on 27 February.

Adult threecornered alfalfa hoppers were collected from alfalfa fields at the Dean Lee and Red River Research Stations near Alexandria and Shreveport, La., respectively. The insects were brought into the laboratory, separated by sex, and placed in 0.95-liter glass jars containing three green bean pods (*Phaseolus vulgaris* L.). Insects were maintained at 27° C, under a 16:8 (L:D) photoperiod. After 10 d, the sexes were recombined in fresh glass jars. Each mating jar contained 10 females and 3 males with two fresh bean pods. Pods were removed from the jars, replaced, and enclosed individually in small plastic bags every 2 d for 10 d after the sexes were combined. Nymphs emerged from the bean pods in plastic bags after approximately 12 d.

Before the test, plants were clipped at the bloom stage on 27 February, 29 March, 30 April, and 20 May. On 15 June, all test plants were clipped to 7 cm. On 25 June, the number of stems that had begun to regrow was recorded. Selected numbers of first-instar threecornered
alfalfa hopper nymphs were removed from the bean pods in the plastic bags and placed directly on the test plants with a small camel's-hair brush. The nymphs were allowed to move freely about the plant. To eliminate nymphal movement from plant to plant, pots were spaced approximately 20 cm apart. Every 2 d, missing or dead nymphs were replaced with nymphs of approximately the same age.

When the control plants reached 10% bloom, the number of girdles present on each test plant was counted, plant heights were measured, and plants were clipped to 7 cm. As the plants were clipped, the number of stems that were at a harvestable height (>= 7 cm) was counted. After clipping, all plants were sprayed with malathion (50% EC at 2.5 ml/liter; Ferti-lome, Voluntary Purchasing Groups, Bonham, Tex.) to remove all nymphs from the test plants. Ten days later, the number of stems was again recorded, and first instars were placed onto the stems. Procedures during the second and third harvest periods were identical to that of the first period.

In addition to the measurements taken during the first harvest, chlorosis and plant maturity ratings were recorded on all plants just before the second and third harvests. The chlorosis rating system used was based on potato leafhopper (Empoasca fabae (Harris)) yellowing ratings: 1, 0-10% of leaves show yellowing; 2, between 10 and 20% yellow; etc. (Kehr and Manglitz 1984). The
maturity rating used was a 0-9 rating system (Kalu & Fick 1981) based on morphological stage of development where 0 was early vegetative and 9 was ripe seed pod.

All harvested plant material was placed in small paper bags and dried at 60°C for 48 h. The dry weight of the harvested forage from each plant was then recorded. Measurements of forage quality were taken using near-infrared spectroscopy calibrated to the respective tests: neutral detergent fiber, acid detergent fiber, cellulose, and acid-insoluble lignin contents as described by Goering (1970); crude protein content by the improved Kjeldahl method (Association of Official Agricultural Chemists 1980); and in-vitro dry digestible matter by the modified Van Soest procedure (Nelson et al. 1976).

Destructive root samples were taken at each harvest to determine root carbohydrate concentration. Roots were washed, and all secondary roots were removed. The remaining tap root was oven dried at 60°C for 48 h, then ground to pass through a 1-mm screen. Samples (100 mg) were taken from each root, and carbohydrate levels were determined by the phenol-sulfuric acid technique described by Whistler and Wolfrom (1962, 388-389) using a Beckman Model 35 Spectrophotometer (Beckman Scientific Instruments Division, Irvine, Calif.) set at 490 nm. Absorbance readings were compared with a standard curve determined using dextrose standards of 0, 50, 100, 150, and 200 parts per million.
Data were analyzed as a split-plot-over-time design with harvest as the main plot and population as the subplot with more than one observation per treatment per block (Steel & Torrie 1980). The Bartlett’s test of homogeneity of sampling error variances (plants) did not indicate heterogeneity; thus, the sampling error variances were pooled. Analysis of variance was computed using PC-SAS (SAS Institute 1989) general linear models. Treatment means were separated using Tukey’s studentized range test ($P = 0.05$) (Steel & Torrie 1980).

Results

The number of girdles per plant was significantly greater on plants infested with six nymphs compared to those infested with three nymphs ($F = 89.16\ df = 1,40; \ P = 0.0001$) (Fig. 1.1). The number of girdles increased through the test period until an average of a $15.2 \pm 0.97$ (mean ± SE) girdles per plant were found on plants infested with six nymphs at the third harvest period. This corresponds to an average of two girdles per stem for these heavily infested plants.

Mean values by individual harvest and across all harvests for stem counts and root carbohydrate concentrations are given in Table 1.1. There were no significant differences in the number of stems per plant among the three treatments at the first harvest ($F = 1.05;\ df = 2, 16; \ P = 0.3726$). The plants under the high infestation had significantly fewer stems reaching a
Figure 1.1. Mean number of girdles (± SEM) produced per plant by nymphal threecornered alfalfa hopper populations over three harvest periods.
Table 1.1. Stem regrowth and root carbohydrate content for each harvest and overall harvests as affected by *S. festinus* populations.

<table>
<thead>
<tr>
<th>Harvest</th>
<th>No. nymphs</th>
<th>No. live stems at infestation (mean ± SE)</th>
<th>No. stems at harvest (mean ± SE)</th>
<th>% Total avail. carbohydrates (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>9.6±0.4a</td>
<td>9.1±0.4a</td>
<td>40.3±2.1a</td>
</tr>
<tr>
<td>3</td>
<td>9.6±0.4a</td>
<td>9.5±0.5a</td>
<td>37.8±2.6a</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>9.3±0.3a</td>
<td>9.9±0.5a</td>
<td>34.9±3.0a</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>11.4±0.4a</td>
<td>8.4±0.4a</td>
<td>47.5±1.7a</td>
</tr>
<tr>
<td>3</td>
<td>11.4±0.5a</td>
<td>7.2±0.6ab</td>
<td>42.4±1.6ab</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>11.9±0.6a</td>
<td>6.4±0.4b</td>
<td>38.8±2.1b</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>11.0±0.5a</td>
<td>9.3±0.6a</td>
<td>53.3±2.0a</td>
</tr>
<tr>
<td>3</td>
<td>11.0±0.5a</td>
<td>8.1±0.8ab</td>
<td>49.0±1.9ab</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>8.0±0.7b</td>
<td>6.8±0.5b</td>
<td>45.8±2.7b</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>0</td>
<td>10.7±0.3a</td>
<td>9.0±0.3a</td>
<td>47.6±1.3a</td>
</tr>
<tr>
<td>3</td>
<td>9.9±0.3a</td>
<td>8.3±0.4a</td>
<td>43.3±1.3b</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>9.7±0.3a</td>
<td>7.7±0.4a</td>
<td>39.9±1.6c</td>
<td></td>
</tr>
</tbody>
</table>

Means followed by the same letter within harvest and column are not significantly different (P ≥ .05; d.f. = 2,16; Tukey's studentized multiple range test [Steel & Torrie 1980]).
harvestable height than the control plants at the second
\((F = 6.88; \text{df} = 2, 16; \ P = 0.007)\) and third \((F = 4.30; \text{df} = 2, 16; \ P = 0.032)\) harvests. Before the third harvest period, infested plants had significantly \((F = 6.59; \text{df} = 2, 16; \ P = 0.0082)\) fewer live stems than the control plants.

Root carbohydrate concentrations in plants under the high-infestation treatment were significantly lower than concentrations in control plants at the second \((F = 6.21; \text{df} = 2, 16; \ P = 0.0101)\) and third \((F = 3.24; \text{df} = 2, 16; \ P = 0.0658)\) harvests. At each individual harvest, the moderately infested plants had root carbohydrate levels intermediate to, but not significantly different from, control plants and plants under the heavy infestation. Over all three harvests, each addition of three nymphs significantly \((F = 9.62; \text{df} = 2, 16; \ P = 0.0018)\) reduced the carbohydrate concentration of the roots.

Mean values for plant heights, maturity ratings, and chlorosis ratings are given in Table 1.2. Heavy infestations of threecornered alfalfa hopper nymphs significantly \((F = 6.90; \text{df} = 2, 16; \ P = 0.0069)\) accelerated plant maturity at the third harvest and across the second and third harvests \((F = 4.31; \text{df} = 2, 16; \ P = 0.0319)\). Maturity ratings were not taken at the first harvest.

No chlorosis was seen at the first harvest, so ratings were not taken. At the second harvest, plants
Table 1.2. Plant height, maturity rating, and chlorosis rating for each harvest and overall harvests as affected by *S. festinus* populations.

<table>
<thead>
<tr>
<th>Harvest</th>
<th>No. nymphs</th>
<th>Ht (cm) (mean ± SE)</th>
<th>Maturity&lt;sup&gt;a&lt;/sup&gt; (mean ± SE)</th>
<th>Chlorosis&lt;sup&gt;b&lt;/sup&gt; (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>40.4±1.3a</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>44.4±1.2a</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>40.8±1.3a</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>45.3±1.8a</td>
<td>3.7±0.3a</td>
<td>2.3±0.3b</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>42.1±1.9a</td>
<td>4.2±0.3a</td>
<td>3.8±0.4a</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>45.7±2.0a</td>
<td>4.6±0.3a</td>
<td>4.6±0.5a</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>46.1±2.2a</td>
<td>4.6±0.4b</td>
<td>2.7±0.4b</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>44.1±1.8a</td>
<td>4.9±0.4b</td>
<td>3.2±0.5ab</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>47.4±1.8a</td>
<td>5.8±0.3a</td>
<td>4.7±0.6a</td>
</tr>
<tr>
<td>Overall</td>
<td>0</td>
<td>43.9±1.0a</td>
<td>4.1±0.2b</td>
<td>2.4±0.2b</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>43.5±0.9a</td>
<td>4.6±0.2ab</td>
<td>3.6±0.3ab</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>46.6±1.0a</td>
<td>5.1±0.2a</td>
<td>4.6±0.4a</td>
</tr>
</tbody>
</table>

Means followed by the same letter within harvest and column are not significantly different (P ≥ .05; d.f. = 2,16; Tukey's studentized multiple range test [Steel & Torrie 1980]). a (Kalu and Fick 1981); b Yellowing rating (Kehr and Manglitz 1984).
infested by either three or six threecornered alfalfa hopper nymphs showed a significant ($F = 8.72; df = 2, 16; P = 0.0027$) increase in leaf chlorosis compared with plants in the control. At the third harvest, only the heavily infested plants had chlorosis ratings significantly ($F = 4.08; df = 2, 16; P = 0.0370$) higher than the plants in the control. The presence of threecornered alfalfa hopper nymphs did not affect plant height at each individual harvest or over the three harvests.

Tables 1.3 and 1.4 give the mean values for the forage parameters measured. Forage dry weight per plant was not significantly ($F = 0.05; df = 2, 16; P = 0.950$) affected by the presence of threecornered alfalfa hopper nymphs. Crude protein in the infested plants was significantly lower than in plants in the control over all harvests ($F = 34.04; df = 2, 16; P < 0.0001$). Neutral detergent fiber, which reduces forage quality, was significantly ($F = 15.22; df = 2, 16; P = 0.0002$) higher in the infested plants than in control plants at the first harvest. At the second harvest, only the heavily infested plants had a significant ($F = 7.94; df = 2, 16; P = 0.004$) increase in neutral detergent fiber. Acid detergent fiber, cellulose, acid-insoluble lignin, and in-vitro dry digestible matter were all unaffected by the presence of threecornered alfalfa hopper nymphs.
Table 1.3. Forage dry weight, in-vitro digestibility and crude protein content for each harvest and overall harvests as affected by S. festinus populations.

<table>
<thead>
<tr>
<th>Harvest</th>
<th>No. Nymphs</th>
<th>Dry weight (g/plant) (mean ± SE)</th>
<th>% IVDDM&lt;sup&gt;a&lt;/sup&gt; (mean ± SE)</th>
<th>% Protein (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>1.6±0.1a</td>
<td>77.6±0.3a</td>
<td>21.5±0.3a</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.0±0.1a</td>
<td>77.3±0.3a</td>
<td>20.0±0.2b</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.9±0.1a</td>
<td>76.7±0.2a</td>
<td>19.9±0.2b</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>1.5±0.1a</td>
<td>75.1±0.4a</td>
<td>21.3±0.3a</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.3±0.1a</td>
<td>75.8±0.2a</td>
<td>19.6±0.3b</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.4±0.1a</td>
<td>75.0±0.4a</td>
<td>18.5±0.3c</td>
</tr>
<tr>
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<td>1.4±0.1a</td>
<td>73.9±0.5a</td>
<td>18.2±0.3a</td>
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<tr>
<td></td>
<td>3</td>
<td>1.3±0.1a</td>
<td>74.3±0.3a</td>
<td>16.7±0.3b</td>
</tr>
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<td>1.2±0.1a</td>
<td>74.2±0.2a</td>
<td>16.9±0.4b</td>
</tr>
<tr>
<td>Overall</td>
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<td>75.4±0.2a</td>
<td>20.3±0.2a</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.5±0.1a</td>
<td>75.6±0.2a</td>
<td>18.8±0.2b</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.5±0.1a</td>
<td>75.6±0.2a</td>
<td>18.4±0.2b</td>
</tr>
</tbody>
</table>

Means followed by the same letter within harvest and column are not significantly different (P > .05; d.f. = 2,16; Tukey's studentized multiple range test [Steel & Torrie 1980]). <sup>a</sup> IVDDM, in-vitro dry digestible matter
Table 1.4. Neutral detergent fiber, acid detergent fiber, cellulose and acid insoluble lignin content for each harvest and overall as affected by *S. festinus* populations.

<table>
<thead>
<tr>
<th>Harvest</th>
<th>No. Nymph</th>
<th>% neutral detergent fiber (mean ± SE)</th>
<th>% Acid detergent fiber (mean ± SE)</th>
<th>% cellulose (mean ± SE)</th>
<th>% Acid insoluble lignin (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>29.0±0.4a</td>
<td>20.6±0.4a</td>
<td>16.3±0.3a</td>
<td>4.0±0.1a</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>31.3±0.4a</td>
<td>20.8±0.5a</td>
<td>16.4±0.2a</td>
<td>4.0±0.1a</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>30.7±0.3a</td>
<td>20.6±0.4a</td>
<td>15.6±0.2a</td>
<td>3.7±0.1a</td>
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<tr>
<td>2</td>
<td>0</td>
<td>30.3±0.6b</td>
<td>22.0±0.5a</td>
<td>17.3±0.3a</td>
<td>5.6±0.1a</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>31.5±0.6ab</td>
<td>22.2±0.6a</td>
<td>16.8±0.3a</td>
<td>4.3±0.2a</td>
</tr>
<tr>
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<td>6</td>
<td>32.8±0.5a</td>
<td>22.7±0.7a</td>
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<td>32.0±0.8a</td>
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<td>3.4±0.2a</td>
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<tr>
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<td>22.8±0.4a</td>
<td>17.2±0.4a</td>
<td>3.9±0.2a</td>
</tr>
<tr>
<td>Overall</td>
<td>0</td>
<td>30.4±0.3b</td>
<td>21.9±0.3a</td>
<td>17.1±0.2a</td>
<td>4.5±0.1a</td>
</tr>
<tr>
<td></td>
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<td>22.2±0.3a</td>
<td>16.7±0.2a</td>
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<tr>
<td></td>
<td>6</td>
<td>32.5±0.3a</td>
<td>22.0±0.3a</td>
<td>16.7±0.2a</td>
<td>4.0±0.1a</td>
</tr>
</tbody>
</table>

Means followed by the same letter within harvest and column are not significantly different (P ≥ .05; d.f. = 2,16; Tukey's studentized multiple range test [Steel & Torrie 1980]).
Discussion

The large number of girdles recorded during the study indicates that threecornered alfalfa hopper nymphs were feeding heavily on the infested plants. Most nymphs were found feeding near the girdle site. Hicks et al. (1984) found that the girdle disrupts translocation, creating a nutrient sink just above the girdle. It is in this sink that the insect usually feeds (Mitchell & Newsom 1984). As stated previously, under the high infestations, an average of two girdles were found on each stem at the third harvest. Snyder (1935) also found multiple girdles per stem on black locust seedlings.

The reduction in root carbohydrate concentration indicates that nymphal populations can reduce the amount of carbohydrates that is translocated to the roots. Reductions of root carbohydrate concentration can weaken the plant vigor for regrowth and leave the plant in a condition that is more susceptible to other environmental stresses (Kitchen et al. 1990). Although this study was not designed to correlate carbohydrate levels with stem regrowth, these factors may be related because both are adversely affected by nymphal populations. After two harvest periods under heavy infestation, the number of stems reaching a harvestable height was reduced by 23% as compared with the plants in the control. After three harvest periods, there was almost a 27% reduction in the number of stems in the heavy-infestation treatment. In
Louisiana, alfalfa may be harvested six times in a growing season. Thus, reductions in root carbohydrate concentration and stem regrowth could become much more severe under normal growing conditions.

Threecornered alfalfa hopper populations have been linked with leaf chlorosis in earlier studies (Miner 1959, Duggar 1929), although the amount of chlorosis had not been quantified. The increase in leaf chlorosis in the infested alfalfa plants indicates that the plants were under considerable stress. Yellowing of the leaves may be caused by a variety of factors including deficiencies in plant nutrients such as phosphorous, boron, and magnesium (Graham et al. 1979). The exact cause of the chlorosis, however, is unknown and needs to be studied further.

The enhancement of flower development found in this study conflicts with the results Wilson & Quisenberry (1987) found under field conditions. In that study, threecornered alfalfa hopper populations delayed alfalfa maturity by 10-14 d. A delay in plant maturity could reduce yields by reducing the number of harvests during a growing season. An increased rate of flower development also could reduce yields by slowing the amount of growth before flower development. Further study is needed to understand why flower development would be affected by threecornered alfalfa hopper damage and how this could affect alfalfa production.
Significant reductions in forage quality because of lower crude protein levels or higher neutral detergent fiber content (or both) occurred at all three harvests. Because leaf chlorosis was not observed until the second harvest, reductions in forage quality can occur before any visual effects of girdle damage are noticed. No reductions in forage dry weight were found throughout the experiment. This could possibly be caused by high variability of single plant measurements. Over larger areas, threecornered alfalfa hopper populations have been found to reduce alfalfa yields significantly (Wildermuth 1915, Wilson & Quisenberry 1987).

The increase of leaf chlorosis, the enhancement of flower development, and the reduction of forage quality found in our study show that threecornered alfalfa hopper nymphs stress the plant by their feeding activity. Results of this experiment also show that girdle damage by threecornered alfalfa hopper nymphs reduces stem regrowth, possibly by reducing the amount of total root carbohydrates available. Reductions in root carbohydrate concentration and stem regrowth could weaken the plant stand, making the stand more susceptible to weed invasion (Buntin & Pedigo 1986), disease pressure, or other detrimental environmental conditions (Kitchen et al. 1990).
References Cited


CHAPTER II

Fusarium Crown-rot Development in Alfalfa
Stressed by Threecornered Alfalfa Hopper
(Homoptera: Membracidae) Feeding

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submitted to the Journal of Economic Entomology and
accepted for publication on 30 December 1991.
Poor stand persistence is a major problem hindering the profitability of alfalfa (*Medicago sativa* L.) production in Louisiana (Wilson & Quisenberry 1987). Stand decline may be caused by combinations of insect and disease pressures common in alfalfa fields. Damage caused by Fusarium crown-rot (primarily *Fusarium oxysporum* Schlecht.) and the threecornered alfalfa hopper, *Spissistilus festinus* (Say), are two of the most important pest problems encountered in Louisiana (Lee et al. 1990, Wilson & Quisenberry 1987).

Feeding by the threecornered alfalfa hopper often results in a girdle that completely encircles the plant stem (Wildermuth 1915). In soybean (*Glycine max* L.), these girdles block translocation in the phloem, resulting in an increase of carbohydrates above the girdle (Hicks et al. 1984). Moellenbeck & Quisenberry (1991) found that nymphal threecornered alfalfa populations can reduce root carbohydrate reserves and stem regrowth in alfalfa.

Fusarium crown-rot is a serious disease affecting alfalfa production throughout the world (Leath 1990). *Fusarium oxysporum* is commonly isolated from alfalfa roots and crowns (Richard et al. 1980, Leath 1990, Uddin & Knous 1991), and it has been found to be one of the most virulent *Fusarium* species (Leath & Kendall 1978). It is known to cause considerable stand decline and yield reduction (Richard et al. 1980). The causal agent of this disease is present in most alfalfa producing areas.
However, plants are usually damaged when they are under stress from foliar diseases, untimely harvests, severe winter conditions, or insect pressures (Leath 1990, Leath & Byers 1977). Leath and Byers (1977) found an increase in Fusarium root-rot development in alfalfa plants stressed by the pea aphid, Acyrthosiphon pisum (Harris). In addition, Godfrey & Yeargan (1987) showed that alfalfa stressed by both root-rot fungi and clover root curculio, Sitona hispidulus (F.), had lower yields than alfalfa stressed by either of the two pests alone.

The threecornered alfalfa hopper has been shown to increase both Sclerotial blight (Herzog et al. 1975) and soybean stem canker severity (Russin et al. 1986) in soybean; however, it has not been studied in relation to any alfalfa disease. The main purpose of this study was to evaluate the effect of stress caused by threecornered alfalfa hopper damage on Fusarium crown-rot development and severity. In addition, the effects of these two pests, alone and in combination, on plant development, root carbohydrate concentration, forage yield, and forage quality were studied.

**Materials and Methods**

'Florida 77' alfalfa was planted in the greenhouse on 24 November 1989. One plant per pot (4 liters) was maintained in Sunshine Growers Mix No. 1 (Fisons Western, Downers Grove, Ill.). The soil mixture was steam-sterilized for 12 h at 100°C before planting. All seeds
were inoculated with Pelinoc (Nitrogen Company, Milwaukee, Wis.) to ensure the presence of nitrogen-fixing Rhizobium. The plants were grown under greenhouse conditions with a photoperiod of 16:8 (L:D) and temperature was maintained at 27 ± 5°C. Plants were watered to run through every 3 d. Before the testing period, plants were fertilized monthly with a 0-20-20 (P-N-K) solution. The experimental design used was a 2 x 3 factorial arrangement of treatments in a randomized block design with eight replications. Treatments consisted of the presence or absence of F. oxysporum in combination with zero, three, or six threecornered alfalfa hoppers per plant. The study was continued through six harvest periods, each approximately a month in duration. Each combination of factors within a replication consisted of four plants, two of which were destructively sampled at the third and sixth harvests.

Adult threecornered alfalfa hoppers were collected from a soybean field at the Dean Lee Research Station near Alexandria, La. and an alfalfa field at the Northeast Research Station near St. Joseph, La. The insects were brought into the laboratory and reared on green bean pods (Phaseolus vulgaris L.) as described by Moellenbeck & Quisenberry (1991). An isolate of F. oxysporum, collected from field-grown, necrotic, alfalfa roots was used in this experiment. Suspensions were prepared by culturing the isolate on fresh potato-carrot broth (Lee et al. 1990).
Because Fusarium crown-rot develops slowly and is usually associated with stressed alfalfa (Leath 1990), the *F. oxysporum* inoculum was injected directly into the root tissue to ensure inoculation and increase the rate of infection. Plants were inoculated with *F. oxysporum* on 8 March 1990. Soil was removed to expose the plant crowns, and a hypodermic needle was inserted to a depth of 5 mm into each plant. Plants were injected with either 1 ml of fungal suspension containing $1 \times 10^5$ conidia or 1 ml of distilled water (control). Separate hypodermic needles were used for the isolate and the control.

Plants were harvested at 10% bloom (1 wk after inoculation with fungal suspension) and then maintained through one entire harvest period. On 16 April 1990, all plants were clipped to a height of 7 cm. The next day selected numbers of first instar threecornered alfalfa hopper nymphs were placed, using a small camel-hair brush, directly onto the stems of the test plants. Because threecornered alfalfa hopper nymphs are somewhat sedentary, the nymphs were not caged on the plants. However, plants were spaced approximately 20 cm apart to eliminate plant to plant movement.

When the control plants reached 10% bloom, the girdles present on each plant were counted, plant heights were measured, and plant maturity ratings recorded. The maturity rating system used was a 0-9 rating system (Kalu & Fick 1981) based on morphological stage of development.
Plants were then clipped to a 7 cm height. As the plants were clipped, the stems that were at a harvestable height (>7 cm) were counted. After clipping, all plants were sprayed with malathion (50% emulsifiable concentrate at 2.5 ml/liter: Ferti-lome, Voluntary Purchasing Groups, Bonham, Tex.) to remove all nymphs from the test plants. Ten d later, first instar nymphs were placed back onto the stems.

Harvested plant material was placed in small paper bags (12 by 22 cm) and dried at 60° C for 48 h. Dry weight of each plant was recorded, and the forage ground to particles < 1 mm in size. Forage quality was determined using near-infrared spectroscopy calibrated to the respective tests: neutral detergent fiber (NDF) and acid detergent fiber (ADF) as described by Goering (1970); crude protein (CP) content by the improved Kjeldahl method (Association of Official Agricultural Chemists 1980); and in vitro dry digestible matter (IVDDM) by the modified Van Soest procedure (Nelson et al. 1976). Procedures used during the second, fourth, and fifth harvest periods were identical to the first.

At the third and sixth harvest, destructive root samples were taken to determine root carbohydrate concentration and root necrosis severity. Roots were washed, and all secondary roots were removed. Roots were then split longitudinally to expose any necrotic root tissue. Necrosis was categorized by measuring the length
of spread from the point of injection and by a 0-4 rating (Lee et al. 1990) where 0 = no necrosis; 1 = slight
discoloration, 1 - 10% tissue affected; 2 = discoloration
spreading, 11 - 50% tissue affected; 3 = large area
discolored, 51 - 75% tissue affected; and 4 = extensive
necrosis, 75 - 100% tissue affected. A sample (1 cm) from
each diseased root was plated on fresh potato-carrot agar
(Dade & Gunnell 1969) to verify the presence of F.
oxysporum. Roots then were oven dried at 60° C for 48 h
and ground in a cyclone mill to pass through a 1 mm
screen. Carbohydrate levels were determined from root
samples (100 mg) by the phenol-sulfuric acid technique
described by Whistler & Wolfrom (1962) using a Beckman
Model 35 Spectrophotometer (Beckman Scientific Instruments
Division, Irvine, Cal.) set at 490 nm. Absorbance
readings were compared with a standard curve determined
using dextrose standards of 0, 50, 100, 150, and 200 parts
per million.

Data were analyzed by a repeated measures
multivariate approach (Moser et al. 1990) using the
repeated statement of PROC GLM of SAS (SAS Institute
1989). Bartlett's test of homogeneity did not indicate
significant heterogeneity and thus, the sampling error
variances (plants) were pooled. Significance was
determined with the Wilks' criterion (L) (Wilks 1932) and
F statistic approximation. Orthogonal contrasts were used
to determine the average effect of three and six nymphs
per plant versus the non-infested plants and six nymphs per plant versus three nymphs per plant. Individual harvest analyses were performed for dependent variables showing a significant interaction between harvest and insect, harvest and pathogen, or harvest and insect and pathogen. Girdles per plant were analyzed using only plants that had been infested. Further, only inoculated plants were used to determine the effect of insect populations on disease index and length of necrosis.

Results

Platings of diseased root samples indicated that F. oxysporum was present in all samples from the inoculated plants. Some uninoculated plants contained necrotic tissue, but F. oxysporum was not isolated from these tissues. Thus, all uninoculated plants were given zeros for both lesion length and disease index. Mean lesion lengths was 19.81 ± 1.75 mm (± SEM) and the disease index was 2.81 ± 0.14 (± SEM) for inoculated plants at the sixth harvest. The overall multivariate analysis showed a significant interaction between harvest and pathogen for both lesion length ($L = 0.62; F = 20.48; df = 1, 34; P < 0.0001$) and disease index ($L = 0.62; F = 20.88; df = 1, 34; P < 0.0001$). At both the third and sixth harvests, the presence of F. oxysporum caused significant lesion lengths ($F = 66.39; df = 1, 35; P = 0.0001; F = 110.51; df = 1, 34; P = 0.0001$) and disease indices ($F = 164.83; df = 1, 35; P = 0.0001; F = 304.35; df = 1, 34; P = 0.0001$).
Damage caused by the threecornered alfalfa hopper did increase the development and severity of *Fusarium* crown-rot. Nymphal populations significantly (*F* = 4.58; df = 2, 14; *P* = 0.0296) increased disease index at the sixth harvest (Fig. 2.1). In addition, a trend developed where the length of necrosis was increased with each addition of three nymphs at both the third and sixth harvest (Fig. 2.2). At each harvest the number of girdles in inoculated plants was higher than in the uninoculated plants, although the differences were not significant (Fig. 2.3).

The effect of insect infestation and the interaction between harvest and insect were significant for the number of girdles per plant (Table 2.1). The contrasts of the non-infested versus the two infestation levels and three insects versus six insects could not be calculated because only infested plants were included in the analysis of girdle counts. Within individual harvests, six insects caused a significant increase in girdles produced when compared to three insects, with the exception of the first harvest (Fig. 2.4). A maximum of 8.78 ± 0.81 (mean ± SEM) girdles per plant was counted on plants infested with six nymphs at the fifth harvest. Girdle counts on plants infested with three nymphs per plant peaked at the fourth harvest when 5.59 ± 0.47 (mean ± SEM) girdles were found.

The interaction effect between insect and pathogen was significant (*F* = 3.26; df = 2, 34; *P* = 0.0505) for the
Figure 2.1. Mean disease index (± SEM) in plants infested by 0, 3, and 6 nymphs over six harvest periods.
Figure 2.2. Mean length of Necrosis (± SEM) in plants infested by 0, 3, and 6 nymphs over six harvest periods.
Figure 2.3. Mean number of girdles (± SEM) on *F. oxysporum* inoculated and uninoculated plants.
Table 2.1. Multivariate repeated measures analysis of girdles, plant height, maturity, and dry weight yield.

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>No. of Girdles</th>
<th>Height</th>
<th>Maturity</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>L&lt;sup&gt;e&lt;/sup&gt;</td>
<td>F</td>
<td>Pr&gt;F</td>
</tr>
<tr>
<td>H</td>
<td>5,30</td>
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<td>31.74</td>
<td>0.00</td>
<td>0.09</td>
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<tr>
<td>HxB</td>
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<td>2.68</td>
<td>0.04</td>
<td>0.13</td>
</tr>
<tr>
<td>HxI</td>
<td>10,60</td>
<td>0.27</td>
<td>6.10</td>
<td>0.01</td>
<td>0.52</td>
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<tr>
<td>HxP</td>
<td>5,30</td>
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<td>0.25</td>
<td>0.93</td>
<td>0.80</td>
</tr>
<tr>
<td>HxIxP</td>
<td>10,60</td>
<td>0.63</td>
<td>1.29</td>
<td>0.33</td>
<td>0.71</td>
</tr>
<tr>
<td>I</td>
<td>2,34</td>
<td>13.73</td>
<td>0.00</td>
<td>3.91</td>
<td>0.03</td>
</tr>
<tr>
<td>P</td>
<td>1,34</td>
<td>0.60</td>
<td>0.45</td>
<td>2.03</td>
<td>0.16</td>
</tr>
<tr>
<td>IxP</td>
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<td>0.75</td>
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<td>0 vs 3,6</td>
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<td>.</td>
<td>3.81</td>
<td>0.06</td>
</tr>
<tr>
<td>3 vs 6</td>
<td>1,34</td>
<td>.</td>
<td>.</td>
<td>4.19</td>
<td>0.05</td>
</tr>
</tbody>
</table>

a H = Harvest, B = Block, I = Insect, P = Pathogen, 0 vs 3,6 = Contrast between zero insects and the average of three and six insects, 3 vs 6 = Contrast between three insects and six insects.

b df for No. of girdles: HxB = 25,42; HxI = 5,11; HxP = 5,11; HxIxP = 5,11; I = 1,15; P = 1,15; IxP = 1,15

c 0 - 9 rating (Kalu & Fick 1981).

d Dry weight yield (g per plant)

e Wilks' criterion (Wilks 1932)
Figure 2.4. Mean number of girdles (± SEM) on plants infested by 0, 3, and 6 nymphs over six harvest periods.
number of harvestable stems over all harvests. The main
effects of insect infestation ($F = 0.62; df = 2, 34; P = 0.5449$) and pathogen inoculation ($F = 0.12; df = 1, 34; P = 0.7302$) were not significant ($F = Plants infested with six nymphs averaged $5.86 \pm 0.64$ (mean $\pm$ SEM) stems over the six harvests when inoculated with *F. oxysporum* and $6.71 \pm 0.69$ (mean $\pm$ SEM) when not inoculated. Plants infested with three nymphs per plant were not affected by the presence of *F. oxysporum*, averaging $6.63 \pm 0.89$ (mean $\pm$ SEM) stems when inoculated and $6.61 \pm 0.81$ (mean $\pm$ SEM) when not inoculated. Uninfested plants also were not affected by the presence of *F. oxysporum*, averaging $6.76 \pm 0.85$ (mean $\pm$ SEM) when inoculated and $6.20 \pm 0.79$ (mean $\pm$ SEM) when uninoculated.

The interaction between harvest and insect also was significant for plant height (Table 2.1). Individual harvest analyses showed a significant ($F = 9.36; df = 2, 34; P = 0.0006$) insect effect at the sixth harvest (Fig. 2.5). The contrast of three versus six insects was significant over all six harvests (Table 2.1). *Fusarium oxysporum* did not affect plant height overall or at any individual harvest. In addition, neither the presence of threecornered alfalfa hopper nymphs nor *F. oxysporum* significantly affected plant maturity (Table 2.1).

The interactions between harvest and insect and harvest and pathogen were significant for forage dry weight yield (Table 2.1). Individual harvest analyses
Figure 2.5. Average height (± SEM) of plants infested by 0, 3, and 6 nymphs over six harvest periods.
showed a significant ($F = 10.71; \text{df} = 2, 34; P = 0.0002$) insect effect only at the sixth harvest (Fig. 2.6). Yields at the previous harvests were essentially equal among the non-infested and the infested plants. Thus, only after six harvest periods, did threecornered alfalfa hopper populations reduce dry weight yield. The effect of inoculation of *F. oxysporum* was significant at the first ($F = 6.02; \text{df} = 1, 35; P = 0.0193$) and fourth ($F = 3.65; \text{df} = 1, 35; P = 0.0645$) harvests (Fig. 2.7). The inoculated plants had a higher dry weight yield at the first harvest. The yields were essentially equal until the fourth harvest where the uninoculated plants had higher dry weight yields. The yields were again equal at the fifth and sixth harvests.

The interaction between harvest and insect was significant ($P \leq 0.05$) for all of the forage quality parameters measured (Table 2.2). The harvest and pathogen interaction was not significant for any of the parameters. The three-way interaction between harvest, insect and pathogen was significant for ADF concentration.

The individual harvest analyses of CP concentration showed a significant insect effect at harvest one ($F = 4.31; \text{df} = 2, 34; P = 0.0212$), two ($F = 4.44; \text{df} = 2, 35; P = 0.0192$), three ($F = 6.13; \text{df} = 2, 35; P = 0.0052$), five ($F = 22.39; \text{df} = 2, 34; P = 0.0001$), and six ($F = 5.86; \text{df} = 2, 34; P = 0.0065$). The non-infested plants had the highest CP levels at all six harvests (Fig. 2.8). The
Figure 2.6. Average yield (± SEM) of plants infested by 0, 3, and 6 nymphs over six harvest periods.
Figure 2.7. Average yield (± SEM) of *F. oxysporum* inoculated and uninoculated plants.
Table 2.2. Multivariate repeated measures analysis of forage quality parameters.

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>ADF</th>
<th>NDF</th>
<th>IVDDM</th>
<th>CP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L^2</td>
<td>F</td>
<td>Pr&gt;F</td>
<td>L</td>
</tr>
<tr>
<td>H</td>
<td>5,30</td>
<td>0.10</td>
<td>54.25</td>
<td>0.00</td>
<td>0.24</td>
</tr>
<tr>
<td>HxB</td>
<td>35,129</td>
<td>0.14</td>
<td>2.14</td>
<td>0.00</td>
<td>0.19</td>
</tr>
<tr>
<td>HxI</td>
<td>10,60</td>
<td>0.39</td>
<td>3.67</td>
<td>0.00</td>
<td>0.56</td>
</tr>
<tr>
<td>HxP</td>
<td>5,30</td>
<td>0.92</td>
<td>0.51</td>
<td>0.76</td>
<td>0.84</td>
</tr>
<tr>
<td>HxIXP</td>
<td>10,60</td>
<td>0.54</td>
<td>2.19</td>
<td>0.03</td>
<td>0.85</td>
</tr>
<tr>
<td>I</td>
<td>2,34</td>
<td>6.11</td>
<td>0.01</td>
<td>2.77</td>
<td>0.08</td>
</tr>
<tr>
<td>P</td>
<td>1,34</td>
<td>2.40</td>
<td>0.13</td>
<td>0.59</td>
<td>0.45</td>
</tr>
<tr>
<td>IXP</td>
<td>2,34</td>
<td>1.10</td>
<td>0.35</td>
<td>0.22</td>
<td>0.80</td>
</tr>
<tr>
<td>0 vs 3,6</td>
<td>1,34</td>
<td>4.88</td>
<td>0.03</td>
<td>5.39</td>
<td>0.03</td>
</tr>
<tr>
<td>3 vs 6</td>
<td>1,34</td>
<td>7.08</td>
<td>0.01</td>
<td>0.12</td>
<td>0.74</td>
</tr>
</tbody>
</table>

a  H = Harvest, B = Block, I = Insect, P = Pathogen, 0 vs 3,6 = Contrast between zero insects and the average of three and six insects, 3 vs 6 = Contrast between three insects and six insects.
b  ADF = Acid detergent fiber
c  NDF = Neutral detergent fiber
d  IVDDM = In-vitro dry digestible matter
e  CP = Crude Protein
f  Wilks' criterion (Wilks 1932)
Figure 2.8. Percent crude protein (± SEM) on plants infested by 0, 3, and 6 nymphs over six harvest periods.
contrast of the non-infested versus the two population levels was significant (Table 2.2).

The individual harvest analyses of IVDDM showed a significant insect effect at harvests one ($F = 5.20; \text{df} = 2, 35; P = 0.0105$) and five ($F = 5.06; \text{df} = 2, 34; P = 0.0119$). At the first harvest, the heavily infested plants had the highest IVDDM values. At harvest 5, the non-infested plants had the highest values. The IVDDM values were essentially equal among the populations at the remaining harvests (Fig. 2.9).

The contrast between the non-infested and infested plants was significant for NDF concentration (Table 2.2). NDF was significantly affected by nymphal populations at the first ($F = 3.92; \text{df} = 2, 35; P = 0.0291$) and sixth ($F = 4.43; \text{df} = 2, 34; P = 0.0195$) harvests. With the exception of harvest two, the non-infested plants had the lowest NDF concentration throughout the test (Fig. 2.10).

The interaction between insect and pathogen was significant for ADF concentration at the fifth harvest ($F = 4.43; \text{df} = 2, 34; P = 0.0195$) where the non-infested and heavily infested plants had higher percentages when inoculated with \textit{F. oxysporum}. The plants under the low infestation level, however, had a lower ADF concentration when inoculated. Overall, inoculated plants infested with three nymphs had the highest ADF concentration ($33.31 \pm 0.20\%$, mean $\pm$ SEM) and the control plants had the lowest concentration ($32.13 \pm 0.17$, mean $\pm$ SEM). Significant
Figure 2.9. Percent in-vitro digestible dry matter in plants infested by 0, 3, and 6 nymphs over six harvest periods.
Figure 2.10. Percent neutral detergent fiber in plants infested by 0, 3, and 6 nymphs over six harvest periods.
effects from insects were found at the first ($F = 6.99$; df $= 2$, 35; $P = 0.0028$), second ($F = 3.99$; df $= 2$, 35; $P = 0.0275$), fifth ($F = 5.88$; df $= 2$, 34; $P = 0.0061$), and sixth ($F = 6.26$; df $= 2$, 34; $P = 0.0048$) harvests. At the first two harvests, the non-infested plants were intermediate to the two infestation levels. At the fifth and sixth harvest, the non-infested plants had lowest ADF concentrations (Fig. 2.11).

Root carbohydrate concentration was significantly ($F = 22.99$; df $= 2$, 35; $P = 0.0001$) reduced by insect populations. In addition, both the contrast of absence versus presence of insects ($F = 37.21$; df $= 1$, 35; $P = 0.0001$) and the low versus the high population ($F = 8.76$; df $= 1$, 35; $P = 0.0055$) were significant. The non-infested, low population, and high population plants averaged root carbohydrate concentrations of 51.57 ± 2.94%, 44.54 ± 3.31%, and 39.07 ± 3.46% (mean ± SEM), respectively. Neither the main effect of pathogen ($F = 0.86$; df $= 1$, 35; $P = 0.3592$) nor the interaction between insect and pathogen ($F = 1.38$; df $= 2$, 35; $P = 0.2651$) were significant.

Discussion

The number of girdles, length of necrosis, and disease index indicated heavy pressure on the test plants. A 2.81 disease index in inoculated plants at the sixth harvest indicates that approximately 50% of the root tissue was necrotic. The stabilization of girdle counts
Figure 2.11. Percent acid detergent fiber in plants infested by 0, 3, and 6 nymphs over six harvest periods.
at the fifth and sixth harvests may have been caused by the reduction in the number of stems for those two harvest periods. At harvest five, plants infested with six nymphs per plant averaged 8.78 girdles and 6.72 stems which produced a girdle to stem ratio of 1.31. At the sixth harvest, this ratio was 1.49. Thus, although fewer girdles were counted, the number of girdles per stem was greater. This may indicate girdling was reduced due to a lack of suitable sites.

The only significant yield reduction in plants inoculated with *Fusarium oxysporum* was for harvestable stems in plants infested with six threecornered alfalfa hoppers. These results suggest that Fusarium crown-rot severity is affected by an interaction with stress caused by insects. Stress caused by the pea aphid has been previously shown to increase *Fusarium* crown-rot severity (Leath 1990). Leath & Byers (1977) also showed that Fusarium crown-rot reduced plant longevity when the plants were subjected to aphid feeding. Our data show stress caused by high threecornered alfalfa hopper populations increase the effect of *F. oxysporum* on alfalfa plants in terms of stem regrowth.

The threecornered alfalfa hopper has previously been linked to increased disease severity in soybean (Herzog et al. 1975, Russin et al. 1986). In our study, damage caused by threecornered alfalfa hoppers increased Fusarium crown-rot severity after six harvest periods. This may be
very important in establishing the relationship between threecornered alfalfa hopper damage and stand decline. As alfalfa stands mature, Fusarium crown-rot becomes much more important (Richard et al. 1980). In association with threecornered alfalfa hopper feeding, Fusarium crown-rot may become more severe or develop more quickly under field conditions.

The effect of threecornered alfalfa hopper damage on plant height has not been fully explained. Moellenbeck & Quisenberry (1991) found that nymphal populations did not affect plant height over three harvest periods in a greenhouse study. However, the threecornered alfalfa hopper has been linked with decreased growth (Duggar 1930), and has been found to reduce plant height at the fifth and sixth harvests under field conditions (Wilson & Quisenberry 1987). The results of this study show that nymphal populations cause a reduction in plant height after repeated infestations. Further study is necessary to better determine the relationship between threecornered alfalfa hopper damage and plant growth both during single population outbreaks and after repeated infestations.

Earlier studies have yielded conflicting results regarding the effect of threecornered alfalfa hoppers on plant maturity. Nymphal populations have been found to accelerate plant maturity under greenhouse conditions (Moellenbeck & Quisenberry 1991), and delay plant maturity under field conditions (Wilson & Quisenberry 1987). In
this study, plant maturity was unaffected by threecornered alfalfa hopper populations. Moellenbeck & Quisenberry (1991) found that threecornered alfalfa hoppers did not reduce forage yield during three harvest periods. The results of this study indicate that these insects can reduce forage yield only after repeated girdle damage. The loss in forage yield may be caused by both a reduction in plant height and fewer harvestable stems.

Fusarium crown-rot is known to reduce yield in alfalfa (Richard et al. 1980); however, it progresses slowly in alfalfa stands (Leath 1990). This test lasted six harvest periods, which corresponds to only one growing season in Louisiana. Over a longer period of time, or in older alfalfa plants, dry weight yields might be reduced more drastically by F. oxysporum.

The reductions in IVDDM and CP concentration and the increases in ADF and NDF concentrations indicate that threecornered alfalfa hopper populations can significantly reduce the quality of the harvested alfalfa forage. Earlier studies also found that threecornered alfalfa hoppers reduce forage quality (Wilson & Quisenberry 1987, Moellenbeck & Quisenberry 1991). The significant interaction among harvest, insect and pathogen effects for ADF concentration indicate that F. oxysporum can also affect forage quality when combined with insect damage.

Threecornered alfalfa hoppers were found to decrease
root carbohydrate concentration. These results agree with those of Moellenbeck & Quisenberry (1991) who also found reduced root carbohydrate concentrations in plants infested with this insect. Reductions in root carbohydrate concentration can weaken plant stands making them more susceptible to severe weather conditions, weed competition or disease (Kitchen et al. 1990).

Adequate control of threecornered alfalfa hoppers may be more important than previously thought. The threecornered alfalfa hopper not only reduces forage yield and quality as previously found, but also affects stand vigor by reducing root carbohydrate reserves and increasing Fusarium crown-rot development in previously infected plants. Field studies are now needed to better determine the long term effects of threecornered alfalfa hopper damage on alfalfa.
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CHAPTER III

Screening Alfalfa for Resistance to the
Threecornered Alfalfa Hopper (Homoptera: Membracidae)

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submitted to the Journal of Economic Entomology and
accepted for publication on 3 March 1992.
Insect resistant alfalfa cultivars (*Medicago sativa* L.) have played an important role in the management of several insect species, including the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), and the pea aphid, *Acyrthosiphon pisum* Harris, (Sorensen et al. 1988). Resistance, however, has not been important in the management of the threecornered alfalfa hopper, *Spissistilus festinus* (Say) on alfalfa.

The threecornered alfalfa hopper is a major pest of alfalfa in the Southeastern United States, reducing both forage yield and forage quality (Isenhour 1985; Miner 1959; Wilson & Quisenberry 1987). Feeding by the threecornered alfalfa hopper often results in a girdle that completely encircles the plant stem (Wildermuth 1915). These girdles on soybean stems interfere with the movement of translocates in the phloem, resulting in an increase of carbohydrates above the girdle (Hicks et al. 1984). In alfalfa, feeding damage has been found to reduce root carbohydrate levels and stem regrowth, possibly leading to stand decline (Moellenbeck & Quisenberry 1991).

Control of threecornered alfalfa hoppers is generally aimed toward adult populations, however, late-instar nymphs are the most damaging stage (Moore & Mueller 1976). Nymphal populations are difficult to control with insecticides because they are found near the base of
plants, protected from spraying by the upper foliage canopy (Miner 1959). Timely harvest may aid in control of nymphal populations (Graham & Ellisor 1940; Miner 1959) though reductions in populations at harvest may take place after damage has already occurred.

Difficulties in controlling nymphal populations by the use of conventional methods warrant the study of alfalfa resistance as a possible means of threecornered alfalfa hopper control. Alfalfa cultivars have previously been screened for antixenosis, antibiosis, and tolerance to the threecornered alfalfa hopper with little success in identifying resistance (Kulash & Hanson 1949; Nielson & Schonhorst 1965; Randolph & Meisch 1970). The work presented here was initiated to screen alfalfa cultivars under more controlled conditions for antixenotic and antibiotic resistance to the threecornered alfalfa hopper.

Materials and Methods

Antixenosis Test. This test was conducted using adult threecornered alfalfa hoppers. Six alfalfa cultivars were used in this study based on the following attributes. 'Florida 77' and 'Cimarron' are recommended for use in Louisiana (Bracy & Allen 1982). Also, 'Florida 77' has been used in studies of damage caused by the threecornered alfalfa hopper (Wilson & Quisenberry 1987; Moellenbeck & Quisenberry 1991). 'Cimarron VR' and 'Dona Ana' have shown resistance and 'Garst 630' has shown moderate resistance to the spotted alfalfa aphid and the pea aphid (Anon. 1989).
'Zia' showed possible resistance to the threecornered alfalfa hopper (Randolph & Meisch 1970).

Seeds were planted in the greenhouse 15 February 1990. One plant per pot (8 by 8 by 8 cm) was maintained in Sunshine Growers Mix No. 1 (Fisons Western, Downers Grove, Ill.). All seeds were inoculated with Pelinoc (Nitrogen Company, Milwaukee, Wis.) prior to planting. Artificial lighting was used to maintain a photoperiod of 16:8 (L:D). Greenhouse temperature was maintained at 27 ± 5° C. Plants were clipped to a height of 7 cm at the flowering stage twice before the initiation of the screening test.

One pot of each cultivar was placed in a circle within metal tubs (28 cm high by 45 cm diam.) filled with sand. The pots were buried in the sand so that neither the pot nor the soil in the pot were visible. The plants were spaced 10 cm apart and 8 cm from the edge of the tub. A 45 by 45 by 97 cm cage made of 14 x 16 mesh nylon screen attached to a PVC pipe frame was placed over the plants and pushed down into the sand. The base of the nylon screen was covered with sand to seal all openings.

Adult threecornered alfalfa hoppers were collected from white clover, *Trifolium repens* L., at the Perkins Road Research Station, Baton Rouge, La. The insects were brought into the laboratory, separated by sex, and placed in 0.95-liter glass jars containing three greenbean, *Phaseolus vulgaris* L., pods. The insects were maintained in a growth chamber set at 27 ± 0.5° C and a 16:8 (L:D)
photoperiod. After 7 d, forty females and twelve males were placed into each of the eight cages, equidistant from the six cultivars.

Insect preference was measured by counting the number of insects on each test plant at 12, 24, 48, and 72 h. Also, after 72 h, plant heights were measured, the number of girdles per plant were counted, and the plants were clipped to soil level. Harvested material was taken into the laboratory where leaf tissue was removed from the stems. The number of eggs within each plant was then determined by direct count using the lactophenol-acid fuchsin staining method described by Simonet & Pienkowski (1977).

All data were analyzed using the general linear models procedure (PROC GLM) of SAS (SAS Institute 1989). A multivariate repeated measures approach (Moser et al. 1990) was used for the four insect counts. Plant height was included in the model as a covariate. Egg and girdle counts were analyzed as a randomized block design. Mean separations were determined using the Student-Newman-Keul's Test (Steel & Torrie 1980).

Antibiosis Test. The antibiosis test was conducted using threecornered alfalfa hopper nymphs and consisted of the same six cultivars listed previously. Seeds were planted 25 October 1991. One plant per pot (4 liters) was maintained as described above. All plants were harvested twice at 10 % bloom prior to the testing period.
On 21 March 1991, adult threecornered alfalfa hoppers were collected on mixed stands of vetch, *Vicia* spp., and crimson clover, *Trifolium incarnatum* L., from Adams and Wilkinson counties in Mississippi. Hoppers were brought into the laboratory and reared on greenbean pods as described by Moellenbeck & Quisenberry (1991). The nymphs used in this test were offspring of collected adults.

Plants were infested in the morning using nymphs that had hatched the previous night. One nymph was placed directly onto each plant using a small, camel's-hair brush. The nymphs were allowed to move freely about the plant. To eliminate nymphal movement from plant to plant, pots were spaced approximately 20 cm apart.

Daily observations of each plant were made to follow the development of the nymphs. Instar determinations were made using a key to the nymphal instars (Jordan 1952) and observing the presence of exuvia. Upon reaching adulthood, insects were taken into the laboratory, weighed and sexed. In addition, the number of girdles present on the plant was counted after each insect was removed.

Preliminary studies showed a high level of nymphal mortality, especially during the first instar. For that reason, 20 replications were used in this study to ensure an adequate sample size. In addition, those preliminary studies included the six cultivars mentioned plus a greenbean pod control. Nymphs were unable to survive on the beans, so they were not included in this study.
Individual instar duration, total time to adult eclosion, adult weight, and girdle counts were analyzed as a randomized block design using PROC GLM of SAS (SAS Institute 1989). Female threecornered alfalfa hoppers are generally larger than the males (Jordan 1952). Thus, adult weights were analyzed separately for male and female insects. Mean separations were performed by the Student-Newman-Keul's test (Steel & Torrie 1980).

Results

Antixenosis Test. A significant cultivar effect for the number of adult threecornered alfalfa hoppers counted on each plant was found in the overall multivariate repeated measures analysis ($F = 2.41; df = 5, 34; P = 0.05$). Because neither a significant time effect ($F = 1.58; df = 3, 32; P = 0.21$) nor a significant time by cultivar interaction effect ($F = 1.27; df = 15, 89; P = 0.24$) were found, insect counts from the four sample periods were pooled and analyzed as a randomized block design. The fewest insects were found on 'Zia' and 'Cimarron VR' and the most were found on 'Cimarron', 'Garst 630', and 'Florida 77' (Table 3.1). In addition, significantly more girdles were produced on 'Cimarron' than on all other cultivars except 'Garst 630' ($F = 4.17; df = 5, 35; P < 0.01$) (Table 3.1). No significant differences in eggs oviposited were found among the cultivars ($F = 0.55; df = 5, 34; P = 0.74$) (Table 3.1).
Table 3.1. Mean number (± SEM) of adult threecornered alfalfa hoppers, eggs, and girdles found on alfalfa cultivars in the antixenosis test

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>No. of adults per plant</th>
<th>No. of eggs per plant</th>
<th>No. of girdles per plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cimarron</td>
<td>4.6 ± 0.5a</td>
<td>6.9 ± 3.3a</td>
<td>5.0 ± 2.1a</td>
</tr>
<tr>
<td>Cimarron VR</td>
<td>1.8 ± 0.4bc</td>
<td>2.9 ± 1.6a</td>
<td>0.6 ± 0.2b</td>
</tr>
<tr>
<td>Dona Ana</td>
<td>3.1 ± 0.5ab</td>
<td>7.6 ± 1.8a</td>
<td>1.9 ± 0.8b</td>
</tr>
<tr>
<td>Florida 77</td>
<td>3.7 ± 0.6a</td>
<td>1.9 ± 0.8a</td>
<td>2.0 ± 0.3b</td>
</tr>
<tr>
<td>Garst 630</td>
<td>4.0 ± 0.6a</td>
<td>6.9 ± 2.6a</td>
<td>3.3 ± 1.2ab</td>
</tr>
<tr>
<td>Zia</td>
<td>1.5 ± 0.3c</td>
<td>8.4 ± 7.7a</td>
<td>0.9 ± 0.4b</td>
</tr>
</tbody>
</table>

Means within columns followed by the same letter are not significantly different (P ≥ .05; Student-Newman-Keul's procedure [Steel & Torrie 1980]).
Antibiosis Test. Nymphal mortality occurred on 27 of the 120 plants in the test. Nymphs were designated as dead if a dead insect was found or if a nymph could not be found on a plant for 10 consecutive d. Mortality occurred within 3 d of infestation in 21 of the 27 cases. The 27 nymphs that did not complete development to adult eclosion were not included in the analyses of nympha development. However, the number of girdles per plant was counted and analyzed for all of the plants in the test.

Total length of nympha development was significantly longer when nymphs were reared on 'Florida 77' as compared with 'Zia' (F= 2.92; df = 5, 63; P = 0.02). All other cultivars were intermediate (Table 3.2). Overall, nympha development averaged 26.3 ± 0.4 (Mean ± SEM) d. No single stadium was significantly affected by the different cultivars (P > 0.05). The first stadium lasted an average of 3.7 ± 0.1 (Mean ± SEM) d. The second through fifth stadia lasted 5.4 ± 0.2, 4.7 ± 0.1, 5.1 ± 0.1, and 7.9 ± 0.2 (Mean ± SEM) d, respectively.

The number of girdles counted per plant did not significantly differ among the six cultivars (F = 2.26; df = 5, 91; P = 0.06). Adult males and females weighed an average of 12.2 ± 0.19 mg and 15.8 ± 0.24 mg (Mean ± SEM), respectively. Male weights differed significantly among the six cultivars (F = 20.85; df = 5, 11; P < 0.01) with the heaviest males developing on 'Florida 77' and the lightest on 'Dona Ana' (Table 3.2).
Table 3.2. Nymphal duration, number of girdles, and adult male and female threecornered alfalfa hopper weights (Mean ± SEM) for alfalfa cultivars in the antibiosis test

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Nymphal duration (d)</th>
<th>No. of girdles per plant</th>
<th>Male wt. (mg)</th>
<th>Female wt. (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cimarron</td>
<td>26.3 ± 1.0ab</td>
<td>0.9 ± 0.2a</td>
<td>11.8 ± 0.3c</td>
<td>17.1 ± 0.8a</td>
</tr>
<tr>
<td>Cimarron VR</td>
<td>26.7 ± 0.8ab</td>
<td>1.8 ± 0.8a</td>
<td>12.3 ± 0.3bc</td>
<td>15.9 ± 0.5a</td>
</tr>
<tr>
<td>Dona Ana</td>
<td>26.4 ± 0.9ab</td>
<td>1.5 ± 0.3a</td>
<td>11.3 ± 0.4d</td>
<td>16.1 ± 0.8a</td>
</tr>
<tr>
<td>Florida 77</td>
<td>28.0 ± 1.0a</td>
<td>1.8 ± 0.3a</td>
<td>13.2 ± 0.6a</td>
<td>15.1 ± 0.6a</td>
</tr>
<tr>
<td>Garst 630</td>
<td>25.5 ± 0.8ab</td>
<td>1.3 ± 0.3a</td>
<td>12.6 ± 0.6b</td>
<td>15.5 ± 0.5a</td>
</tr>
<tr>
<td>Zia</td>
<td>24.7 ± 0.6b</td>
<td>1.9 ± 0.3a</td>
<td>12.3 ± 0.4bc</td>
<td>16.0 ± 0.4a</td>
</tr>
</tbody>
</table>

Means within columns followed by the same letter are not significantly different (P ≥ .05; Student-Newman-Keul's procedure [Steel & Torrie 1980]).
differences in female weights among the cultivars were found $(F = 2.21; \text{df} = 5, 22; P = 0.09)$ (Table 3.2).

**Discussion**

Ovipositional rates monitored by egg counts did not differ among the cultivars due mainly to the high variability within any particular cultivar. The low number of adults and girdles counted on 'Zia' and 'Cimarron VR' during the antixenosis test indicates those two cultivars are exhibiting some resistance to this insect. 'Florida 77' and 'Dona Ana' also had lower levels of girdle incidence than 'Cimarron' or 'Garst 630', indicating possible antixenotic properties in those two cultivars.

Nymphal mortality that occurred within 3 d of infestation was considered to be caused by handling or other unknown factors rather than by a cultivar effect. Only six nymphs died after the first 3 d, thus none of the tested cultivars showed levels of antibiosis high enough to cause significant mortality. Nymphal duration on 'Florida 77' was significantly lengthened in comparison with 'Zia' on which nymphs developed the fastest. Thus, although 'Zia' showed antixenotic properties, it did not show any antibiotic properties. The six cultivars varied greatly in adult male weights, however, female weights were essentially equal. Male weights were lowest on 'Dona Ana' and 'Cimarron' indicating possible antibiotic properties in those two cultivars. The delay in nymphal
development on 'Florida 77' probably does not indicate antibiosis because adult males were heaviest when reared on this cultivar.

Although the exact mechanism(s) of alfalfa resistance to the threecornered alfalfa hopper is unknown, physical factors such as trichomes may play an important role. Simple trichomes are related to alfalfa antixenotic resistance to the potato leafhopper, *Empoasca fabae* (Harris), and the spotted alfalfa aphid (Elden et al. 1986; Manglitz & Kerr 1984). Upon closer study of the test cultivars, variation in trichome density among the six cultivars was observed. Thus, the antixenosis found in 'Zia' and 'Cimarron VR' may be related to the presence of simple trichomes. Further study is needed, however, to better determine the relationship between alfalfa pubescence and antixenosis to the threecornered alfalfa hopper.

The threecornered alfalfa hopper has been reported to feed on a variety of host plants, with a distinct preference for legumes (Mueller & Dumas 1987). Although the threecornered alfalfa hopper is mobile as an adult, nymphs are highly sedentary and seldom wander far from the place of hatching (Jordan 1952). These characteristics indicate that antixenosis may be an effective mode of plant resistance. Antixenotic properties of an alfalfa cultivar may reduce adult threecornered alfalfa hopper populations, moving egg laying to alternative host plant
species. The sedentary and more destructive nymphaal stage would thus be removed from the alfalfa field.

Insect resistant alfalfa cultivars have been used successfully in the management of several important alfalfa pests (Sorensen et. al 1988). The variation present among these six cultivars suggests that alfalfa breeding programs based on selection of resistance to the threecornered alfalfa hopper could be successful. 'Zia' and 'Cimarron VR' exhibited some level of antixenosis to this insect in the form of both lower populations and reduced feeding damage. In addition, 'Dona Ana' and 'Cimarron' exhibited possible antibiotic properties. Use of these cultivars in breeding programs and initiating selection for resistance to this insect may increase the importance of host plant resistance in future threecornered alfalfa hopper management and provide a more economic control method for this important alfalfa pest.
References Cited


Isenhour, D. J. 1985. Efficacy of insecticides against Spissistilus festinus (Say), Empoasca fabae (Harris) and Lygus lineolaris (Palisot de Beauvois) in alfalfa in Georgia. J. Entomol. Sci. 20: 121-128.


CHAPTER IV

Field Screenings of Alfalfa Cultivars for Resistance to the Threecornered Alfalfa Hopper (Homoptera: Membracidae)
The threecornered alfalfa hopper, *Spissistilus festinus* (Say), is an important insect pest of alfalfa, *Medicago sativa* L., throughout most of the southern United States (Duggar 1930; Kulash & Hanson 1949; Miner 1959; Nielson & Schonhorst 1965; Randolph & Meisch 1970; Isenhour 1985; Wilson & Quisenberry 1987). Field and greenhouse studies have shown that threecornered alfalfa hopper populations affect alfalfa production by reducing dry weight forage yield, forage quality, stem regrowth, and root carbohydrate storage (Wilson & Quisenberry 1987; Moellenbeck & Quisenberry 1991). The impact of threecornered alfalfa hopper damage on alfalfa warrants continued research into the development of effective and sustainable control strategies. Control measures for threecornered alfalfa hopper populations rely upon the application of insecticides; however, only one insecticide (carbaryl) is currently registered for threecornered alfalfa hopper control in alfalfa (Anon 1991). Because of the lack of chemical control options facing alfalfa producers in the southern United States, host plant resistance may prove to be an important control strategy.

Earlier studies have screened over 100 alfalfa cultivars for threecornered alfalfa hopper resistance (Kulash & Hanson 1949; Nielson & Schonhorst 1965; Randolph & Meisch 1970). In greenhouse screenings of alfalfa seedlings, Randolph & Meisch (1970) found the highest levels of resistance in 'Socheville', 'Zia', 'Ladak', and
'Hairy Peruvian' based on visual ratings. However, resistance was not expressed under field conditions. Nielson & Schonhorst (1965) in a field study recommended 'Chilean 21-5', 'Kansas 67-1108', 'Rhizoma' and 'Sirsa #9' as sources of resistance based upon both population levels and damage (girdle) incidence. When looking at either screening criteria alone, however, the cultivars tested were ranked much differently. Nielson & Schonhorst (1965) recommended that evaluations must be based on both plant damage and population levels. Since 1970, research efforts in screening alfalfa cultivars and lines for resistance to the threecornered alfalfa hopper have been limited. Currently, no alfalfa cultivars are classified as being resistant to the threecornered alfalfa hopper (Manglitz & Ratcliffe 1988).

In order to better identify resistant and susceptible alfalfa germplasm, standard field screening techniques must be developed. Our current research consists of two major objectives: the identification of threecornered alfalfa hopper-resistant and susceptible alfalfa germplasm and the development of effective and consistent screening techniques under field and greenhouse conditions. The research presented herein reports on a 2 yr study designed to screen alfalfa cultivars under Louisiana field conditions.

Materials and Methods

Screening trials were conducted at the Northeast and
Macon Ridge research stations near St. Joseph and Winnsboro, La., respectively. Plots at each location were arranged in a randomized block design with four replications. The cultivars were planted in 4.1 m ridged rows spaced 1 m apart to facilitate damage (girdle) counts. Approximately 2 g of seed were planted per row. Border rows were planted to 'Florida 77' at ca. 5 kg/ha.

Northeast Station (soil type: Sharkey clay). Alfalfa seed was planted 7 November 1989. Cultivars 'Mesa Sirva', 'Tourneur 501', 'Arizona Indian', and 'Chilean 21-5' originally planted on that date failed to germinate. The following spring those rows were re-tilled, and on 23 March 1990 'Archer', 'Pioneer 5432', 'Condor' and 'Apollo' were planted in their place.

Soil samples taken the fall of 1989 indicated no fertilization was required. Weed control was generally maintained through hand hoeing and tractor-mounted cultivation. However, rescue treatments of sethoxydim (Poast Plus at 1.75 liters/h; BASF Corp. Research Triangle Park, N.C.) were applied 30 November 1989, 15 March 1991, and 13 June 1991 for grass control, and butyric acid (Butyrac 200 at 4.4 l/h; Rhone-Poulenc. Research Triangle Park, N.C.) for henbit, Lamium amplexicaule L., control. Plots were harvested when border rows were at 10% bloom.

Carbofuran (Furadan 4 flowable [F] at 1.2 liters/h; FMC Corporation, Philadelphia, Pa.) was applied on 21 March 1990 and on 26 March 1991 (0.6 liters/h) for alfalfa
weevil, Hypera postica (Gyllenhal), control. These applications were applied before threecornered alfalfa hopper populations were present and did not affect data collection. Thiodicarb (Larvin 3.2 F at 4.8 liters/h; Rhone-Poulenc Ag Company, Research Triangle Park, N.C.) was applied for velvetbean caterpillar, Anticarsia gemmatalis Hubner, and soybean looper, Pseudoplusia includens (Walker) control on 26 Oct. 1990. This insecticide application also did not affect threecornered alfalfa hopper populations. No other insecticide applications were made.

The alfalfa cultivars were screened for threecornered alfalfa hopper resistance by taking girdle and stand counts and monitoring adult populations. Girdle counts were taken bi-weekly from 24 May through 2 November in 1990 and 20 June through 16 October in 1991. Counts were obtained by averaging the number of girdles totally encircling a stem on three randomly sampled plants within each row. Girdles were not counted in rows with fewer than three plants.

Adult threecornered alfalfa hopper populations were monitored by biweekly sweep samples from 24 May through 14 November in 1990 and 30 May through 18 October in 1991. Sweep samples consisted of two sweeps of a standard sweep net across each row. Rows with less than three plants remaining were not sampled. Samples were bagged, frozen, and taken into the laboratory for counting.
Stand counts were made in the spring and fall of 1990 and 1991 to evaluate cultivar persistence under three-cornered alfalfa hopper feeding damage and Louisiana growing conditions. All rows at the first stand count date (spring 1990) remained solid seeded, thus preventing accurate stand counts. At this date, all rows were given a value of 30 plants. Subsequent stand counts were measured by counting the number of plants per row.

Macon Ridge Station (soil type: Gigger silt loam). Alfalfa seed was planted 23 October 1989. Because of uneven stands, all rows were thinned to a maximum of 25 plants per row on 15 May 1990. Cultivars 'Mesa Sirva', 'Tourneur 501', 'Arizona Indian', and 'Chilean 21-5' failed to germinate, but were not replaced at this location. Fertilizer was applied 24 October 1989 and 1 August 1991 (336 kg/h and 448 kg/h of 0-20-20, respectively). Weed control was generally maintained through hand-hoeing and tractor-mounted cultivation; however, sethoxydim (Poast Plus at 1.75 liters/h; BASF Corp. Research Triangle Park, N.C.) was applied on 17 June 1991 and fluazifop-P-butyl (Fusilade 2000, 6.1 liters/h; ICI Americas Inc. Wilmington, Del.) was applied 3 August 1990 for rescue control of grasses. Applications of thiodicarb (Larvin 3.2 F at 4.8 liters/h) were made on 4 September and 16 October 1990 for beet armyworm, Spodoptera exigua (Hubner) control. The test was flood-irrigated on 26 June and 10
July 1990 and 21 August 1991. Plots were harvested when border rows were ca. 10% bloom.

Alfalfa cultivars at the Macon Ridge Station were evaluated by girdle counts and stand counts as described for the Northeast Station. Biweekly girdle counts were taken from 6 July through 24 Oct in 1990 and from 13 June through 29 October in 1991. Stand counts were taken in the spring and fall of 1990 and 1991. Initial stand counts in the spring of 1990 were taken after the rows had been thinned. The lack of solid rows did not allow sweep samples to be taken.

Data Analysis. Because the two trials were sampled on different days and consisted of different cultivars, the two locations were analyzed separately. Girdle and adult population counts were log(x+1) transformed and analyzed in a repeated measures multivariate approach (Moser et al. 1990) using the repeated statement of the general linear models procedure (PROC GLM) of SAS (SAS Institute 1989). All data presented have been back-transformed. Single sample date analyses were performed when a significant ($P \leq 0.05$) interaction between sample date and cultivar was found. Percent stand reductions during the summer of 1990, winter of 1990-1991, summer of 1991, and from spring 1990 to fall 1991 were calculated using the following formula:

$$\text{Percent reduction} = ((\text{stand 1} - \text{stand 2})/\text{stand 1}) \times 100.$$  

Percentages were analyzed as a randomized block design.
Cultivar means for all dependent variables were compared using the least significant difference (LSD) procedure protected at the 0.05 level.

Results and Discussion

All rows initially contained a solid stand of plants at the Northeast station. In the fall of 1991, 60.6 ± 3.2% (mean ± SEM) of that original stand had been lost. The greatest amount of stand loss occurred during the winter of 1990-1991 with a 51.9 ± 4.0% (mean ± SEM) reduction from the previous fall. The large reduction was mainly caused by high rainfall in the spring of 1991 that resulted in standing water across most of the test site. Cultivars differed significantly in percent stand loss at the Northeast station during the summer of 1990 (F = 4.03; df = 16, 52; P = 0.0001), the winter of 1990-1991 (F = 2.47; df = 16, 52; P = 0.007) and overall (F = 6.00; df = 16, 52; P = 0.0001). 'Hairy Peruvian', 'Zia', 'Apollo' and 'Moapa' lost the highest percentage of plants among the cultivars tested (Table 4.1) and do not seem to be adapted for wet conditions. Both 'Hairy Peruvian' and 'WL 515' were reduced to less than three plants per plot in three replications and were not included in the girdle count or adult population analyses. 'Cimarron VR' lost the fewest plants and performed well under the wet conditions. Other cultivars losing few plants were 'GA Plains' and 'Florida 77'.

The heavy rainfall in the spring of 1991 did not

<table>
<thead>
<tr>
<th>CULTIVAR</th>
<th>PERCENT STAND REDUCTION</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>WINTER 90-91</td>
<td>SUMMER 91</td>
<td>TOTAL</td>
<td></td>
</tr>
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<td>APOLLO</td>
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<td>54.9±11.7abcd</td>
<td>23.2±10.7a</td>
<td>85.8±3.7abc</td>
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<tr>
<td>ARCHER</td>
<td>25.0±15.0bcd</td>
<td>52.8±19.9abcd</td>
<td>2.4±10.2a</td>
<td>71.7±11.7abcd</td>
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<td>9.6±11.4a</td>
<td>39.2±7.0efgh</td>
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</tr>
<tr>
<td>CIMARRON VR</td>
<td>0.0±0.0d</td>
<td>0.8±10.5ed</td>
<td>16.4±5.3a</td>
<td>18.3±6.2h</td>
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</tr>
<tr>
<td>CONDOR</td>
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<td>51.4±21.2abcd</td>
<td>31.4±4.8a</td>
<td>75.8±12.0abc</td>
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<tr>
<td>CUF 101</td>
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<td>38.9±7.7a</td>
<td>70.0±8.6abcd</td>
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<td>13.9±7.9a</td>
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<tr>
<td>FLORIDA 77</td>
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<td>-0.5±5.1a</td>
<td>39.6±7.9fgh</td>
<td></td>
</tr>
<tr>
<td>GA PLAINS</td>
<td>2.5±2.5d</td>
<td>12.2±15.9bcde</td>
<td>19.1±7.0a</td>
<td>34.2±8.0gh</td>
<td></td>
</tr>
<tr>
<td>GARST 630</td>
<td>17.5±4.8bcd</td>
<td>13.1±4.8de</td>
<td>15.1±6.9a</td>
<td>39.2±7.0efgh</td>
<td></td>
</tr>
<tr>
<td>HAIRY PERUVIAN</td>
<td>15.0±5.0bcd</td>
<td>89.8±5.1a</td>
<td>36.1±21.7a</td>
<td>95.0±2.9a</td>
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</tr>
<tr>
<td>LADAK</td>
<td>37.5±14.9ab</td>
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<td>23.2±14.0a</td>
<td>70.8±10.6abcd</td>
<td></td>
</tr>
<tr>
<td>MOAPA</td>
<td>25.0±2.9bcd</td>
<td>68.9±7.7ab</td>
<td>16.2±17.4a</td>
<td>83.3±1.4abc</td>
<td></td>
</tr>
<tr>
<td>PIONEER 5432</td>
<td>37.5±18.9ab</td>
<td>14.2±5.8de</td>
<td>-7.5±9.1a</td>
<td>49.2±defg</td>
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<tr>
<td>WL 515</td>
<td>30.0±12.9bc</td>
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<td>27.2±12.6a</td>
<td>66.7±15.4bcd</td>
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<tr>
<td>WL 516</td>
<td>17.5±4.8bcd</td>
<td>44.1±21.0bcde</td>
<td>14.0±5.6a</td>
<td>61.7±11.7cdef</td>
<td></td>
</tr>
<tr>
<td>ZIA</td>
<td>57.5±6.3a</td>
<td>6254±7.0abc</td>
<td>20.0±11.5a</td>
<td>88.3±1.7ab</td>
<td></td>
</tr>
</tbody>
</table>

Means within a column followed by the same letter are not significantly different (LSD; \( P \leq 0.05 \).
cause high stand loss at the Macon Ridge station because the soil at that location drains very quickly and water did not collect on the soil surface. Total stand reduction from the spring of 1990 through the fall of 1991 averaged 19.1 ± 2.6 % (mean ± SEM) (Table 4.2). The difference in water stress at the two locations allowed a comparison of stand persistence among the cultivars under different growing conditions. There was a significant cultivar effect for percent stand loss after the winter of 1990-1991 \((F = 2.08; df = 12, 36; P = 0.044)\), the summer of 1991 \((F = 3.09; df = 12, 36; P = 0.004)\), and overall \((F = 2.92; df=12, 36; P = 0.006)\) at the Macon Ridge Station. 'Ladak' had the highest percent stand loss of all cultivars tested (Table 4.2). 'Moapa', 'WL 516', and 'GA Plains' lost the fewest plants and seem to be highly persistent under the growing conditions found at this location. 'Moapa' germinated poorly, and averaged only 8.0 ± 3.0 plants at the first stand count. The actual increase in plants most likely resulted from late germination. With good stand persistence at both locations, 'GA Plains' seems well adapted to Northeast Louisiana's wide range of growing conditions.

The 1990 and 1991 seasonal distributions of adult threecornered alfalfa hoppers and girdle damage at the two locations are shown in Figure 4.1. Adult populations peaked on 19 September and 17 October in 1990. Adult populations again peaked on 13 September 1991; however,

<table>
<thead>
<tr>
<th>CULTIVAR</th>
<th>SUMMER 90</th>
<th>WINTER 90-91</th>
<th>SUMMER 91</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIMARRON</td>
<td>11.1±4.9a</td>
<td>6.5±3.8abcd</td>
<td>5.4±9.7bcd</td>
<td>23.0±2.9bc</td>
</tr>
<tr>
<td>CIMARRON VR</td>
<td>15.0±3.0a</td>
<td>3.2±1.9bcd</td>
<td>12.9±8.1ab</td>
<td>28.2±7.4ab</td>
</tr>
<tr>
<td>CUF 101</td>
<td>-1.3±1.3a</td>
<td>7.5±4.8abcd</td>
<td>9.0±8.7bcd</td>
<td>13.8±11.7bcd</td>
</tr>
<tr>
<td>DONA ANA</td>
<td>9.4±4.5a</td>
<td>14.8±2.1abc</td>
<td>3.1±6.1bcde</td>
<td>25.4±4.9bc</td>
</tr>
<tr>
<td>FLORIDA 77</td>
<td>2.9±4.4a</td>
<td>13.9±3.2ab</td>
<td>-6.1±2.6cde</td>
<td>12.1±2.8bcd</td>
</tr>
<tr>
<td>GA PLAINS</td>
<td>-0.4±14.0a</td>
<td>15.7±5.8abc</td>
<td>-14.0±19.0de</td>
<td>7.6±7.6bcd</td>
</tr>
<tr>
<td>GARST 630</td>
<td>14.5±3.7a</td>
<td>16.5±8.3abc</td>
<td>-6.7±4.1bcde</td>
<td>24.4±5.1abc</td>
</tr>
<tr>
<td>HAIRY PERUVIAN</td>
<td>17.9±6.9a</td>
<td>4.2±4.2bcd</td>
<td>6.7±3.9bcd</td>
<td>26.2±9.1abc</td>
</tr>
<tr>
<td>LADAK</td>
<td>6.6±12.6a</td>
<td>19.1±7.1a</td>
<td>34.4±11.4a</td>
<td>46.7±16.3a</td>
</tr>
<tr>
<td>MOAPA</td>
<td>4.4±4.4a</td>
<td>8.5±12.6abcd</td>
<td>-18.3±9.8e</td>
<td>-4.4±19.8d</td>
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<td>WL 515</td>
<td>8.8±3.9a</td>
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</tr>
<tr>
<td>WL 516</td>
<td>5.1±11.2a</td>
<td>-2.1±8.2d</td>
<td>1.3±1.3bcde</td>
<td>7.1±2.9cd</td>
</tr>
<tr>
<td>ZIA</td>
<td>9.0±2.4a</td>
<td>-4.4±2.3d</td>
<td>13.5±7.6abc</td>
<td>18.2±5.3bcd</td>
</tr>
</tbody>
</table>

Means within a column followed by the same letter are not significantly different (LSD; P <=0.05).
Figure 4.1. Seasonal Distribution of adult threecornered alfalfa hoppers and girdle damage.
because of the major stand loss in the spring of 1991, the 1991 peak was much lower than 1990. In 1990, girdle damage peaked at both locations on 19 September. This high girdle damage was most likely caused by the nymphal stages that resulted in the subsequent adult peak. Girdle damage gradually diminished after this date. In 1991, girdle damage at the Macon Ridge Station peaked on 20 June and again on 13 September. Girdle damage remained low at the Northeast Station throughout most of the growing season, again probably because of the reduction in plant material caused by water damage. Girdle damage increased later in the season subsequent to the adult population peak.

A significant interaction between cultivar and sample date ($L = 0.00027; F = 1.33; df = 224, 254.5; P = 0.01$) and a significant cultivar effect ($F = 2.09; df = 14, 36; P = 0.04$) was found for girdle counts at the Northeast station. Overall, at the Northeast station 'Ladak' was the most heavily damaged of the cultivars tested (Table 4.3). 'Garst 630' and 'Moapa' were also heavily damaged, while 'Dona Ana' and 'Condor' experienced the least amount of girdle damage. Single sample date analyses resulted in a significant cultivar effect on 20 June 1990 ($F = 2.36; df = 14, 36; P = 0.02$), 10 July 1990 ($F = 2.53; df = 14, 36; P = 0.01$), 2 November 1990 ($F = 1.96; df = 14, 36; P = 0.05$), 25 July 1991 ($F = 2.21; df = 14, 36; P = 0.03$), and 16 October 1991 ($F = 2.31; df = 14, 36; P = 0.02$).
Table 4.3. Overall mean number of girdles per plant for cultivars at the Macon Ridge and Northeast Research Stations and the overall mean number of adult threecornered alfalfa hoppers collected per sample at the Northeast Research Station.

<table>
<thead>
<tr>
<th>CULTIVAR</th>
<th>GIRDLES/PLANT MACON RIDGE&lt;sup&gt;a&lt;/sup&gt;</th>
<th>GIRDLES/PLANT NORTHEAST&lt;sup&gt;b&lt;/sup&gt;</th>
<th>ADULTS/2 SWEEPS NORTHEAST&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOLLO</td>
<td>.</td>
<td>1.5±0.2ab</td>
<td>1.3±0.2ef</td>
</tr>
<tr>
<td>ARCHER</td>
<td>.</td>
<td>1.2±0.2abcd</td>
<td>1.8±0.2bcd</td>
</tr>
<tr>
<td>CIMARRON</td>
<td>1.4±0.1bc</td>
<td>1.4±0.2abc</td>
<td>2.0±0.2abc</td>
</tr>
<tr>
<td>CIMARRON VR</td>
<td>1.4±0.2bc</td>
<td>1.1±0.1abcd</td>
<td>1.4±0.2def</td>
</tr>
<tr>
<td>CONDOR</td>
<td>.</td>
<td>1.0±0.1cd</td>
<td>1.6±0.2cde</td>
</tr>
<tr>
<td>CUF 101</td>
<td>1.4±0.1bc</td>
<td>1.2±0.1abed</td>
<td>2.4±0.3ab</td>
</tr>
<tr>
<td>DONA ANA</td>
<td>1.4±0.2bc</td>
<td>0.9±0.1d</td>
<td>2.0±0.2abc</td>
</tr>
<tr>
<td>FLORIDA 77</td>
<td>1.3±0.1bc</td>
<td>1.1±0.1bcd</td>
<td>2.3±0.2ab</td>
</tr>
<tr>
<td>GA PLAINS</td>
<td>1.2±0.2c</td>
<td>1.3±0.1abcd</td>
<td>2.3±0.3ab</td>
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<td>GARST 630</td>
<td>1.7±0.2b</td>
<td>1.5±0.2a</td>
<td>1.8±0.2bcde</td>
</tr>
<tr>
<td>HAIRY PERUVIAN</td>
<td>1.6±0.2b</td>
<td>.</td>
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</tr>
<tr>
<td>LADAK</td>
<td>2.2±0.2a</td>
<td>1.5±0.1a</td>
<td>1.1±0.2f</td>
</tr>
<tr>
<td>MOAPA</td>
<td>1.3±0.2bc</td>
<td>1.4±0.1ab</td>
<td>2.5±0.2a</td>
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<tr>
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<td>.</td>
<td>1.4±0.2abc</td>
<td>1.5±0.3def</td>
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<td>WL 515</td>
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<td>.</td>
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<td>1.0±0.1bcd</td>
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</tr>
<tr>
<td>ZIA</td>
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<td>1.2±0.1abed</td>
<td>1.3±0.2def</td>
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</tbody>
</table>

Means within a column followed by the same letter are not significantly different (LSD; P <=0.05). Significance levels based on log-transformed data.

<sup>a</sup> Based on 18 sample dates.
<sup>b</sup> Based on 17 sample dates.
<sup>c</sup> Based on 21 sample dates.
Cultivar rank varied greatly among the sample dates at the Northeast station (Table 4.4). For example, 'Pioneer 5432' was the most heavily damaged cultivar on 25 September 1991, however, it also had the least amount of girdle damage on 10 July 1990. Cultivars that reflected the least amount of girdle damage on several sample dates included 'Pioneer 5432', 'Cimarron VR', 'Condor', and 'Dona Ana'.

Analysis of girdle counts from the Macon Ridge did not result in a significant interaction between cultivar and sample date ($L = 0.1357; F = 0.91; \text{df} = 204, 184.2; \text{P} = 0.42$), however, there was a significant cultivar effect ($F = 3.10; \text{df} = 12, 32; \text{P} = 0.005$). Overall, 'Ladak' was damaged significantly more than any other cultivar (Table 4.3). 'GA Plains' had the least amount of girdle damage, however, it was not significantly different from many of the other cultivars tested.

The analysis of adult population data at the Northeast station resulted in a significant interaction between cultivar and sample date ($L = 0.000001; F = 1.3; \text{df} = 294, 169.8; \text{P} = 0.03$) and a significant cultivar effect ($F = 5.04; \text{df} = 14, 31; \text{P} = 0.0001$). Cultivars varied significantly in the individual harvest analyses on 24 May 1990 ($F = 2.83; \text{df} = 14, 39; \text{P} = 0.01$), 6 July 1990 ($F = 1.92; \text{df} = 14, 39; \text{P} = 0.05$), 3 August 1990 ($F = 1.93; \text{df} = 14, 39; \text{P} = 0.05$), 14 November 1990 ($F = 2.93; \text{df} = 14, 39; \text{P} = 0.004$), 22 August 1991 ($F = 2.35; \text{df} = 14, 39; \text{P} = 0.004$), 24 December 1991 ($F = 2.53; \text{df} = 14, 39; \text{P} = 0.01$), 31 December 1991 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 1 January 1992 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 15 January 1992 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 1 April 1992 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 12 April 1992 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 1 May 1992 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 1 June 1992 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 1 July 1992 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 1 August 1992 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 1 September 1992 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 1 October 1992 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 1 November 1992 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 1 December 1992 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 1 January 1993 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 1 February 1993 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 1 March 1993 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 1 April 1993 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 1 May 1993 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 1 June 1993 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 1 July 1993 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 1 August 1993 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 1 September 1993 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 1 October 1993 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 1 November 1993 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$), 1 December 1993 ($F = 2.33; \text{df} = 14, 39; \text{P} = 0.004$).
Table 4.4. Mean number of girdles per plant on cultivars tested at the Northeast Research Station.

<table>
<thead>
<tr>
<th>CULTIVAR</th>
<th>SAMPLE DATES WITH A SIGNIFICANT CULTIVAR EFFECT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6/20/90</td>
</tr>
<tr>
<td>APOLLO</td>
<td>0.5±0.1c</td>
</tr>
<tr>
<td>ARCHER</td>
<td>0.7±0.4abc</td>
</tr>
<tr>
<td>CIMARRON</td>
<td>1.7±0.2ab</td>
</tr>
<tr>
<td>CIMARRON VR</td>
<td>1.1±0.4bc</td>
</tr>
<tr>
<td>CONDOR</td>
<td>0.5±0.1c</td>
</tr>
<tr>
<td>CUF 101</td>
<td>1.8±0.4ab</td>
</tr>
<tr>
<td>DONA ANA</td>
<td>1.8±0.4ab</td>
</tr>
<tr>
<td>FLORIDA 77</td>
<td>0.9±0.2bc</td>
</tr>
<tr>
<td>GA PLAINS</td>
<td>1.0±0.4bc</td>
</tr>
<tr>
<td>GARST 630</td>
<td>2.2±0.4a</td>
</tr>
<tr>
<td>LADAK</td>
<td>1.8±0.6abc</td>
</tr>
<tr>
<td>MOAPA</td>
<td>1.3±0.7abc</td>
</tr>
<tr>
<td>PIONEER 5432</td>
<td>0.5±0.2c</td>
</tr>
<tr>
<td>WL 516</td>
<td>0.8±0.2bc</td>
</tr>
<tr>
<td>ZIA</td>
<td>0.6±0.2c</td>
</tr>
</tbody>
</table>

Means within a column followed by the same letter are not significantly different (LSD; $P \leq 0.05$). Significance levels based on log-transformed data.
14, 30; $P = 0.02$), and 28 August 1991 ($F = 3.56; \text{df} = 14, 30; P = 0.001$). Excessive rainfall probably reduced the number of sample dates with a significant cultivar effect in the early part of 1991 because significant cultivar effects were found early in the 1990 growing season. Overall, 'Ladak' and 'Apollo' supported the lowest adult populations, while 'Moapa' had the highest populations (Table 4.3). However, cultivar rankings did vary significantly among sample dates (Table 4.5). 'Pioneer 5432', 'Garst 630', and 'Archer' are among the cultivars that had the lowest adult populations at several sample dates. In general, there was no significant variation among cultivars during population peaks. Analyses of sweep samples taken on 19 September and 17 October 1990 and 13 September 1991 did not show a significant cultivar effect. This may indicate that any antixenosis exhibited in these cultivars is low level and not effective against major threecornered alfalfa hopper outbreaks.

Monitoring nymphal populations may be a better screening technique, because they are considered the most damaging stage (Moore and Mueller 1976). However, nymphs are normally found near the crown of alfalfa plants and are almost impossible to monitor in the field, even with plants arranged in ridged rows. Time-consuming direct visual counts are the only sampling technique recommended in monitoring threecornered alfalfa hopper nymphal populations (Mueller 1980). Unless an easier method is
Table 4.5. Mean number of adult threecornered alfalfa hoppers per sample for cultivars tested at the Northeast Research Station.

<table>
<thead>
<tr>
<th>CULTIVAR</th>
<th>SAMPLE DATES WITH A SIGNIFICANT CULTIVAR EFFECT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5/24/90</td>
</tr>
<tr>
<td>APOLLO</td>
<td>0.3±0.3de</td>
</tr>
<tr>
<td>ARCHER</td>
<td>0.0±0.0e</td>
</tr>
<tr>
<td>CIMARRON</td>
<td>2.0±0.7abc</td>
</tr>
<tr>
<td>CIMARRON VR</td>
<td>0.5±0.3cde</td>
</tr>
<tr>
<td>CONDOR</td>
<td>0.3±0.3de</td>
</tr>
<tr>
<td>CUF 101</td>
<td>1.0±0.4abcd</td>
</tr>
<tr>
<td>DONA ANA</td>
<td>1.5±0.5ab</td>
</tr>
<tr>
<td>FLORIDA 77</td>
<td>1.1±0.4abcd</td>
</tr>
<tr>
<td>GA PLAINS</td>
<td>1.8±0.5a</td>
</tr>
<tr>
<td>GARST 630</td>
<td>0.5±0.3cde</td>
</tr>
<tr>
<td>LADAK</td>
<td>0.8±0.5bcde</td>
</tr>
<tr>
<td>MOAPA</td>
<td>0.5±0.3cde</td>
</tr>
<tr>
<td>PIONEER 5432</td>
<td>0.0±0.0e</td>
</tr>
<tr>
<td>WL 516</td>
<td>0.3±0.3de</td>
</tr>
<tr>
<td>ZIA</td>
<td>1.0±0.4abcd</td>
</tr>
</tbody>
</table>

Means within a column followed by the same letter are not significantly different (LSD; \( P \leq 0.05 \)). Significance levels based on log-transformed data.
developed, nymphal population levels can not be used as a screening criterion in a large scale selection program because of time constraints.

The significant interaction between sample date and cultivar and the difference in cultivar performance between the two locations indicate that alfalfa screening trials for threecornered alfalfa hopper resistance must include multiple damage evaluations under several growing conditions. 'Ladak' was considered resistant in an earlier greenhouse study (Randolph and Meisch 1970), however, it was consistently the most susceptible cultivar in our field tests. Because perceived resistance found in tested cultivars can be affected by various environmental conditions such as soil type, air temperature, or soil moisture conditions, multiple location field trials are necessary to determine the stability and range of insect resistance (Smith 1989). This indicates the importance of multiple environments and repeated damage evaluations.

'Dona Ana', 'GA Plains', and 'Cimarron VR' were alfalfa cultivars that were consistently less damaged across the two locations and showed good stand persistence. Thus, these cultivars are recommended as selection sources. Interestingly, aphid-resistant cultivars 'Zia' and 'Moapa' (Sorenson et al. 1988) did not show cross-resistance to the threecornered alfalfa hopper. 'Ladak' showed the greatest amount of damage, and thus, is recommended for use as a susceptible check. Further
experimentation and screening trials are needed to better define the mechanism(s) of resistance found in these cultivars. Results of these studies show that variation in threecornered alfalfa hopper susceptibility does exist among alfalfa cultivars and that selection for increased resistance is possible. Earlier studies have shown the importance of threecornered alfalfa hopper damage on alfalfa production and the need for effective control measures (Wilson & Quisenberry 1987; Moellenbeck & Quisenberry 1991). Alfalfa insect resistance has been successful in the control of several insect pests including the pea aphid, Acyrthosiphon pisum Harris, and the spotted alfalfa aphid, Therioaphis maculata Buckton (Sorenson et al. 1988). The development of alfalfa cultivars resistant to the threecornered alfalfa hopper may also prove to be an effective and sustainable control strategy.
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Isenhour, D. J. 1985. Efficacy of insecticides against Spiessistilus festinus (Say), Empoasca fabae (Harris) and Lygus lineolaris (Palisot de Beauvois) in alfalfa in Georgia. J. Entomol. Sci. 20: 121-128.


SUMMARY

This series of studies was designed to identify alfalfa, *Medicago sativa* L., cultivars resistant to the threecornered alfalfa hopper, *Spissistilus festinus* (Say), and to develop effective screening techniques that could be used in a plant breeding program. In order to do so, it was first necessary to determine the effects of threecornered alfalfa hopper damage on alfalfa growth.

Damage caused by threecornered alfalfa hopper nymphs was found to significantly reduce root carbohydrate concentration, indicating that nymphal populations can reduce the amount of carbohydrates that is translocated to the roots. In addition, plants infested with this insect had a significant reduction in stem regrowth. Although direct correlations between root carbohydrate levels and stem regrowth could not be made, these factors may be related because both are adversely affected by nymphal populations. After three harvest periods, there was almost a 27% reduction in the number of stems on heavily infested plants. Reductions in stem regrowth and root carbohydrate concentration can weaken a plant and lead to poor alfalfa stand persistence.

Damage caused by threecornered alfalfa hoppers was also shown to increase Fusarium crown-rot severity after six harvest periods. This further demonstrates the importance of threecornered alfalfa hopper damage in increasing crown-rot severity that may lead to a reduction
in stand persistence. As Fusarium crown-rot increases in alfalfa stands, considerable stand decline can occur (Richard et al. 1980). In association with threecornered alfalfa hopper feeding damage, Fusarium crown-rot may be enhanced and further impact stand persistence.

Threecornered alfalfa hopper damage was found to significantly reduce forage quality by reducing crude protein and in-vitro digestible dry matter and increasing acid detergent fiber and neutral detergent fiber concentrations. Threecornered alfalfa hoppers also were found to reduce forage yield after repeated infestations and girdle damage. The loss in forage yield may be caused by both a reduction in plant height and reduced stem regrowth. Reductions in forage quality and forage yield can greatly reduce the profitability of alfalfa production. These reductions, in combination with reduced stand persistence caused by threecornered alfalfa hoppers and Fusarium crown-rot, show the impact threecornered alfalfa hoppers have on alfalfa production and demonstrate the need for effective control strategies.

After the effects of threecornered alfalfa hopper damage on alfalfa growth were determined, host plant resistance studies were initiated to develop field and greenhouse screening techniques and to identify alfalfa cultivars with potential resistance to this insect pest. No-choice and choice tests were conducted in the greenhouse in conjunction with two separate field
screening trials to identify resistant cultivars and determine the mechanism(s) of the resistance. While conducting these tests, procedures used and variables measured were compared to help develop consistent and practical screening techniques.

The choice and no-choice tests were conducted using six alfalfa cultivars, 'Cimarron', 'Cimarron VR', 'Dona Ana', 'Florida 77', 'Garst 630', and 'Zia'. Ovipositional rates, monitored by egg counts, did not differ among the cultivars due mainly to the high variability within any particular cultivar. The low number of adults and girdles counted on 'Zia' and 'Cimarron VR' during the choice test indicated some resistance to the threecornered alfalfa hopper. 'Florida 77' and 'Dona Ana' also had lower levels of girdle incidence than 'Cimarron' or 'Garst 630', indicating possible antixenotic properties.

In the no-choice test of antibiosis, only six nymphs died after the first 3 d, and thus, none of the tested cultivars showed levels of antibiosis high enough to cause significant mortality. Nymphal duration on 'Florida 77' was significantly lengthened in comparison with 'Zia', on which nymphal duration was the shortest. Although 'Zia' showed antixenotic properties, it did not show any antibiotic properties. Male weights were lowest on 'Dona Ana' and 'Cimarron' indicating possible antibiotic properties.
Field screening trials were conducted at the Northeast and Macon Ridge Research Stations. Cultivars were evaluated by girdle counts, adult population monitoring, and stand counts. Adult populations peaked on 19 September and 17 October in 1990 and on 13 September 1991. Overall, 'Ladak' and 'Apollo' supported the lowest adult populations, while 'Moapa' had the highest populations. However, cultivar rankings did vary significantly among sample dates. 'Pioneer 5432', 'Garst 630', and 'Archer' had the lowest adult populations on several sample dates. In general, there was no significant variation among cultivars during population peaks. This may indicate that any antixenosis exhibited in these cultivars is low level and not effective against high threecornered alfalfa hopper outbreaks.

Cultivar rank varied greatly in girdle damage among the sample dates at the Northeast station. 'Ladak' was the most heavily damaged of the cultivars tested. Cultivars that reflected the least amount of girdle damage on several sample dates included 'Pioneer 5432', 'Cimarron VR', 'Condor', and 'Dona Ana'. At the Macon Ridge Station, 'Ladak' was again damaged significantly more than any other cultivar. 'GA Plains' had the least amount of girdle damage, however, it was not significantly different from many of the other cultivars tested.

When looking at stand persistence 'Cimarron VR', 'GA Plains', and 'Florida 77' performed well under the wet
conditions at the Northeast Station. At the Macon Ridge Station, 'Ladak', the most heavily damaged cultivar, had the highest percent stand loss of all cultivars tested. 'Moapa', 'WL 516', and 'GA Plains' lost the fewest plants and were highly persistent. With good stand persistence at both locations, 'GA Plains' seems well adapted to Northeast Louisiana's wide range of growing conditions.

'Dona Ana', 'GA Plains', and 'Cimarron VR' were the alfalfa cultivars that were consistently less damaged across the two locations and showed good stand persistence. Thus, these cultivars are recommended as sources of germplasm resistant to the threecornered alfalfa hopper. 'Ladak' showed the greatest amount of damage and thus, is recommended for use as a susceptible check. Significant interactions between sample date and cultivar and differences in cultivar performance between the two locations indicate that alfalfa screening trials for resistance to the threecornered alfalfa hopper must include multiple damage evaluations under several growing conditions.

The identification of resistance in alfalfa cultivars 'Dona Ana' and 'Cimarron VR' to the threecornered alfalfa hopper in the greenhouse studies agrees with the field studies where they also performed well. This suggests that preliminary germplasm screening can be conducted under greenhouse conditions. Greenhouse evaluations can be conducted quickly and with less expense than full-scale
field evaluations. The development of accurate greenhouse screenings would be an important step towards including threecornered alfalfa hopper resistance as a selection criterion in a large-scale breeding program.

The studies reported herein show the importance of threecornered alfalfa hopper control in alfalfa production. Results of these studies also show that variation in threecornered alfalfa hopper susceptibility does exist among alfalfa cultivars and that selection for increased resistance in alfalfa is possible. In addition, greenhouse and field screening techniques have been identified that could be used in the evaluation of alfalfa germplasm for resistance to the threecornered alfalfa hopper. Further experimentation and screening trials are needed to better define the mechanism(s) of resistance found in the alfalfa cultivars identified in this study. The development of alfalfa cultivars resistant to the threecornered alfalfa hopper may prove to be an effective and sustainable control strategy that can ensure the profitability of alfalfa production in Louisiana and the southeastern United States.
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APPENDIX A

Variation in Resistance to the Threecornered Alfalfa Hopper and the Alfalfa Weevil in Alfalfa Somaclones
Somaclonal variation developed through tissue culture has many potential uses in developing insect resistant crop plants (Smith. 1989. Plant Resistance to Insects: A Fundamental Approach. Wiley). Plants regenerated from cell culture usually differ from the cultivar from which they were developed. Fall armyworm, Spodoptera frugiperda (J. E. Smith), resistance in bermudagrass, Cynodon dactylon (L.) Pers., has been enhanced through the use of somaclonal variation (Croughan and Quisenberry. 1989. J. Econ. Entomol. 82: 236-238). The use of somaclonal variation has also led to increased disease tolerance in sugarcane, Saccharum officinarum L., and alfalfa, Medicago sativa L., (Hartman et al. 1984. Plant Science Letters. 34: 183-184; Larkin and Scowcroft. 1983. Plant Cell Tissue Organ Culture 2: 111-121).

The alfalfa weevil, Hypera postica Gyllenhal, and the threecornered alfalfa hopper, Spissistilus festinus (Say), are two important alfalfa insect pests in the southern United States. Insect resistant alfalfa cultivars are being looked to as a means of reducing the damage caused by these economic pests. Tissue culture derived alfalfa plants are one source of germplasm that is being utilized in the search for insect resistant alfalfa. The purpose of our studies was to screen alfalfa somaclones against these insect species.

Alfalfa somaclones derived from the cultivar 'Apollo' were screened for resistance to the threecornered alfalfa
hopper and the alfalfa weevil. The tests were conducted using two cuttings each of 30 different somaclones. All screening trials were conducted in a greenhouse with a 16:8 (L:D) photoperiod illuminated by 40-W fluorescent lights. The temperature was maintained at 27 ± 5°C.

Alfalfa weevil eggs were collected in early March from an alfalfa field near Alexandria, LA. Alfalfa stems were cut at ground level and brought into the laboratory. Leaves were removed and stems were slit longitudinally. Eggs were removed from the stems by washing them into petri dishes. Upon eclosion, six larvae were placed directly onto each test plant using a camel's-hair brush. Larvae were allowed to feed on the plants until pupation. At that time, plants were assessed for defoliation damage.

Threecornered alfalfa hopper nymphs were obtained from a laboratory colony maintained at Louisiana State University. Four first instar nymphs were applied on each test plant. Nymphs were allowed to feed until adult eclosion. Damage was assessed by counting the number of girdles found on each plant. After girdle counts were taken, all plants were clipped to a height of 7 cm and sprayed with malathion (50% EC at 2.5 ml/liter; Fertilome, Voluntary Purchasing Groups, Inc., Bonham, Tex.) to remove the remaining nymphs. The experiment was repeated on the same test plants after the somaclones had cycled through two harvest periods (ca. 2 mo). The procedures used in the second experiment were identical to the first.
The alfalfa weevil data were analyzed as a completely randomized design with two replications. The threecornered alfalfa hopper data were analyzed as a randomized block design with experiment being a blocking factor. Duncan's (1955. Biometrics. 11: 1-42) multiple range test was used for mean separation.

A significant somaclone effect was found for both the alfalfa weevil ($F = 2.01; df = 30,30; P = 0.03$) and threecornered alfalfa hopper ($F = 1.67; df = 30,53; P = 0.05$) data, indicating variation among the different somaclones in their susceptibility to these two insect species. Table A.1 shows the mean number (± SEM) of damaged leaves and girdles for the 30 somaclones and the 'Apollo' check. The highest levels of damage from the two insect species were observed on the AP9 and AP16 somaclones. AP17 and AP23 had the lowest number of leaves damaged by the alfalfa weevil and were significantly different from the check. AP1 and AP19 exhibited the fewest girdles; however, none of the somaclones differed significantly from the 'Apollo' check.

The variation found in these somaclones indicates that insect resistance can be increased in alfalfa through the use of tissue culture. Two alfalfa somaclones showed a significant increase in resistance to the alfalfa weevil by exhibiting less leaf damage than the parent check. When looking at girdle damage, none of the somaclones differed significantly from the check; however, variation
Table A.1. Mean (± SEM) number of leaves damaged by alfalfa weevil larvae and the number of girdles caused by threecornered alfalfa hopper nymphs on 'Apollo' somaclones

<table>
<thead>
<tr>
<th>Somaclone</th>
<th>No. of Damaged Leaves</th>
<th>No. of Girdles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apollo check</td>
<td>29.0 ± 2.0 abc</td>
<td>3.0 ± 0.4 abcde</td>
</tr>
<tr>
<td>AP1</td>
<td>20.5 ± 1.5 bcde</td>
<td>1.0 ± 1.0 e</td>
</tr>
<tr>
<td>AP2</td>
<td>19.5 ± 1.5 bcde</td>
<td>3.5 ± 1.5 abcde</td>
</tr>
<tr>
<td>AP3</td>
<td>15.5 ± 2.5 cde</td>
<td>4.7 ± 0.7 abc</td>
</tr>
<tr>
<td>AP4</td>
<td>21.0 ± 3.0 bcd</td>
<td>3.8 ± 1.0 abcde</td>
</tr>
<tr>
<td>AP5</td>
<td>21.3 ± 2.7 bcd</td>
<td>4.0 ± 0.7 abcde</td>
</tr>
<tr>
<td>AP6</td>
<td>27.0 ± abc</td>
<td>2.0 ± 0.0 bcde</td>
</tr>
<tr>
<td>AP7</td>
<td>24.5 ± 1.5 abcd</td>
<td>3.0 ± 1.2 abcde</td>
</tr>
<tr>
<td>AP8</td>
<td>15.0 ± 3.0 cde</td>
<td>3.8 ± 1.3 abcde</td>
</tr>
<tr>
<td>AP9</td>
<td>31.5 ± 7.5 ab</td>
<td>5.0 ± 0.5 ab</td>
</tr>
<tr>
<td>AP10</td>
<td>22.0 ± 3.0 bcd</td>
<td>2.3 ± 0.8 bcde</td>
</tr>
<tr>
<td>AP11</td>
<td>23.0 ± 0.0 abcd</td>
<td>2.3 ± 0.6 bcde</td>
</tr>
<tr>
<td>AP12</td>
<td>19.5 ± 4.5 bcde</td>
<td>4.0 ± 1.5 abcde</td>
</tr>
<tr>
<td>AP13</td>
<td>20.0 ± 0.0 bcde</td>
<td>4.0 ± 1.5 abcde</td>
</tr>
<tr>
<td>AP14</td>
<td>15.5 ± 6.5 cde</td>
<td>3.8 ± 1.2 abcde</td>
</tr>
<tr>
<td>AP15</td>
<td>20.0 ± 2.0 bcde</td>
<td>3.8 ± 1.4 abcde</td>
</tr>
<tr>
<td>AP16</td>
<td>37.5 ± 12.5 a</td>
<td>5.5 ± 1.8 a</td>
</tr>
<tr>
<td>AP17</td>
<td>14.0 ± 1.0 de</td>
<td>2.0 ± 0.0 bcde</td>
</tr>
<tr>
<td>AP18</td>
<td>19.0 ± 5.0 bcde</td>
<td>2.5 ± 0.6 abcde</td>
</tr>
<tr>
<td>AP19</td>
<td>24.0 ± 4.0 abcd</td>
<td>1.0 ± 0.7 e</td>
</tr>
<tr>
<td>AP20</td>
<td>29.0 ± 4.0 abc</td>
<td>2.5 ± 1.0 abcde</td>
</tr>
<tr>
<td>AP21</td>
<td>28.0 ± 3.0 abcd</td>
<td>3.0 ± 0.4 abcde</td>
</tr>
<tr>
<td>AP22</td>
<td>21.5 ± 1.5 bcd</td>
<td>1.5 ± 0.5 de</td>
</tr>
<tr>
<td>AP23</td>
<td>5.5 ± 1.5 e</td>
<td>4.3 ± 1.3 abcd</td>
</tr>
<tr>
<td>AP24</td>
<td>17.0 ± 3.0 bcde</td>
<td>2.5 ± 1.3 abcde</td>
</tr>
<tr>
<td>AP25</td>
<td>21.0 ± 2.0 bcd</td>
<td>1.8 ± 0.9 cde</td>
</tr>
<tr>
<td>AP26</td>
<td>15.5 ± 3.5 cde</td>
<td>3.0 ± 0.6 abcde</td>
</tr>
<tr>
<td>AP27</td>
<td>26.5 ± 1.5 abcd</td>
<td>4.0 ± 0.7 abcde</td>
</tr>
<tr>
<td>AP28</td>
<td>27.0 ± 1.0 abcd</td>
<td>2.5 ± 0.9 abcde</td>
</tr>
<tr>
<td>AP29</td>
<td>19.5 ± 1.5 bcde</td>
<td>4.3 ± 1.4 abcd</td>
</tr>
<tr>
<td>AP30</td>
<td>24.5 ± 9.5 abcd</td>
<td>2.3 ± 0.8 bcde</td>
</tr>
</tbody>
</table>

a Means within a column followed by the same letter are not significantly different (P > 0.05; Duncan's [1955] multiple range test)

b Only one clone available for this test so standard error could not be calculated.
among the somaclones was significant. Somaclonal variation is an important source of insect resistant germplasm which can be used in plant breeding programs. Variation derived from tissue culture has led to increased disease and insect resistance. The resistant somaclones found in this study are being studied further and will be incorporated into an alfalfa breeding program aimed at increasing insect resistance in commercial alfalfa cultivars.
APPENDIX B

Simple Trichome Density and Alfalfa Antixenosis to the Threecornered Alfalfa Hopper
Simple trichomes have been shown to deter potato leafhopper, *Empoasca fabae* Harris, feeding on alfalfa, *Medicago sativa* L. (Taylor. 1956. Agron. J. 48: 78-81). In order to determine the role of trichomes in alfalfa antixenosis to the threecornered alfalfa hopper, *Spissistilus festinus* (Say), a choice test similar to that described in Chapter 3 was conducted.

**Materials and Methods**

The cultivars 'Cimarron', 'Cimarron VR', 'Florida 77', and 'Zia' were used in a choice test consisting of eight replications. Threecornered alfalfa hopper adults were collected from an alfalfa field at the Northeast Research Station in St. Joseph, LA. The four cultivars were placed in cages as described in Chapter 3, and 40 female and 20 male adult threecornered alfalfa hoppers were placed into each cage. The number of adults on each cultivar was counted at 24, 48, and 72 h.

After 72 h, three stems from each plant were removed, and the number of simple trichomes at the base, middle, and apex of the stem were counted. Trichome counts at the base of the plant were taken by splitting stems longitudinally and counting the number of trichomes within a 2 mm section randomly selected from an internode within the bottom one-fourth of the stem. Similarly, trichome counts at the middle of the stem were taken using a 2 mm section selected from the middle portion of the stem. The number of trichomes at the apex were counted using a 1 mm²
adjacent to the midvein on the underside of the last fully expanded trifoliate leaf. Total pubescense was computed by adding the base, middle and apex counts.

Trichome counts were analyzed as a nested design with stems being nested within each cultivar and replication. Cultivar mean separations were performed using least significant difference (LSD). Threecornered alfalfa hopper counts were analyzed using a multivariate repeated measures approach. The relationships between the number of trichomes and population counts was determined using Pearson correlation coefficients between the average number of trichomes at the base, middle, apex, and total and the number of adults per plant averaged over the three counts.

Results and Discussion

The cultivars varied significantly in the number of trichomes found at the base (F = 5.43; df = 3, 77; P = 0.002), middle (F = 3.79; df = 3, 77; P = 0.01), apex (F = 3.41; df = 3, 77; P = 0.02), and total (F = 7.16; df = 3, 77; P = 0.0003). 'Florida 77' had the most trichomes at the base, middle, and total, and 'Cimarron' had the most at the apex (Table B.1).

The repeated measures analysis did not yield a significant time effect or a significant interaction between time and cultivar, and thus, the three counts were pooled and analyzed as a randomized block design. Using the pooled counts, there was significant variation among
Table B.1. Mean number (± SEM) of simple trichomes on the base, middle, and apex of a stem, total number of trichomes counted, and the number of threecornered alfalfa hopper adults found on four alfalfa cultivars.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Trichomes</th>
<th>Adult TCAH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Base</td>
<td>Middle</td>
</tr>
<tr>
<td>Cimarron</td>
<td>4.5±1.0b</td>
<td>11.3±1.4b</td>
</tr>
<tr>
<td>Cimarron VR</td>
<td>2.8±0.5b</td>
<td>12.0±1.7b</td>
</tr>
<tr>
<td>Florida 77</td>
<td>7.5±1.0a</td>
<td>17.3±1.6a</td>
</tr>
<tr>
<td>Zia</td>
<td>4.3±0.7b</td>
<td>10.5±1.4b</td>
</tr>
</tbody>
</table>

1 Means within a column followed by the same letter are not significantly different (LSD).

Table B.2. Pearson correlation coefficients (Prob > |R|) between trichome counts and adult populations.

<table>
<thead>
<tr>
<th>Trichome Counts</th>
<th>Base</th>
<th>Middle</th>
<th>Apex</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult Populations</td>
<td>0.0494</td>
<td>0.2111</td>
<td>-0.0876</td>
<td>0.1028</td>
</tr>
<tr>
<td></td>
<td>(0.79)</td>
<td>(0.25)</td>
<td>(0.63)</td>
<td>(0.58)</td>
</tr>
</tbody>
</table>
the four cultivars ($F = 6.87; df = 3, 85; P = 0.0003$). Overall, 'Florida 77' had the highest number of adults present and 'Cimarron' had the fewest (table B.1). This disagrees with the results of Chapter 3 which showed 'Cimarron' as having the highest population levels. Because alfalfa cultivars are a mixture of genotypes, variation within a cultivar can be expected and may be the cause of these conflicting results. Pearson correlation coefficients are given in Table B.2. None of the correlations between trichome counts and adult populations were found to be significant. Because 'Florida 77' had the most trichomes and the highest populations present, obviously, some other factor is involved in the antixenosis found in this study. Other factors such as the presence or absence of surface waxes, the thickness of plant tissue, or the presence of secondary metabolites may be involved.
APPENDIX C

A Bibliography of the Threecornered Alfalfa Hopper (Homoptera: Membracidae)


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APPENDIX D

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January 14, 1992

Dr. Daniel J. Moellenbeck
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Moellenbeck, D. J. & S. S. Quisenberry. Screening alfalfa for resistance to the threecornered alfalfa hopper (Homoptera: Membracidae). (Submitted to the Journal of Economic Entomology as manuscript # J91-317).


Moellenbeck, D. J., S. S. Quisenberry & P. D. Colyer. Fusarium crown-rot development in alfalfa stressed by threecornered alfalfa hopper (Homoptera: Membracidae) feeding. (Submitted to the Journal of Economic Entomology as manuscript # J91-243).
VITA

Daniel John Moellenbeck was born on February 25, 1964, in Davenport, Iowa. He grew up on a family farm outside of Walcott, Iowa. In 1982, he graduated from Davenport West High School and began study at Iowa State University. He received his B.S. in 1986 with a major in Biometry and a minor in Pest Management. After graduation, he worked in Slater, Iowa, as a statistician/agronomist for the Garst Seed Company. In 1988, he received a Lousiana State University Board of Regents Fellowship and began his PhD program at Louisiana State University. He worked in the host plant resistance to insects laboratory under Dr. Sharron Quisenberry studying alfalfa resistance to the threecornered alfalfa hopper. In 1990, he married Theresa Ann Ott, originally of Greene, Iowa.
DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Daniel J. Moellenbeck

Major Field: Entomology

Title of Dissertation: The Effect of Threecornered Alfalfa Hopper Populations on Alfalfa Growth and the Development of Host Plant Resistance Screening Techniques

Approved:

[Signature]
Major Professor and Chairman

[Signature]
Dean of the Graduate School

EXAMINING COMMITTEE:

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Date of Examination:

February 21, 1992