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POTATO LEAFHOPPER (*EMPOASCA FABAE*) AND ALFALFA WEEVIL (*HYPERA POSTICA*) DENSITY AND DAMAGE IN BINARY MIXTURES OF ALFALFA AND FORAGE GRASSES

By

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ABSTRACT

POTATO LEAFHOPPER (*EMPOASCA FABAE*) AND ALFALFA WEEVIL (*HYPERA POSTICA*) DENSITY AND DAMAGE IN BINARY MIXTURES OF ALFALFA AND FORAGE GRASSES

By

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Density and damage of potato leafhopper and alfalfa weevil in intercropped alfalfa/forage grass fields and in monoculture alfalfa fields were compared. In 1990, alfalfa weevil larval density (using sweep net sampling) and alfalfa tip damage was lower in fields intercropped with smooth brome, orchardgrass and timothy. On several sampling dates in 1989 and 1990, fewer potato leafhoppers (using suction sampling) were found in plots intercropped with brome and orchardgrass than in monoculture alfalfa. Potato leafhoppers were unable to reproduce on monocultures of smooth brome, orchardgrass, or timothy in the laboratory. Adult leafhoppers emigrated in higher numbers from caged pots of orchardgrass and smooth brome grass in both monocultures and mixtures with alfalfa than from alfalfa alone. Intercropping did not alter forage quality (crude protein, acid detergent fiber, and neutral detergent fiber) of alfalfa in any of the three harvests in 1990.

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CHAPTER 1

REVIEW OF LITERATURE

The potato leafhopper, *Empoasca fabae* (Harris) (Homoptera: Cicadellidae), is a serious pest of alfalfa, soybeans, beans (dry, edible, and fresh), potatoes and other crops in Michigan. Potato leafhoppers overwinter in the southern United States (Decker 1959) and migrate northward as adults in April, May, and June (Medler 1957). These adults are carried in warm air masses at elevations of 0.8 to 1.6 km (Glick 1939, 1960) and are deposited on the landscape with rains, at fronts and cooler air masses, and when exhausted or inactive (Wellington 1945, Pienkowski and Medler 1965). Storms most favorable for transport are typical in spring. They are characterized by high winds, rapid movement directly from the Mississippi Valley, ending in rain in the upper Midwest. The synoptic conditions that produce these patterns (high pressure to the east, low pressure to the west) are well known (Carlson et al. 1991, Huff 1963, Pienkowski and Medler 1964). Potato leafhoppers are believed to be incapable of tolerating winter in the northern states where they feed. Decker and Cunningham (1967), after caging adults on alfalfa fields in fall, could find no surviving leafhoppers in spring. Also, potato leafhoppers cannot survive at temperatures below -15 degrees C (Decker and Maddox 1967).

Following migration, adults can breed on diverse interim host plants, such as oaks and honeysuckle (Gyrisco 1958). They then migrate to field crops where they oviposit. In alfalfa, eggs are laid in stem pith (Simonet and Pienkowski 1977), usually at night (Kieckhefer and Medler 1964). The average life span from egg to death as an adult is 33 days (DeLong 1938). The time required for eggs to hatch as nymphs is 136 $\pm$ 39.7 degree days (base 7.6 degrees C) (Simonet and Pienkowski 1980). Nymphs and adults feed on the phloem of host plants, leaving salivary sheath material in the phloem and occasionally xylem (Gyrisco 1958, Womak 1984). This sheath material causes interrupted photosynthate transport, leading to build-up in tissues distal to the feeding site (Smith and Poos 1931). Photosynthetic rates and, in turn, build up of carbohydrate reserves in the tap root, decline due to reduced transpiration rates (Poos and Johnson 1936, Davis and Wilson 1953, Wilson et al. 1955, Zaky 1981, Womak 1984, Shaw and Wilson 1986). Feeding traps higher levels of total nonstructural carbohydrate in infested leaves (Flinn et al. 1990) and prevents elongation of stem internodes (Oloumi-Sadeghi et al. 1988), resulting in a smaller plant. "Hopperburn," the visually observable result of the blockage and resultant damage, can be used as an indicator of infested and damaged alfalfa (Oloumi-Sadeghi et al. 1988). Feeding damage results in decreased forage dry weight and nitrogen content (Smith and Ellis 1983, Lamp et al. 1985, Flinn and Hower 1984, Kouskoulekas and Decker 1968).

Why immigrants chose particular stands of host plants is not understood. Long distance immigrants tend to be female (Glick 1960); they appear better able to survive without food and water during the long flight (Decker and Cunningham 1967). As the season progresses, in-field sex ratios shift, eventually reaching 1:1 by fall (Medler et al. 1966). Immigrants appear to cluster at field edges (Flinn et al. 1990) and elevated portions of the field (Kieckhefer and Medler 1966). Female potato leafhoppers may have a greater tendency to disperse out of alfalfa (Flinn et al. 1990).

It is hypothesized by Flinn et al. (1990) that potato leafhoppers to go through three steps during spatial distribution. Long-distance immigrants land on alfalfa or nonhost plants as the cue for long distance flight ceases. Host-searching behavior begins, and short flights take the leafhoppers to alfalfa. When alfalfa is found the leafhoppers stop even the short flights and they accumulate at field edges (Flinn et al. 1990). Some researchers have found the presence of grass weeds reduces oviposition and increases flight of potato leafhoppers (Lamp et al. 1984, Smith 1987). The presence of nonhost plants, such as forage grasses, might prolong the flight of migrating leafhoppers and render the field unacceptable to potential immigrants.

Currently, controls for potato leafhopper include insecticide sprays and cutting. To be effective, insecticides must be applied before nymphal populations are large enough to have caused yellowed leaves (hopperburn) (Womak 1984). Frequent cutting of alfalfa reduces the damage of potato leafhopper by killing the nymphs and killing or disturbing the adults (Simonet and Pienkowski 1979, Cuperus et al. 1986). Harvests which leave low stubble provide a poor recolonizing substrate for potato leafhoppers (Cuperus et al.1986).

A second major pest of alfalfa in Michigan is the alfalfa weevil, *Hypera postica* (Gyllenhal), (Coleoptera: Curculionidae). This weevil was introduced from Europe and appeared first in Utah in 1904 (Titus 1910). By 1966, 41 states had established weevil populations (USDA 1966). Adult beetles chew holes in alfalfa stems and lay eggs in clusters of 2-25 within the hole, usually no more than seven to 10 cm above ground (Hamlin et al. 1943). When the eggs hatch, first instar larvae (0.5 mm long) feed inside the plants for three to four days, then climb to growing leaf buds at the tips of the plants to feed for the remainder of the first instar and part of the second (Titus 1910). For the rest of their larval life, the weevils feed on leaves, leaving leaf veins and the lower epidermis untouched. This feeding gives infested fields a frosted appearance (Hamlin et al. 1943). Full-grown larvae are green with a white stripe down the middle of the back. They spin white cocoons, pupate and emerge as adults in two to three weeks. Adults which survive cutting remain active until July (Hower 1982). Most of these adults will migrate to sheltered areas to overwinter and reach sexual maturity the following spring (Landis and Haas 1990).

Alfalfa weevil adults apparently use humidity, vision, and olfaction to detect host plants (Meyer 1975, Meyer and Raffensperger 1974a, b, c). The adults are not strong flyers and usually fly only with the wind, but can travel as far as 136 km each year (Prokopy and Gyrisco 1965, Blickenstaff et al. 1972, Blickenstaff 1965). Weevils cannot distinguish alfalfa in chambers from empty controls when the air in both has been humidified (Meyer and Raffensperger 1974a). However, they "respond more actively" to living alfalfa than to dead alfalfa, even when air currents are pulled away from the plants, suggesting the use of visual cues (Meyer and Raffensperger 1974c). However, adults cannot visually discriminate between alfalfa and red clover, oats, bluegrass, or beans (Meyer 1975). Although Meyer and Raffensperger minimize the importance of olfaction in weevil host plant orientation, adults do turn more frequently when exposed to alfalfa leaf odor (Golik and Pienkowski 1969). A combination of all senses probably serves to get weevils to a planted field (humidity detection), then to move toward plants resembling alfalfa (vision), and finally to incite them to taste the plants (olfaction). Feeding and oviposition would then occur.

Alfalfa weevils damage the first cutting of alfalfa (Hintz et al. 1976), but can also weaken the stand, limiting subsequent cuttings. Overwintering mortality of alfalfa can be increased due to weevil damage (Godfrey and Yeargan 1989), due to reductions in nonstructural root carbohydrates (Fick and Liu 1976) on which the plant depends for regrowth and overwintering. Alfalfa weevil can be controlled with integrated pest management, including early harvests, pesticides, and introduced parasitoids *Microctonus aethiopoides* (Loan)(Hymenoptera: Braconidae), *Bathyplectes anurus* (Thomson)(Hymenoptera: Braconidae) and *Bathyplectes curculionis* (Thomson)(Hymenoptera: Ichneumonidae) (Landis and Haas 1990). These three parasitoids are listed in order of importance in Michigan, but in other states these and other parasitoids may perform differently; for example *B. curculionis* is reported to be more important in Illinois (Onstad and Shoemaker 1984). Areas with well-established parasitoid populations frequently have no need for chemical control of alfalfa weevil (Day 1981). Fungal pathogens, such as *Erynia phytonomi* (Zygomycetes: Entomophthorales), can also be a major cause of mortality in weevils during moist springs (Goh et al. 1989). Fungal epizootics, which can thrive in wet springs, often occur after first-cutting alfalfa matures and after the weevil populations have peaked (Puttler et al. 1980, Brandenburg 1985, Nordin et al. 1983).

Intercropping with forage grasses or allowing weeds into the sward is a potential method of managing alfalfa herbivores. The presence of grass weeds reduced potato leafhopper density (Lamp et al. 1984). Grass weeds or grass weed volatiles in alfalfa reduced female fecundity and increased flight (Smith 1987). The nymphs of the few eggs laid in grass weeds, especially yellow nutsedge (*Cyperus esculentus* L.), crabgrass (*Digitaria sanguinalis* (L.) Scop.), and foxtail (*Setaria faberi* Herrm), generally do not survive (Lamp et al. 1984). Since extracts of grass applied to alfalfa also reduced oviposition (Smith 1987) and increased flight, the presence of non-food biomass in an alfalfa-grass field is not the only deterrent to potato leafhoppers.

Intercropping grass or weeds may also affect alfalfa weevil. Anecdotal evidence from alfalfa growers suggests weedy or grassy fields contain fewer potato leafhopper and alfalfa weevil. Titus noticed in 1910 that alfalfa fields in Summit County, Utah with "considerable" (sic) timothy seem to experience less alfalfa weevil damage. He noticed the timothy appeared to shade the alfalfa. Alfalfa weevils feed only on legumes, prefering alfalfa. They must taste or eat alfalfa to oviposit; other plants probably contain oviposition deterrents (Byrne 1969). Since alfalfa weevil must taste alfalfa before ovipositing, and they will not eat grass, intercropped grasses in alfalfa fields could serve to mask food and oviposition sites, reducing alfalfa weevil numbers and damage. Buntin (1989) did not find grass weeds to alter alfalfa weevil defoliation of alfalfa, and grass root dry weight increased when alfalfa was removed by alfalfa weevil, but this caged greenhouse experiment did not allow for choices or migration. Weevils use vision and odor to locate host plants, and thus could be distracted by grass odors, colors, or shapes. Finally, higher concentrations of preferred food resources exist in monocultures. Root's (1973) "resource concentration hypothesis" states that herbivores inhabiting monocultures of host plants will exhibit greater colonization rates, greater reproduction and longer tenure time as compared to the same herbivore inhabiting a diverse community. If greater concentrations of food exist in monocultures, both potato leafhoppers and alfalfa weevil populations may be reduced in intercrops.

The "enemies impact hypothesis" (Price 1984) may also serve as a partial explanation of why potato leafhoppers and alfalfa weevils may be less prevalent in intercropped alfalfa. Price's clarified version of the "enemies hypothesis" states that crop diversification creates an environment more favorable than monocultures for natural enemies of pests by providing additional resources. Nabid bugs, (especially *Nabis roseipennis*) a known predator of potato leafhopper, prefer to oviposit in grasses (Martinez and Pienkowski 1983, Mundinger 1922). Significantly more nabids were found in grassy alfalfa than in "uninfested" alfalfa in studies by

Barney, et al. (1984). The practical impact of nabids on potato leafhopper is questionable, however, because nabids prefer to eat pea aphids, *Acrythosiphon pisum* (Harris) (Homoptera: Aphididae), a fairly sessile creature, and they have not been shown to be an effective control agent of potato leafhopper (Rensner et al. 1983).

Alfalfa, *Medicago sativa* L., is a popular forage crop because it produces hay more than once a year and it contains more nitrogen than most other field crops. In addition, because it fixes atmospheric nitrogen in underground nodules, it adds nitrogen to the soil. A stand of alfalfa can last four to six years, depending on fertility, soil, climate, and weeds or companion crops (Scheaffer and Marten 1986). Alfalfa has a crown and a tap root which stores nonstructural carbohydrates used to replenish photosynthetic tissue in times of defoliation, stress, and cutting (Hodgkinson 1969, Reynolds 1971, Chatterton et al. 1974, and Smith 1975).

As a forage, alfalfa excels in providing nitrogen. However, too high of a percentage of crude protein in the diet of cattle can cause problems in reproduction, bloat, and waste, as more energy is used to excrete the additional urea created. Too much protein causes high surface tension in the rumen and forms bubbles which will not burst, causing bloat (Howarth 1975). A high rate of release of soluble protein from alfalfa is positively correlated with incidence of bloat (Howarth et al. 1978). A balance must be reached between degradable proteins, which feed rumen microbes, and undegradable proteins, which provide proteins directly to the cow. Adding grass to the cow's rations can increase efficiency by aiding in the formation of the rumen mat, which holds particles for microbe fermentation, and by decreasing bloat by diluting soluble proteins (Howarth et al. 1978, Michael Allen, Animal Scientist, Michigan State University, personal communication). Alfalfa grown for grazing is often planted with a forage grass to reduce bloat potential.

Weeds or grass mixed in the ration or grown with the alfalfa can reduce the problems mentioned above by adding fiber to the diet. Many annual weeds also supply nitrogen and other nutrients to the forage. Marten and Andersen (1975) found that three common weeds were equivalent to alfalfa in nutrient composition and digestibility at early maturity: Pigweed (*Amaranthus retroflexus*), common lambsquarters (*Chenopodium album*), and common ragweed (*Ambrosia artemisiifolia*). Nine of the 12 weeds tested by Marten and Andersen contained more crude protein than oats and ten were more digestible.

Dutt et al. (1979) determined that quackgrass (*Agropyron repens*) reduced digestibility and protein percentage in alfalfa forage. Milking cows fed alfalfa treated with the herbicide pronamide to control quackgrass had nearly 20% higher milk production than those fed weedy forage. The incidence of the problems of high nitrogen feed listed above, however, was not discussed. Also, dandelions grew in the place of the damaged quackgrass. Dandelions can contain as much crude protein as alfalfa but are exceptionally high in potassium, they increase drying time in hay and create agronomic problems (Doll 1986).

This performance of quackgrass in terms of adding fiber and indigestible matter to the forage is probably typical of grasses in alfalfa. Forage grasses, such as those planted with alfalfa in this study, will also lower the highly digestible components, partly digestible components, and percent protein. But for the reasons listed earlier, this apparent decrease in forage quality may not be detrimental.

Some weeds do decrease forage quality, milk production and taste, and palatability for cattle and goats. Temme et al. (1979) found Pennsylvania smartweed (*Polygonum pensylvanicum*), yellow foxtail (*Setaria lutescens*) and shepherd's purse (*Capsella bursa-pastoris*) were the most damaging to laboratory-determined forage quality. Yellow rocket (*Barbarea vulgaris*) is refused by cattle and goats and significantly reduced all qualitative parameters in alfalfa hay containing it (Dutt et al. 1982).

Pure alfalfa is difficult and expensive to maintain and may not be profitable (Cosgrove and Barrett 1988). Seeding alfalfa with a companion crops is a recognized method for establishing alfalfa without herbicides. Goodwin and Morrison (1984) observed wheat companion seeding of alfalfa suppressed dry matter production of redroot pigweed and lambsquarters by more than 50%, night flowering catchfly by 75%, and wild oat and green foxtail by 60 to 70%. Other broadleaf weeds, however, were not significantly reduced. Also, companion cropping reduced light available to alfalfa seedlings and reduced second season regrowth by 50%. In Michigan, many farmers companion seed alfalfa with oats to provide early forage harvest, as oats can be harvested before alfalfa (Temme et al. 1979). Recently, studies have shown that the same benefits of an oat companion crop can be achieved by intercropping alfalfa with forage grasses (Brothers 1991, Schmidt 1991).

Adding grass to alfalfa may help outcompete weeds (Drolsom and Smith 1976) and should not add to hay drying time as with dandelion-infested hay (Doll 1985). Dutt et al. (1982) found that alfalfa forage with 29% weeds, including quackgrass, smooth brome, and orchardgrass, did not suffer reduced feeding value. Casler and Walgenbach (1990) found legume/forage grass mixtures suppressed weeds, aided in preventing erosion, and increased stand duration.

The negative interactions of insects and weed pests may also be improved with alfalfa-grass intercropping. Buntin (1989) reported that alfalfa weevil feeding on alfalfa regrowth following harvest allowed weeds to outcompete alfalfa in the second cutting. In addition, Berberet et al. (1987) found the combination of alfalfa weevil and weeds resulted in poor yield and stand retention. In this study, it is hypothesized that intercropping forage grasses into alfalfa stands will reduce insect pest damage. Since adding grass to alfalfa stands has been shown to suppress weeds, and if intercropping also helps control insects, these stands could produce greater quantities of good quality forage for longer duration than monoculture alfalfa.

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CHAPTER 2

ALFALFA WEEVIL, *HYPERA POSTICA*, LARVAL DENSITY AND DAMAGE IN ALFALFA INTERCROPPED WITH FORAGE GRASSES

ABSTRACT

The effect of alfalfa (*Medicago sativa* L.) intercropped with three forage grass species on alfalfa weevil numbers, damage, and plant biomass was determined in a randomized complete block design field experiment. Eight treatments consisting of alfalfa alone (18 and 14.6 kg/ha) and in mixtures with the forage grasses: smooth brome grass (*Bromus enermis* Leyss.), orchardgrass (*Dactylis glomerata* I.), and timothy (*Phleum pratense* L.). Each grass species was intercropped with alfalfa (14.6 kg/ha) at two seeding rates. Weekly insect sampling showed significantly ($p \leq 0.05$ using two way ANOVA and contrasts) more alfalfa weevil larvae in alfalfa monocultures than in alfalfa-forage grass mixtures on two sampling dates (immediately prior to and at first cutting). Alfalfa weevil damage was also greater in alfalfa monocultures than in four out of six mixtures at harvest. Weevil density was not correlated with alfalfa, grass, or total plant biomass density. Intercropping forage grasses shows promise for reducing alfalfa weevil damage and producing long-lasting stands of high-quality forage.

INTRODUCTION

Alfalfa weevil, *Hypera postica* (Gyllenhal) is a serious pest of first-cutting alfalfa in Michigan. The weevil's feeding removes leaf tissue, giving the field a silvery appearance and reducing the quantity and quality of alfalfa harvested (Hamlin et al. 1943). Alfalfa weevil feeding on regrowth following cutting hinders alfalfa's ability to compete with weeds (Buntin 1989) and can reduce subsequent yields (Edwards et al. 1978). The weevil can be managed using integrated pest management techniques, including cultural, chemical, and biological controls.

Timely cutting of hay (early to mid-bud stage) is recommended as the primary method for limiting weevil damage. In combination with the introduced parasitoids, *Microctonus aethiopoides* (Loan)(Hymenoptera: Braconidae), *Bathyplectes anurus* (Thomson), and *Bathyplectes curculionis* (Thomson)(Hymenoptera: Ichneumonidae), these two management practices largely control weevil populations. In Michigan, *M. aethiopoides*, a parasitoid of adults, is probably the primary controlling parasitoid, followed by the larval parasitoid, *B. anurus* (Landis and Haas 1990). Insecticides are used when these methods fail to provide adequate suppression of weevil populations.

An additional cultural method of managing alfalfa weevil may be through intercropping alfalfa with forage grasses. Forage grass/legume mixtures are commonly grown for beef and horse feed and have other well-known advantages including erosion control, weed control, and increased stand longevity (Drolsom and Smith 1976). The effect of such mixtures on alfalfa weevil, however, is not known.

Alfalfa weevil feeds only on legumes and prefers alfalfa. Titus (1910) noticed alfalfa and timothy intercrop fields in Summit County, Utah had less alfalfa weevil damage than in other fields. He believed this was due to shading of the alfalfa by the taller timothy. Anecdotal evidence from Michigan producers also indicates that alfalfa weevil damage is lower in fields with a grass intercrop or grass weeds, but rigorous testing of the effect on pests of intercropped forage grasses has not been conducted.

Intercropping may reduce the concentration of food available to alfalfa weevil. Root's (1973) "resource concentration hypothesis" states that herbivores inhabiting monocultures of host plants will exhibit greater colonization rates, greater reproduction and longer tenure time as compared to the same herbivore inhabiting a diverse community. If greater concentrations of food exist in monocultures, alfalfa weevil populations may be reduced in intercrops. In addition, alfalfa weevils use vision and olfaction to orient to alfalfa (Meyer 1975, Meyer and Raffensperger 1974a, b, c, Golik and Pienkowski 1969). They must taste or eat alfalfa to oviposit as other plants probably contain oviposition deterrents (Byrne 1969).

After feeding on alfalfa tips and leaves in the early spring, full-grown larvae spin white cocoons, pupate and emerge as adults in two to three weeks (Titus 1910). In Michigan, most of these adults will migrate to sheltered areas to overwinter (Landis and Haas 1990). In spring, they reach sexual maturity, move back into alfalfa fields, and oviposit. The effects of intercropping on weevils might be mainifested in differences in numbers of eggs, larvae, or recolonizing adults, and altered feeding damage. It is possible that intercropping grasses could interrupt the life cycle of the weevil at four important junctions: feeding, recolonization, oviposition, and larval development. Here, only the overall effect of lifecycle interruption, using density and damage as indicators, was studied.

In this study, the effect of intercropping stands of alfalfa with three common, cool-weather forage grasses, smooth brome (*Bromus enermis* Leyss.), orchardgrass (*Dactylis glomerata* I.), and timothy (*Phleum pratense* L.); on alfalfa weevil density and damage was investigated. If intercropping these grasses reduces alfalfa weevil damage, this technique could reduce pesticide usage and lower the cost of producing high quality forage.

METHODS

Field preparation.

A 1.0 hectare plot (Kalamazoo sandy loam) on the Kellogg Biological Station, Hickory Corners, Michigan, was selected for this field study. The field was previously in corn and soybeans, over-seeded with various legumes. For several years prior, the field was in alfalfa. In the fall prior to planting, the field was limed (4482 kg/ha on November 3, 1988), and chisel plowed. No further amendments were called for by the soil test for establishing alfalfa. The following spring, the field was disked (April 28, 1989), field-cultivated (May 1), and cultipacked to prepare a seed bed (May 8).

Two levels of alfalfa (cultivar 'Big Ten'), orchardgrass (cultivar 'Potomac'), timothy, and smooth brome grass (cultivar 'VNS') were planted in eight treatments with five replications in a randomized complete block design on May 10, 1989 (Table 2.1). The grasses were obtained from Scott Farm Seed Company, Mechanicsburg, Ohio, and the alfalfa from Great Lakes Hybrids, Ovid, Michigan. Each treatment plot was 9.88 m by 12.16 m with borders and edges planted in low density (14.6 kg/ha) alfalfa. Five replications of eight treatments were arranged in a randomized complete block design. In the spring following establishment, potassium fertilizer (0-0-60, 258 kg/ha on April 24, 1990) was applied to the entire field as recommended by soil test results. Post-emergence herbicides were used as needed to control broadleaf weeds in all treatments and to remove weed grasses from alfalfa monocultures (Table 2.2).

Table 2.1. Planting densities of alfalfa and three forage grasses, May 10, 1989, at Kellogg Biological Station.

TREATMENT NUMBER	TREATMENT	GRASS PLANTED kg/ha	GRASS PLANTED (lb/A)	ALFALFA PLANTED kg/ha	ALFALFA PLANTED (lb/A)
1	alfalfa (alfalfa alone-high)	none		18	(16)
2	alfalfa (alfalfa alone-low)	none		14.6	(13)
3	alfalfa and brome (brome-high)	5.6	(5.0)	14.6	(13)
4	alfalfa and brome (brome-low)	2.8	(2.5)	14.6	(13)
5	alfalfa and orchard (orchard-high)	1.1	(1.0)	14.6	(13)
6	alfalfa and orchard (orchard-low)	0.6	(0.5)	14.6	(13)
7	alfalfa and timothy (timothy-high)	4.5	(4.0)	14.6	(13)
8	alfalfa and timothy (timothy-low)	2.2	(2.0)	14.6	(13)

Table 2.2. Chemicals applied to field at Kellogg Biological Station field over course of study.

DATE	TARGET PEST	CHEMICALS AND RATES	COMMENTS
June 3, 1989	quackgrass[1]	Roundup® 33% (isopropylamine salt of glyphosphate 41%)	applied with ropewick on quackgrass. Did not complete field and rain immediately followed.
June 6, 1989	quackgrass	Roundup® 33%	ropewick, entire field
June 16, 1989	broadleaves, primarily lambsquarters[2] and pigweed[3]	Butyrac® (2,4-D) 2 qts/Acre	backpack sprayer, entire field
June 28, 1989	quackgrass	Roundup® 33%	ropewick, non-plot areas
May 13, 1990	quackgrass, other grasses	Poast® 1 pint/Acre (plus crop oil and 28% urea ammonium nitrate)	backpack sprayer, applied to alfalfa monoculture plots (treatments 1 & 2)
June 21, 1990	potato leafhopper[4]	Cygon® (dimethoate) 1 pint/Acre	backpack sprayer, on leafhopper exclusion subplots only
July 17, 1990	potato leafhopper	Cygon® 1 pint/Acre	backpack sprayer, on new exclusion subplots

[1] *Agropyron repens*
[2] *Chenopodium album*
[3] *Amaranthus retroflexus*
[4] *Empoasca fabae*

Sampling and Analysis.

From May 2, 1990 until June 6, 1990, alfalfa weevil density was estimated using sweep sampling (10 sweeps per plot) (Table 2.3). Sweeps were taken using the "pendulum" sweep technique (net 37 cm diameter). Both adults and larvae were counted, however adult numbers were too low to analyze statistically. Numbers of larvae in each instar were determined on the June 6 sampling date by examining head capsule diameter.

Alfalfa weevil larvae damage was assessed on June 6, the day prior to cutting. Twenty alfalfa tips were randomly selected in each plot, examined for holes and other signs of weevil feeding damage, and designated damaged or undamaged. The tips in each category were totalled and percent damage in each plot was calculated.

Between May 19 and June 6, 1990, plots were sampled weekly to determine biomass of alfalfa, planted grass, and weeds (Table 2.3). Quadrats of 1/16 meter square area were randomly tossed into a plot and the vegetation within the quadrat was clipped, removed and sorted into groups consisting of alfalfa, planted grasses, and weeds. Sorted material was dried, weighed, and ground. The effect of alfalfa weevil feeding on alfalfa and grass quality was analyzed by the Hach procedure (Hach et al. 1987, Watkins et al. 1987) to determine nitrogen content. Acid detergent fiber and neutral detergent fiber procedures were performed to determine total fiber content (Van Soest and Wine 1967).

All dependent variables (damage, insect density, plant biomass) were analyzed using two-way ANOVA (Systat, Evanston, IL). Significant treatment effects ($p \leq 0.05$) were further explored using planned comparisons (contrasts). Contrasts used to compare differences in insect numbers, and biomass (alfalfa, weed, and total) were high rate alfalfa against low rate alfalfa, both alfalfa monocultures together against all mixtures combined, and low rate of alfalfa against each individual intercrop treatment. The low alfalfa rate was used to compare because each mixture was planted with alfalfa at the low rate. Contrasts used to compare grass biomass were the treatment with the most grass against all other mixtures, and high rate of each grass against the low rate of the same grass for each grass. Differences in biomass and forage quality between sprayed and unsprayed areas were tested with t-tests. Correlations between each of biomass and damage, and larvae numbers were determine.

Table 2.3. Sampling types and dates on field at Kellogg Biological Station in 1990.

	Sampling type			
Date	Larval sweep	Vegetation	Larval damage	Harvest
May 2	X			
May 9	X			
May 18	X			
May 19		X		
May 23	X			
May 25		X		
May 31	X			
June 2		X		
June 6	X	X	X	
June 7				X

RESULTS

Alfalfa weevil surveys performed on May 2 and May 9 revealed almost no alfalfa weevil larvae and are not presented in further analysis. On May 18, alfalfa weevil numbers were low [under 0.5 per sweep (Figure 2.1)], with no significant differences in alfalfa weevil numbers between treatments. Total forage biomass on May 19 was fairly uniform across treatments and not significantly different (Table 2.4). Alfalfa biomass was also uniform with no significant differences between treatments, however treatment 3 (brome at the high rate) had numerically the lowest amount of alfalfa and significantly the most grass (Figure 2.2, Table 2.5). There was significantly more weed biomass in the alfalfa monocultures than in the intercrops except treatment 6 (orchardgrass at the low rate) (Table 2.6).

On May 23, the alfalfa weevil larval population began to rise (Figure 2.1). The high-rate alfalfa monoculture had the most weevil larvae and the high-rate brome and orchard mixtures had the least, however, differences were not significant. The vegetation survey for May 25 showed the high-rate of brome mixture had the highest total biomass, and lowest alfalfa biomass, but these differences were not significant (Figure 2.2). The high brome mixture did have significantly more grass biomass than in all other mixtures (Table 2.5). The difference in weed biomass seen one week earlier disappeared as the mixtures yielded more weeds.

By May 30, the numbers of weevil larvae in the alfalfa monocultures (both seeding rates) were significantly greater than in any of the mixtures (Figure 2.1, Table 2.7). The number of weevils in mixtures rose uniformly; numbers in the high rates of brome and orchard were still the lowest. The biomass survey taken on June 2 (Figure 2.2) showed no significant differences in grass biomass. Alfalfa did differ significantly, however, as the high brome treatment had less alfalfa than in the low alfalfa monoculture (Table 2.6). The low rate of alfalfa were compared with the intercrops because the planting rate of alfalfa was the same as in the mixtures.

On June 6, the day before cutting, alfalfa weevils in the alfalfa monocultures continued to be more numerous than in the mixtures. The number of weevils between the two monoculture treatments was not significantly different (Table 2.7), but weevils were significantly less numerous in all six mixtures than in alfalfa monocropped at the low rate. The amounts of alfalfa in the mixture treatments varied, but not between the two alfalfa monocultures (Figure 2.2). Alfalfa biomass in the two alfalfa monocultures was significantly greater than in the mixtures (Table 2.6). Alfalfa biomass in the low rate alfalfa monoculture was significantly greater than in all mixtures except the orchardgrass treatments ($p=0.060$ for orchard at the high rate contrasted against alfalfa monocropped at the low rate, $p=0.065$ for low orchard contrasted to low alfalfa).

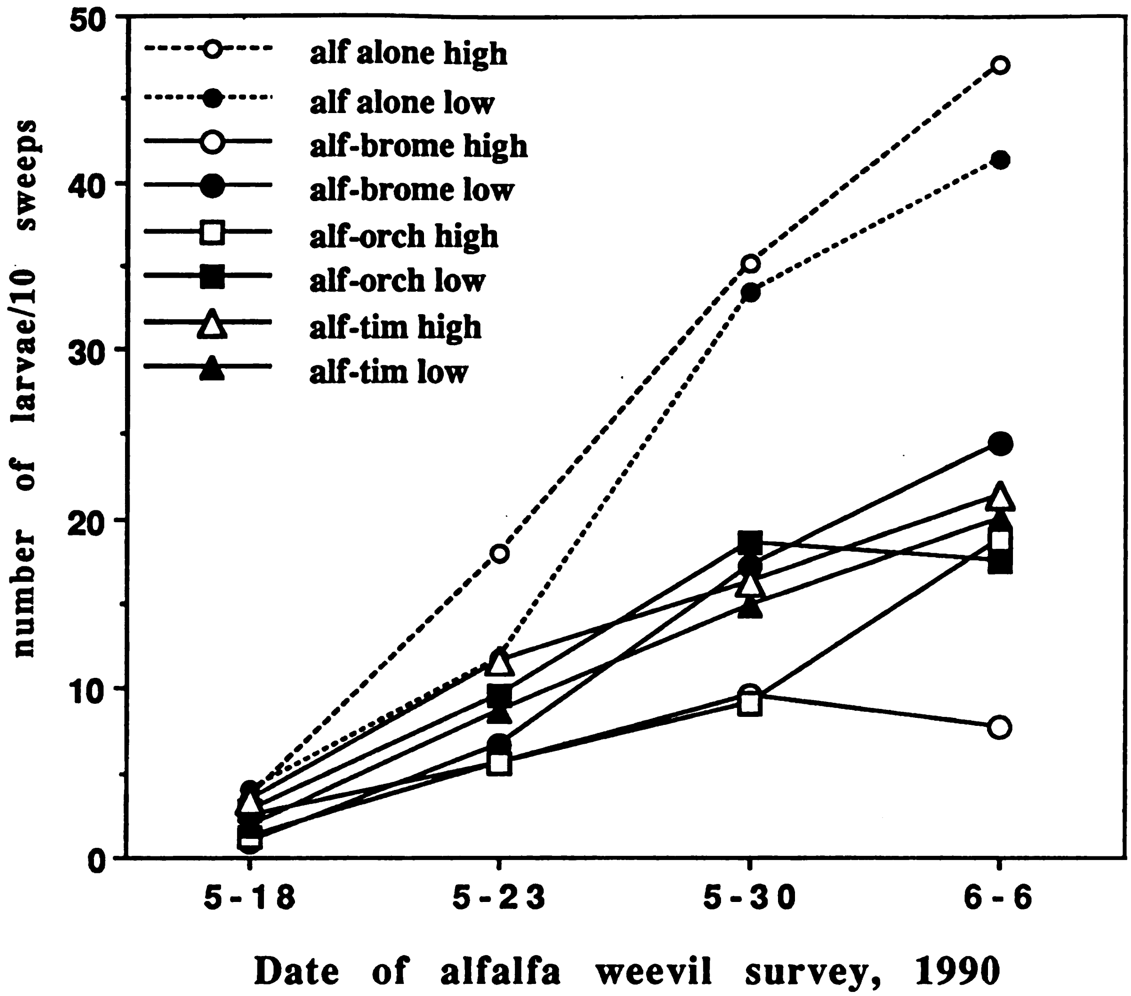
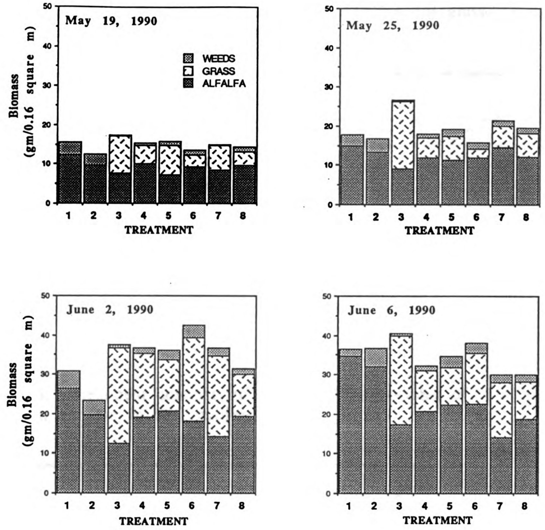


Figure 2.1. Alfalfa weevil larvae density per treatment on four sampling dates; ten sweeps per replication, five replications per treatment.



Treatment legend

1=alfalfa alone high	2=alfalfa alone low
3=alfalfa-brome high	4=alfalfa-brome low
5=alfalfa-orchard high	6=alfalfa-orchard low
7=alfalfa-timothy high	8=alfalfa-timothy low

Figure 2.2. Biomass survey results for first cutting, 1990. Biomass equals vegetation within one 1/16th meter quadrat per replication, averaged over replications.

Table 2.4. Results of ANOVA treatment effects on biomass from first cutting vegetation surveys, 1990. ANOVA for grass portions excluded monoculture treatments.

	p values[a]			
DATE	TOTAL	ALFALFA	GRASS	WEEDS
May 19	ns	ns	0.027*	0.001**
May 25	ns	ns	0.003**	ns
June 2	ns	0.007**	ns	0.016**
June 6	ns	0.002**	ns	ns

Table 2.5. Treatment contrasts of grass biomass portions of vegetation surveys in first cutting, 1990.

	p values[a]	
Contrasts	May 19	May 25
Brome high vs. other mixtures	0.006**	<0.001***
Brome high vs. brome low	0.020*	0.001***
Orchard high vs. orchard low	0.029*	ns
Timothy high vs. timothy low	ns	ns

[a]Treatments are considered significantly different by ANOVA and contrasts if $p \leq 0.05$.

*p value ≤ 0.05
**p value ≤ 0.01
***p value ≤ 0.001

Table 2.6. Treatment contrasts of biomass surveys, alfalfa and weed portions, in first cutting.

Contrasts	p values[a]			
	May 19 weeds	June 2 alfalfa	June 2 weeds	June 6 alfalfa
Alfalfa high vs. rest	<0.001***	0.001**	0.010**	0.001***
Monocultures vs. rest	<0.001***	0.003**	0.002**	<0.001***
alfalfa low vs. all mixtures	0.003**	ns	ns	0.001***
alfalfa low vs. brome high	0.004**	0.027*	0.014*	0.005**
alfalfa low vs. brome low	0.005**	ns	ns	0.027*
alfalfa low vs. orchard high	0.029*	ns	ns	ns
alfalfa low vs. orchard low	ns	ns	ns	ns
alfalfa low vs. timothy high	0.003**	ns	ns	0.001***
alfalfa low vs. timothy low	ns	ns	ns	0.003**

[a]Treatments are considered significantly different by ANOVA and contrasts if $p \leq 0.05$.

*p value $\leq$ 0.05
**p value $\leq$ 0.01
***p value $\leq$ 0.001

Table 2.7. Treatment contrasts of alfalfa weevil larvae numbers in pure and mixed forage stands in 1990.

Contrasts	p Values[a] May 30[b]	June 6
ANOVA treatment effect	<0.001***	0.001***
Alfalfa high vs. alfalfa low	ns	ns
Alfalfa alone (both) vs. all mixtures	<0.001***	<0.001***
Alfalfa low vs. Brome high	<0.001***	<0.001***
Alfalfa low vs. Brome low	0.008**	0.007**
Alfalfa low vs. Orchard high	<0.001***	0.002**
Alfalfa low vs. Orchard low	0.026*	0.002**
Alfalfa low vs. Timothy high	0.005**	0.004**
Alfalfa low vs. Timothy low	0.003**	0.002**

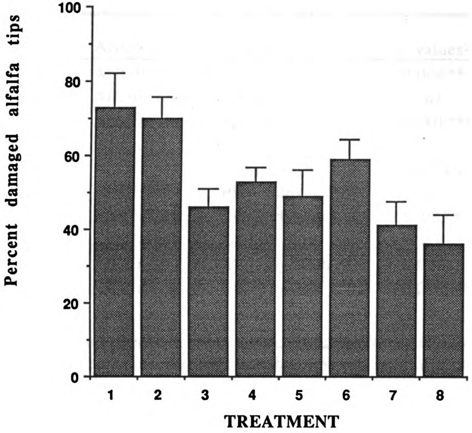
[a]Numbers of alfalfa weevil larvae in different treatments are considered significantly different by ANOVA and contrasts where $p \leq 0.05$.

[b] No significant differences between treatments were found on the first four sampling dates, May 2, May 9, May 18, and May 23.

*p value ≤ 0.05
**p value ≤ 0.01
***p value ≤ 0.001

The damage estimate taken on June 6 resembled the weevil larvae survey and alfalfa biomass survey for the same date (Figure 2.3). The alfalfa monocultures together were significantly more damaged than the mixtures as a whole (Table 2.8), but when comparing each mixture against alfalfa at the low rate, alfalfa intercropped with brome and orchard at the low rates sustained damage comparable to alfalfa alone (low rate). The least damaged treatment was the low rate of timothy (Figure 2.3). This treatment also had one of the lowest amounts of grass (Figure 2.2). The percentage of larvae in each instar for this date was not significantly different across treatments, suggesting that grass presence did not alter development rates and/or sampling effectiveness.

Alfalfa biomass at the earliest vegetation survey date (May 19) was not strongly correlated with alfalfa weevil density at harvest (r^2= 0.176) (Table 2.9). The strongest correlation, damage at harvest (June 6) against alfalfa weevil at the same date was not high (r^2=0.415). Grass biomass on May 19 was also not strongly correlated with weevil density or damage at harvest (r^2=0.356 and 0.028), nor was total biomass at May 19 (at harvest, with weevil density r^2=0.064, damage r^2=0.067). It appears that biomass partitioning early in the season does not directly affect weevil numbers and damage later. Likewise, plant proportions at harvest do not seem to predict weevil numbers and damage. This reduced the possibility of any effect on alfalfa weevil abundance due only to plant biomass.



Treatment legend

1=alfalfa alone high	2=alfalfa alone low
3=alfalfa-brome high	4=alfalfa-brome low
5=alfalfa-orchard high	6=alfalfa-orchard low
7=alfalfa-timothy high	8=alfalfa-timothy low

Figure 2.3. Percentage of twenty randomly selected alfalfa tips damaged by alfalfa weevil larvae by first cutting, June 6, 1990. Error bars are standard error of the mean.

Table 2.8. Treatment contrasts of percent alfalfa weevil damage in pure and mixed forage stands on June 6, 1990.

ANOVAs and Contrasts	p values[a]
ANOVA treatment effect	0.006**
Alfalfa high vs. alfalfa low	ns
Alfalfa alone (both) vs. all mixtures	<0.001***
Alfalfa low vs. Brome high	0.019*
Alfalfa low vs. Brome low	ns
Alfalfa low vs. Orchard high	0.037*
Alfalfa low vs. Orchard low	ns
Alfalfa low vs. Timothy high	0.005**
Alfalfa low vs. Timothy low	0.001***

[a]Numbers of alfalfa weevil larvae in different treatments are considered significantly different by ANOVA and contrasts where $p \leq 0.05$.

*p value ≤ 0.05
**p value ≤ 0.01
***p value ≤ 0.001

Table 2.9. Results of regressions comparing biomass, alfalfa weevil numbers, and damage.

X vs. Y	r^2
May 19 plant data	
forage biomass vs. 5-19 weevil numbers	0.002
forage biomass vs. 6-6 weevil numbers	0.064
forage biomass vs. 6-6 damage	0.067
alfalfa biomass vs. 6-6 weevil numbers	0.176
grass biomass vs. 6-6 weevil numbers	0.356
grass biomass vs. 6-6 damage	0.028
June 6 plant data	
forage biomass vs. 6-6 damage	0.044
alfalfa biomass vs. 6-6 weevil numbers	0.241
grass biomass vs. 6-6 damage	0.073
grass biomass vs. 6-6 weevil numbers	0.270
damage vs. 6-6 weevil numbers	0.415

The vegetation surveys were similar across sampling dates. Brome grass was the dominant grass in early spring and appeared to compete with alfalfa (Figure 2.2). With regard to the alfalfa planting rates, one point became clear: the amount of alfalfa between the alfalfa-alone treatments differed significantly on only one date, June 2, and the number of insects between the two treatments never differed significantly. The biomass of weeds in this second year alfalfa field was not very high, but again it was never different between the two alfalfa-alone treatments. On most dates, weed biomass in the alfalfa-alone treatments was greater than in the intercropped treatments, significantly on May 19. Total biomass in the first cutting was not significantly different between treatments.

Vegetation biomass from harvest surveys was analyzed for Kjeldahl nitrogen as an indicator of forage quality. Forages with high protein are of better quality than those with low protein. There were no significant differences found between any treatments at first cutting (Figure 2.4). Significant differences were found for acid detergent fiber (ADF) and neutral detergent fiber (NDF). Forages with high fiber percentages are of lower quality than those with low fiber. Table 2.10 shows fiber content in alfalfa portions of low rates of orchard and timothy differ significantly from the high rates of those grasses. The high rate of orchard has more fiber than the low rate of orchard, but the high rate of timothy has less fiber than the low rate of timothy. There were no significant differences in the fiber percentages between the alfalfa monocultures or the brome treatments.

There were some differences, however, between the results of the two fibers (Figure 2.5). Alfalfa in treatment 8, the alfalfa-timothy low treatment had the highest percentage of ADF and NDF, and the highest nitrogen content (Figure 2.4). This treatment had the lowest percentage of larval feeding damage (Figure 2.3).

On both protein and fiber analyses, alfalfa in treatment 6 (alfalfa-orchard low) was of highest quality, with the lowest fiber fractions and the highest protein. The rest of the treatments are not as consistent across quality tests.

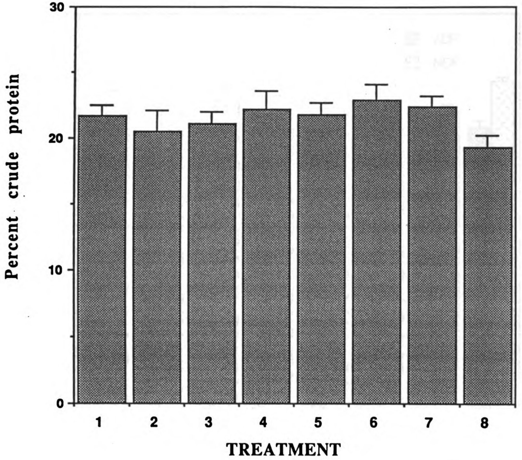
The quality of the grasses in the treatments was also measured. There were no significant differences between nitrogen content of the grasses (ANOVA p=0.166), and likewise with fiber fractions (ADF p=0.073, NDF p=0.067) (Figure 2.6).

Table 2.10. Contrasts of alfalfa biomass fiber analysis treatments, June 6, 1990.

	p values[a]	
Contrast and ANOVA	ADF	NDF
ANOVA treatment effect	0.018	0.010
Monocultures vs. mixtures	ns	ns
Alfalfa high vs. alfalfa low	ns	ns
Brome high vs. brome low	ns	ns
Orchard high vs. orchard low	0.001***	0.002**
Timothy high vs. timothy low	0.037*	0.005**

[a] Treatments are considered significantly different by ANOVA and contrasts where $p \leq 0.05$.

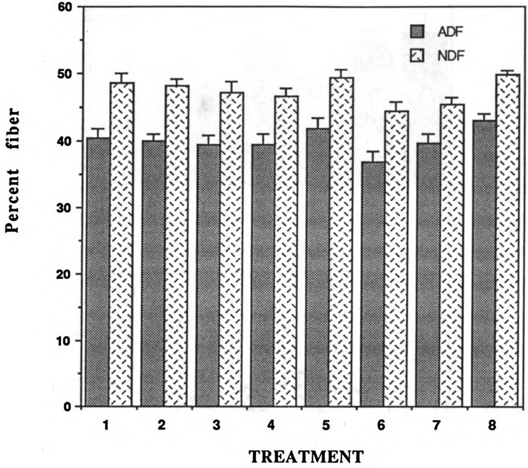
*p value ≤ 0.05
**p value ≤ 0.01
***p value ≤ 0.001



Treatment legend

1=alfalfa alone high | 2=alfalfa alone low
3=alfalfa-brome high | 4=alfalfa-brome low
5=alfalfa-orchard high | 6=alfalfa-orchard low
7=alfalfa-timothy high | 8=alfalfa-timothy low

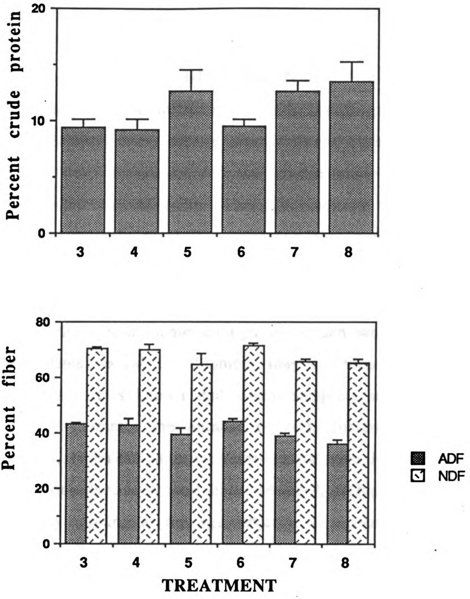
Figure 2.4. Percent crude protein of alfalfa gathered in vegetation surveys on June 6, 1990. Error bars are standard error of the mean.



Treatment legend

1=alfalfa alone high	2=alfalfa alone low
3=alfalfa-brome high	4=alfalfa-brome low
5=alfalfa-orchard high	6=alfalfa-orchard low
7=alfalfa-timothy high	8=alfalfa-timothy low

Figure 2.5. Percent fiber (acid detergent [ADF] and neutral detergent [NDF] fiber) of alfalfa gathered in vegetation surveys on June 6, 1990. Error bars are standard error of the mean.



Treatment legend

3=alfalfa-brome high	4=alfalfa-brome low
5=alfalfa-orchard high	6=alfalfa-orchard low
7=alfalfa-timothy high	8=alfalfa-timothy low

Figure 2.6. Percent crude protein and fiber (acid detergent [ADF] and neutral detergent [NDF] fiber) of grass gathered in vegetation surveys on June 6, 1990. Error bars are standard error of the mean.

DISCUSSION

In this study, alfalfa intercropped with forage grasses contained significantly fewer alfalfa weevil larvae than alfalfa monocultures. These differences did not emerge until May 23 and were not significant until May 30. The rapid increase in grass biomass and concurrently grass height during this period raised some questions about sampling artifacts. First, grass emerging above the alfalfa canopy shaded larval feeding sites in alfalfa tips. If shading altered the microclimate enough to reduce weevil developmental rates, larval development may be retarded in intercropped plots. It is known that sweep sampling in alfalfa consistently underestimates the number of early instars (Higgins et al. 1991). Thus, if larvae in intercropped plots were less developed on average, a consistent bias may have occurred. This possibility was examined by looking at the age structure of larvae from sweeps of all plots on June 6. Since the age structures were not significantly different, it is unlikely that this could explain the results.

A second potential artifact of grass height could be a reduction in sweeping efficiency in these plots. Alfalfa in monocultures was approximately 40 cm tall but grass height was up to 150 cm tall. No direct tests of sweeping efficiency were made. However, the stem samples collected to determine percent damage paralleled the results of larval sweep samples and would not be influenced by grass height.

Planting intercrops appears to reduce alfalfa biomass within first cutting. Other researchers, however, have found mixtures to

produce more total biomass than monocultures (Chamblee and Collins 1988). In later cuttings of the same plots in this study, the mixtures did produce greater harvests than the monocultures. The high rate of brome grass continually produced the most grass of any of the mixtures. Determining composition of orchardgrass plots proved challenging. The tussock (clump) growth pattern of orchardgrass, and a quadrat size which was similar to tussock size, resulted in entire quadrats filled or devoid of orchardgrass. This resulted in the variance of orchardgrass measurement being somewhat higher than other grasses on June 2, and on three dates in later cuttings. Because the other grasses also had high variances at times, quadrat sampling probably did not unduly bias the orchardgrass surveys. Increasing quadrat number or size might increase accuracy of the survey and decrease variability between replications.

The plant biomass present on May 19 did not seem to influence later weevil numbers or damage. Grass and alfalfa biomass, both early in spring and at harvest, was not correlated with weevil density or damage, nor was total forage biomass (Table 2.9). This reduced the possibility of any effect on alfalfa weevil abundance due only to plant biomass. However, the combination of plants could have created different odor plumes than pure alfalfa, as detected by colonizing insects, and may have elicited a different response by the insect. Stanton (1983) described decreasing olfactory attractiveness of hypothetical intercropped patches. Golik and Pienkowski (1969) showed alfalfa leaf odor more than doubled turning rates of hungry adult alfalfa weevils in an arena. If pure alfalfa odor incites weevils to cease flying like it directs kinetic orientation on land, a masked or altered odor plume could result in insects failing to detect alfalfa and continuing flight over "hidden" alfalfa fields. Once the insects are cued to land in an intercropped plot, the amount of grass they encounter could impair oviposition. Weevils must taste alfalfa to oviposit (Byrne 1969); it is possible repeatedly tasting grass could trigger flight or delay oviposition. A study directly investigating the choices made by recolonizing and ovipositing females would further clarify the behavior of weevils in intercrops and explain the lowered numbers we found.

Forage quality parameters did not show significant differences between most treatments, suggesting that these forage quality tests are not sensitive enough to measure differences caused by alfalfa weevil feeding. Some aspect of the crude protein could be flawed as protein is higher than expected for late first cutting alfalfa with 40 to 50 percent fiber (National Research Council 1982), but as all treatments are consistent, it is valid to compare treatments. The fiber data for treatment 8 (timothy at the low rate) alfalfa indicates a low quality forage, but the protein data indicates the best quality of all the treatments. This date had the lowest percentage of visual damage, and it would be reasonable to have the highest percentage of crude protein. It is not reasonable, however, to also have the highest percentage of fiber. This could be an isolated incident, as the other treatments do correspond in general. The forage quality parameters measure different aspects of quality, and it is likely alfalfa weevil feeding, by removing plant matter rather than sucking plant juices, does not alter protein greatly but does increase fiber content. Insect feeding has been shown to increase plant quality in some plants by causing a compensation response (Owen 1980, Owen and Wiegert 1976), but examining this aspect is beyond the scope of this study.

Alfalfa is a popular forage crop because it produces hay more than once a year, it contains more nitrogen than most other field crops, and, because it fixes atmospheric nitrogen in underground nodules, it adds nitrogen to the soil. Because alfalfa is so high in protein, it can cause bloat in animals feeding on it (Howarth 1975). Alfalfa grown for grazing is often planted with a forage grass to reduce bloat. Total crude protein in the feed decreases when grass is added, decreasing the quality parameters of the feed (Van Soest 1982), but this may not be detrimental because of the resulting bloat protection. Planting mixtures could lead to more forage and less potential for bloat.

Adding grass to alfalfa may help outcompete weeds (Drolsom and Smith 1976), prevent erosion, and increase stand duration (Casler and Walgenbach 1990). In this study, intercropping forage grasses into alfalfa stands reduced insect pest damage and weeds. Since adding grass to alfalfa stands has been shown to suppress weeds, and intercropping here helped control alfalfa weevil, intercropped stands could produce greater quantities of good quality forage for longer duration than monoculture alfalfa. For monocultures, planting the low rate could be more profitable than planting the high rate of alfalfa. Intercropping smooth brome grass at the high rate appeared to limit alfalfa weevil numbers and damage more than other mixtures while providing the most total biomass.

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CHAPTER 3

POTATO LEAFHOPPER, *EMPOASCA FABAE*, POPULATION DYNAMICS AND DAMAGE IN ALFALFA/FORAGE GRASS MIXTURES

ABSTRACT

Potato leafhopper (*Empoasca fabae*) (Harris) numbers and damage in alfalfa/forage grass binary mixtures were determined in a field experiment. Treatments consisting of alfalfa alone (14.6 kg/ha and 18 kg/ha) and in mixtures with three forage grasses were planted. The grasses, smooth bromegrass (*Bromus enermis* Leyss.), orchardgrass (*Dactylis glomerata* I.), and timothy (*Phleum pratense* L.), were each planted at two seeding rates with 14.6 kg/ha of alfalfa. There was an overall trend for more adult leafhoppers to be present in alfalfa monocultures, however, this was significant on only one of 12 dates sampled in 1990. Reduced potato leafhopper nymph density in many mixtures occurred on two dates, one immediately prior to and one following second cutting.

In laboratory experiments, adult potato leafhoppers were found to be able to survive but not reproduce on smooth brome, timothy, or orchardgrass monocultures, indicating that the presence of grass in alfalfa plantings may delay or inhibit oviposition by

potato leafhoppers. In a laboratory assay measuring emigration, leafhoppers left alfalfa-grass mixtures (alfalfa-brome, alfalfa-orchardgrass) significantly more than from alfalfa monocultures.

INTRODUCTION

Potato leafhopper, *Empoasca fabae* (Harris) (Homoptera: Cicadellidae), is a serious pest of alfalfa, soybeans, dry beans, potatoes and other crops in Michigan. This insect cannot survive midwestern winters and each year must migrate from the gulf states in late spring (Decker and Cunningham 1967). Typically the first cutting alfalfa crop receives little leafhopper damage, however, new seedings and the second and third cuttings of alfalfa frequently sustain serious damage. Phloem feeding by potato leafhoppers on alfalfa results in visual symptoms termed "hopperburn", decreased dry weight yield and decreased crude protein content (Smith and Ellis 1983, Lamp et al. 1985, Flinn and Hower 1984, Kouskoulekas and Decker 1968). The feeding traps higher levels of total nonstructural carbohydrate in infested leaves (Flinn et al. 1990). The feeding of potato leafhoppers also prevents elongation of stem internodes (Oloumi-Sadeghi et al. 1988), resulting in stunted plants.

Currently, controls for potato leafhopper include insecticide sprays and timely cutting. An additional method of managing potato leafhopper may be cultivating non-host plants (grass or weeds) with alfalfa. Potato leafhopper adults can survive but cannot reproduce on smooth brome, orchardgrass or timothy (Coggins, this volume). The presence of grass weeds in alfalfa has been reported to reduce potato leafhopper density (Lamp et al. 1984). Grass weeds or grass-weed volatiles reduce oocytes and eggs laid per female in alfalfa and increase flight activity (Smith 1987). Since extracts of grass applied to alfalfa also reduce oviposition (Smith 1987) and increase flight, the presence of non-food biomass in an alfalfa-grass field is not the only deterrent to potato leafhoppers. Some Michigan alfalfa growers have noticed reduced potato leafhopper damage in weedy and grassy alfalfa fields but testing the effect of intercropped forage grasses on pests has not previously been conducted.

Although some studies of potato leafhopper movement have been performed, why immigrants chose to move into particular stands of host plants is not understood. Long distance immigrants tend to be female (Glick 1960); they appear to be better able to survive without food and water during the long flight (Decker and Cunningham 1967). As the season progresses, in-field sex ratios change, eventually reaching 1:1 by fall (Medler et al 1966). Immigrants appear to cluster at field edges (Flinn et al. 1990) and elevated portions of the field (Kieckhefer and Medler 1966), with female leafhoppers having a greater tendency to disperse out of alfalfa (Flinn et al. 1990). Flinn, Hower and Taylor (1990) believe potato leafhoppers to go through three steps during spatial distribution: long-distance immigrants land on alfalfa or nonhost plants as the cue for long distance flight ceases, host-searching behavior begins, and short flights take the leafhoppers to alfalfa. Apparently, when alfalfa is found the leafhoppers cease even short flights, causing accumulation at field edges (Flinn et al. 1990). If leafhoppers discriminate between fields of pure alfalfa and alfalfa mixed with nonhosts, intercropping alfalfa could alter these patterns. Furthermore, if the presence of grass weeds reduces oviposition and increases flight of potato leafhoppers (Lamp et al. 1984, Smith 1987), the presence of nonhost plants could render the field less acceptable to potential immigrants.

Prior to the current emphasis on alfalfa monoculture, producers in Michigan typically grew alfalfa/grass mixtures. Mixtures have certain positive agronomic characteristics including weed suppression, increased stand persistence, increased resistance to lodging, decreased drying time of cut forage, and decreased potential for causing bloat in cattle (Casler and Walgenbach 1990, Howarth et al. 1978). These mixtures, although not typically as high in crude protein as alfalfa monocultures, provide an acceptable forage for many classes of animals. Dutt et al. (1982) found that alfalfa forage with 29% weeds, including quackgrass, smooth brome, and orchardgrass, did not suffer reduced feeding value for cattle. New varieties of forage grasses, improved production practices, and the potential to reduce insect damage have caused renewed interest in the production of alfalfa/forage grass mixtures.

The objectives of the field portion of this study were to investigate the effect of intercropping stands of alfalfa with three common, cool-weather forage grasses, smooth brome (*Bromus enermis* Leyss.), orchardgrass (*Dactylis glomerata* I.), and timothy (*Phleum pratense* L.), on potato leafhopper density and damage. The laboratory objectives were to determine if potato leafhoppers could survive and reproduce on the forage grasses listed above, and to analyze emigration attempts from alfalfa, alfalfa/forage grass mixtures, and nonhosts (grass monocultures and soil).

METHODS

Field Studies.

A 1.0 hectare plot (Kalamazoo sandy loam) on the Kellogg Biological Station, Hickory Corners, Michigan, was selected for this field study. The field was previously in corn and soybeans, over-seeded with various legumes. For several years prior, the field was in alfalfa. In the fall prior to planting, the field was limed (4482 kg/ha on November 3, 1988), and chisel plowed. No further amendments were called for by the soil test for establishing alfalfa. The following spring, the field was disked (April 28, 1989), field-cultivated (May 1), and cultipacked to prepare a seed bed (May 8).

Two levels of alfalfa (cultivar 'Big Ten'), orchardgrass (cultivar 'Potomac'), timothy, and smooth brome grass (cultivar 'VNS') were planted in eight treatments with five replications in a randomized complete block design on May 10, 1989 (Table 3.1). The grasses were obtained from Scott Farm Seed Company, Mechanicsburg, Ohio, and the alfalfa from Great Lakes Hybrids, Ovid, Michigan. Each treatment plot was 9.88 m by 12.16 m with borders and edges planted in low density (14.6 kg/ha) alfalfa. In the spring following establishment, potassium fertilizer (0-0-60, 258 kg/ha on April 24, 1990) was applied to the entire field as recommended by soil test results. Post-emergence herbicides were used as needed to control broadleaf weeds in all treatments and to remove weed grasses from alfalfa monocultures (Table 3.2).

Table 3.1. Planting densities of alfalfa and three forage grasses, May 10, 1989, at Kellogg Biological Station.

TREATMENT NUMBER	TREATMENT	GRASS PLANTED kg/ha	GRASS PLANTED (lb/A)	ALFALFA PLANTED kg/ha	ALFALFA PLANTED (lb/A)
1	alfalfa (alfalfa alone-high)	none		18	(16)
2	alfalfa (alfalfa alone-low)	none		14.6	(13)
3	alfalfa and brome (brome-high)	5.6	(5.0)	14.6	(13)
4	alfalfa and brome (brome-low)	2.8	(2.5)	14.6	(13)
5	alfalfa and orchard (orchard-high)	1.1	(1.0)	14.6	(13)
6	alfalfa and orchard (orchard-low)	0.6	(0.5)	14.6	(13)
7	alfalfa and timothy (timothy-high)	4.5	(4.0)	14.6	(13)
8	alfalfa and timothy (timothy-low)	2.2	(2.0)	14.6	(13)

Table 3.2. Chemicals applied to field at Kellogg Biological Station field over course of study.

DATE	TARGET PEST	CHEMICALS AND RATES	COMMENTS
June 3, 1989	quackgrass[1]	Roundup® 33% (isopropylamine salt of glyphosphate 41%)	applied with ropewick on quackgrass. Did not complete field and rain immediately followed.
June 6, 1989	quackgrass	Roundup® 33%	ropewick, entire field
June 16, 1989	broadleaves, primarily lambsquarters[2] and pigweed[3]	Butyrac® (2,4-D) 2 qts/Acre	backpack sprayer, entire field
June 28, 1989	quackgrass	Roundup® 33%	ropewick, non-plot areas
May 13, 1990	quackgrass, other grasses	Poast® 1 pint/Acre (plus crop oil and 28% urea ammonium nitrate)	backpack sprayer, applied to alfalfa monoculture plots (treatments 1 & 2)
June 21, 1990	potato leafhopper[4]	Cygon® (dimethoate) 1 pint/Acre	backpack sprayer, on leafhopper exclusion subplots only
July 17, 1990	potato leafhopper	Cygon® 1 pint/Acre	backpack sprayer, on new exclusion subplots

[1]*Agropyron repens*
[2]*Chenopodium album*
[3]*Amaranthus retroflexus*
[4]*Empoasca fabae*

Field Sampling and Analysis.

In July 1989, after establishing the plots, preliminary insect sampling using a D-vac® (D-vac vacuum insect net, Riverside, California) and biomass sampling was done. The D-vac procedure was similar to that described in Flinn et al. (1990), except here researchers walked in a zigzag pattern while taking 10 suctions per plot (10 suctions = 0.9 m^2 per plot). For biomass sampling in this study, counting stems within five 1/16 meter quadrats was attempted (July 26), but proved too time-consuming. Quadrat harvests (August 10) provided the most information in the least amount of time, and this method was chosen for 1990 vegetation surveys. Quadrats of 1/16 meter were randomly tossed into a plot and the vegetation within the quadrat was clipped, removed and sorted by vegetation type (alfalfa, planted grass, weed). Sorted material was dried and weighed. Weights were compared on a per quadrat basis for statistical analysis.

Between May 30 and August 13, 1990, weekly insect D-vac samples were used to assess potato leafhopper density (Table 3.3). Between May 19 and August 13, 1990, plots were sampled weekly to determine biomass of alfalfa, planted grass, and weeds. Numbers of quadrats per plot varied with plant height (more quadrats on dates with little vegetation). Sorted material was dried, weighed, and ground with a Wiley mill (1 mm screen) and then with a Udy mill (1 mm screen). Vegetation biomass from harvest surveys was analyzed for Kjeldahl nitrogen as an indicator of forage quality (Hach et al. 1987, Watkins et al. 1987). Acid detergent fiber and neutral detergent fiber procedures were performed at harvests to determine total fiber content (Van Soest and Wine 1967). These measurements were used to compare potato leafhopper feeding damage on alfalfa and grass forage quality between treatments. To compare insect-infested plants to non-infested controls, subplots were sprayed with Cygon® (dimethoate, American Cyanamid) insecticide using a backpack sprayer (Table 3.2). Vegetation samples from these areas were also collected (as above), and analyzed for forage quality.

It was hypothesized that feeding damage would be greatest in monoculture alfalfa, less in intercropped plots, and least in insecticide treated subplots. All dependent variables (plant biomass, plant quality, insect numbers) were analyzed using two-way ANOVA (Systat, Evanston, IL). Significant treatment effects ($p \leq 0.05$) were further explored using planned comparisons (contrasts). Contrasts used were high rate alfalfa against low rate alfalfa, both alfalfa monocultures together against all mixtures combined, and low rate of alfalfa against each mixture treatment individually. This contrast schedule was used to compare differences in insect numbers, and alfalfa, weed, and total biomass. The low alfalfa rate was used to compare because each mixture was planted with alfalfa at the low rate. Contrasts used to compare grass biomass were the treatment with the most grass against all other mixtures, and high rate of each grass against the low rate of the same grass for each grass. Differences in biomass and forage quality between sprayed and unsprayed areas were tested with t-tests. Correlations were determined between alfalfa and grass biomass, and potato leafhopper nymphs and adults.

Table 3.3. Sampling types and dates on field at Kellogg Biological Station in 1989.

Date	Sampling type: Insect	Sampling type: Vegetation	Harvest
July 10	x		
July 13			x
July 24	x		
July 26		counted stems	
August 1	x		
August 8	x		
August 10		destructive	
August 17			x

Table 3.4. Sampling types and dates on field at Kellogg Biological Station in 1990.

Date	Sampling type: Insect	Sampling type: Vegetation	Harvest
May 30	x		
June 2		x	
June 5	x		
June 6		x	
June 7			x
June 12	x		
June 17	x		
June 18		x	
June 25	x	x	
July 1	x	x	
July 9	x	x	
July 11			x
July 16	x		
July 23	x		
July 24		x	
July 30	x	x	
August 7	x		
August 13	x	x	
August 14			x

Laboratory portion

Reproduction and survival on grass

In a preliminary experiment, three species of grass (brome, timothy and orchardgrass) in alfalfa-grass mixtures were planted, five grass plants with five alfalfa plants. Pots were 32 ounce food containers (Sweetheart Cup Company, Chicago, IL) and soil was Baccto® Professional planting mix (Michigan Peat Company, Houston, TX). Monocultures of 10 plants each of alfalfa and each grass were also planted. When the plants were approximately six weeks old, three unsexed adult potato leafhoppers were placed in each pot and caged with a pop-bottle cage (Figure 3.1). Cages were made from clean, plastic two-liter soda bottles with tops cut off and holes cut in the side. Holes were covered with saran screening (0.23 mm mesh, Chicopee Manufacturing Company, New Brunswick, NJ). The open end was placed over the pot and taped securely. Adult leafhoppers were gathered from a laboratory culture originally collected on alfalfa and reared on fava beans (*Vicia fava* L., long pod, improved; Harris Seeds, Rochester, NY). As a control, three potato leafhoppers were placed on barren soil in a pot. After three weeks of potato leafhopper infestation, the plant matter was harvested, dried, weighed, and analyzed for crude protein.

This experiment was repeated eight months later, using only monocultures of alfalfa, brome and orchardgrass with ten plants each. Timothy did not thrive in greenhouse conditions and was not used in this or future laboratory experiments. Plants of heterogeneous age and width within each pot (same as previous experiment) were used to control for the possibility of egg desiccation due only to the thinness of young grass. Whereas the preliminary experiment was unreplicated, in the second run, five replications of each monoculture were used. The number of potato leafhoppers was increased to six in each caged pot including both males and females. Plant quality was not analyzed for this experiment.

Leaving assay

In a second laboratory study, movement from alfalfa, alfalfa-forage grass mixtures, and nonhosts were measured by trapping leafhoppers at the only exit of a cage. Flats of eight-week-old alfalfa, brome, and orchardgrass plants were carefully dug from a new seeding at Kellogg Biological Station, Hickory Corners, Michigan. Twenty plants for monocultures and ten plants each, alfalfa and grass, for treatment mixtures were transferred to clay pots (12 cm tall, 12 cm top diameter) and allowed to grow. Plants were watered, fertilized (10-20-20) and clipped regularly as needed. Treatments were bare moist soil, alfalfa alone, brome alone, orchardgrass alone, alfalfa-brome, and alfalfa-orchard. Pots with similar biomass, height, and plant condition were selected, caged and subjected to 20-30 adult potato leafhoppers (same number of leafhoppers were used within replications). Sex ratios of potato leafhopper used were estimated by collecting, killing and sexing a randomly selected subset of leafhoppers gathered at the same time as the experimental insects. Insects were gently blown into the cages and the cages were transferred to temperature and light controlled environmental chambers (I-35 series, Percival Manufacturing Company, Boone, Iowa) for four days. Cages, cylinders of wood and saran screening, were designed to fit snugly on top of pots leaving only a 3 cm diameter hole at the top (Figure 3.2). Daylight in the chamber lasted 14 hours at 26 ^{o} C, and nights were ten hours at 19 ^{o} C. Dawn and dusk were simulated by keeping one set of lights on for two hours in the morning and four hours in the evening. For the rest of the "day," two sets of lights were on. Moonlight was approximated by a foil-covered night-light lit above the cages at all times (including night when all other lights were off). Insects emigrating through the 3 cm diameter hole were caught in diet cups (Fill-Rite Corporation, Newark, New Jersey) sprayed with Tangletrap® (The Tanglefoot Company, Grand Rapids, Michigan) attached above the hole. Leafhoppers leaving the arena were counted at 6 pm and 10 pm the first night of each replicate, and at 8 am and 6 pm the next two. At 8 am of the fourth day, the cages were emptied of leafhoppers and the plant matter was clipped and dried to compare biomass. During each replication, sticky cups were replaced every 24 hours and the captives sexed. Cups were allowed to dry for 5-12 hours after spraying to dissipate Tangletrap® odors.

Insect numbers were totalled for each treatment in each replication, percentages transformed using the arcsin square root transformation, and compared using two-way ANOVA and contrasts.

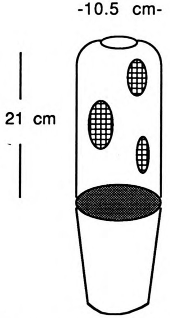


Figure 3.1. Pop-bottle cage used for potato leafhopper reproduction and survival experiments. Screened areas are holes covered with saran screening.

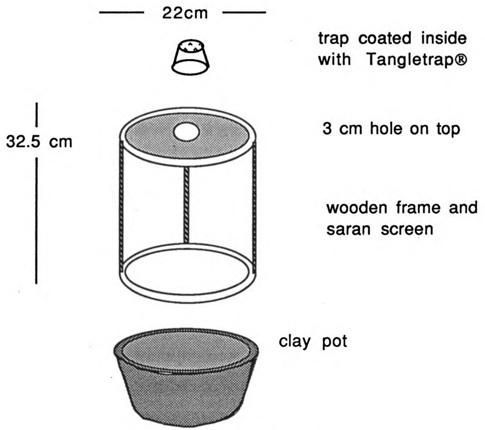


Figure 3.2. Cage used for leaving assay experiments.

RESULTS

Field portion

1989

Lack of selective pre-emergence herbicides for use in mixed plots led us to attempt to establish the entire test without herbicides. As expected from a spring planting, the field had moderate weed pressure. Following crop and weed emergence, treatment with a selective post-emergence herbicide (Buctril®) removed the broadleaf weeds effectively, however, patches of quackgrass (*Agropyron repens*) remained. These were suppressed in alfalfa monocultures using Poast® (a selective post-emergence grass herbicide), but could not be removed from the mixed plots in this fashion without harming the forage grasses. In mixed plots when quackgrass emerged about the crop canopy, a rope-wick applicator was used to achieve partial control (Table 3.2).

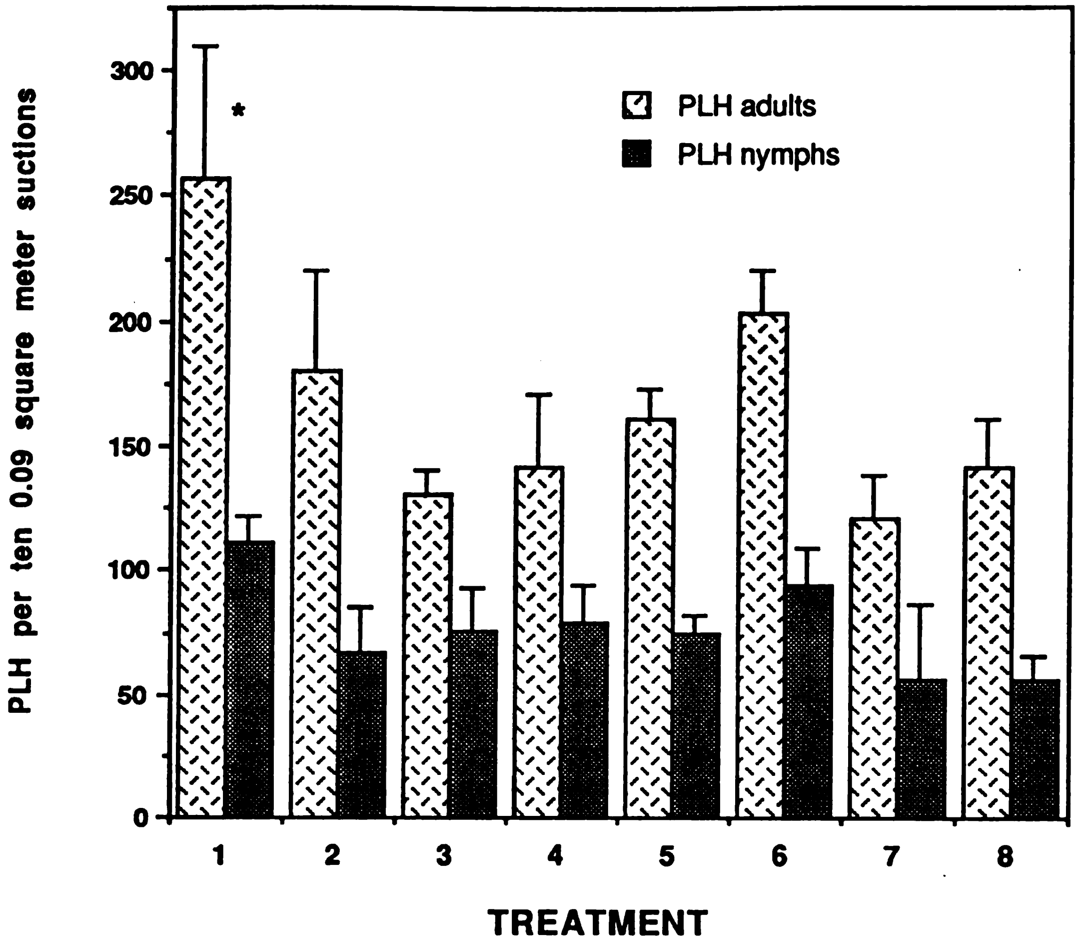
Three cuttings were planned for the establishing alfalfa stand as per typical agronomic practice, however, only two were taken. The first cutting was July 13, 1989, 64 days following seeding. The second cutting was taken on August 17. The forage grasses grew very slowly in this establishment year, generally evident only as a part of the crop understory. Following cutting, the mixed plots were noticeably more green than the alfalfa monoculture plots. The low growth habit of the grasses at this time allowed more grass biomass to escape cutting compared to the taller alfalfa.

The biomass surveys supported these observations. There were no differences in grass biomass in either the stem or the destructive harvest surveys, and the treatments had less grass biomass than alfalfa. Both surveys showed significantly more alfalfa in the alfalfa-high monoculture than in low monoculture and in all other treatments (contrast $p=0.002$ for low vs. high monoculture stems, $p=0.011$ for low vs. high monoculture biomass, and $p<0.001$ for high monoculture vs. all other treatments for both stems and destructive sampling). The stem count revealed no differences between the low rate of alfalfa and the mixtures. The destructive harvest had significantly more alfalfa in alfalfa-low treatments than in brome-low and timothy-low (contrast $p=0.029$ and 0.016).

Spring storms, particularly on May 29, 1989, brought large numbers of migrating potato leafhoppers into Michigan. These leafhoppers infested the establishing alfalfa and caused considerable hopperburn and stunting. Three days prior to first cutting, July 10, 1989, the high rate of pure alfalfa contained significantly ($p<0.001$) higher numbers of adult leafhoppers than all intercropped treatments, except alfalfa mixed with orchardgrass at the low rate (Figure 3.3). Nymph numbers were not significantly different between treatments.

During the regrowth of second cutting, there were no significant treatment differences detected in leafhopper numbers. On July 18, there was a trend for higher leafhopper numbers (adults and nymphs combined) on alfalfa monoculture and alfalfa-timothy intercrops, and low leafhopper numbers on alfalfa-brome treatments (Figure 3.4). On July 24, adult numbers declined in many plots while nymph numbers increased. On August 1, total leafhopper numbers (adults and nymphs combined) in alfalfa monocultures tended to be higher than any of the intercropped treatments.

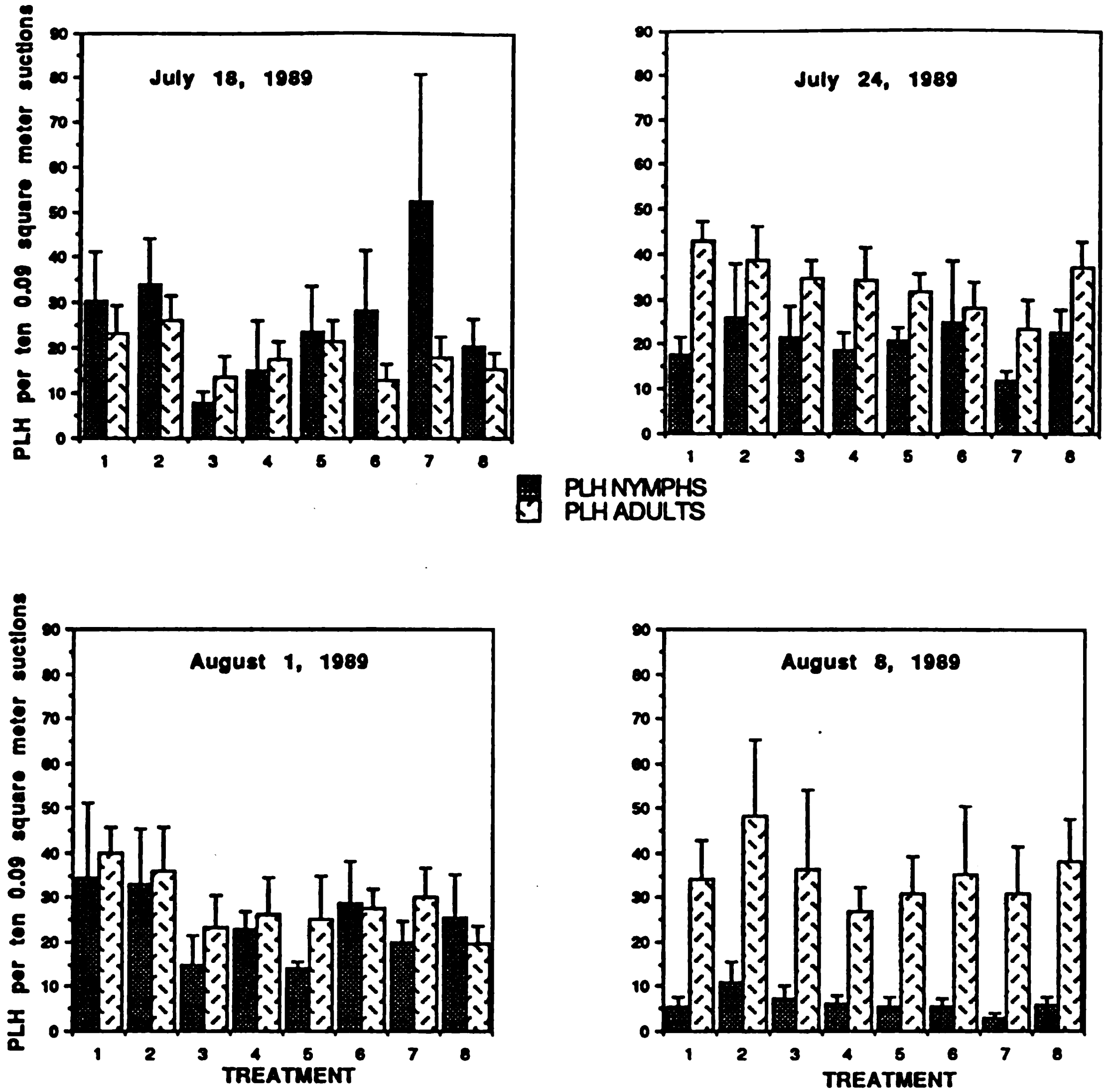
By August 8, adult leafhopper numbers had declined noticeably in all treatments while nymph numbers generally rose slightly. The sudden drop in adult numbers was likely due to an epizootic of *Zoophthora radicans* (Brefeld) Batko, a fungal pathogen of potato leafhopper. Cool, wet conditions in the previous weeks are believed to have triggered the epizootic which was widespread across lower Michigan.



Treatment legend

1=alfalfa alone high	2=alfalfa alone low
3=alfalfa-brome high	4=alfalfa-brome low
5=alfalfa-orchard high	6=alfalfa-orchard low
7=alfalfa-timothy high	8=alfalfa-timothy low

Figure 3.3. Potato leafhopper (PLH) D-vac sampling on July 10, 1989. Treatment 1 (*) contained significantly more adults (p=0.05 or less by ANOVA) than any other treatment except 6. Error bars are standard error of the mean.



Treatment legend

1=alfalfa alone high	2=alfalfa alone low
3=alfalfa-brome high	4=alfalfa-brome low
5=alfalfa-orchard high	6=alfalfa-orchard low
7=alfalfa-timothy high	8=alfalfa-timothy low

Figure 3.4. Adult and nymphal potato leafhopper densities in 1989. Error bars are standard error of the mean, and no significant differences exist between treatments on any date.

1990

For the first half of second cutting, June 11 through June 25, adult potato leafhopper numbers rose uniformly as a group, with no significant differences between treatments (Figure 3.5). Nymph numbers did not increase during this time (Figure 3.6). On June 18, there were significantly more weeds in the alfalfa monocultures than in the other treatments (Figure 3.7, Tables 3.6 and 3.7). On June 25, there was significantly more alfalfa and total biomass in the monocultures than in the mixtures (Figure 3.7, Tables 3.6 and 3.7).

On July 1, 1990, numbers of potato leafhopper adults were significantly greater in the alfalfa alone-low treatment than in brome mixtures (both high and low rates) and in the high rate of orchard mixture (Figure 3.5, Table 3.5). In addition, there were more adults in the alfalfa alone-high treatment than in all other treatments, but no contrasts against this treatment were made. Nymph numbers began to increase on July 1 (Figure 3.6) There was significantly more alfalfa biomass in the monocultures than in the mixtures on July 1 (contrast $p < 0.001$) (Figure 3.7, Table 3.7).

On July 9, there were no significant differences in adult numbers among treatments. The highest numbers were found in low orchard mixtures and the lowest in high orchard mixtures. Nymph numbers showed a significant treatment ANOVA, however, none of our standard contrasts were significant. Numerically more leafhoppers were found in low orchard mixtures and the least in high plantings of all three grasses. Brome at the low rate had more nymphs than brome at the high rate and orchard-low had more than orchard-high (Figure 3.6). Timothy-low also had more nymphs than timothy-high, but here the difference was not significant (p=0.061). There were no significant differences between alfalfa biomass on July 9 (Table 3.6), but there was less alfalfa in the brome-high and orchard-high treatments than in the other mixtures and in the monocultures (Figure 3.7). The alfalfa biomass was fairly uniform across the other treatments. There was significantly more grass in the brome-high and orchard-high treatments when contrasted against the timothy-high treatment (p=0.022 and 0.001 respectively), but there was no difference between brome and orchard biomass. These differences were more pronounced in the controlled (sprayed) subplots.

Immediately following second cutting, there were significant differences in nymph abundance on July 16. Orchard-low and both timothy rates contained more nymphs than the other treatments (Table 3.5, Figure 3.6). Adult numbers on this date were low and did not differ, similar to what occurred after first cutting as well (Figure 3.5). No biomass survey was conducted on this date.

On July 23, nymph numbers decreased from the previous date (Figure 3.6). Adult numbers increased slightly, with no separation between treatments. Alfalfa biomass on July 24 was uniform (Table 3.6), but orchardgrass in both treatments was significantly greater than the other grasses (p<0.001, Table 3.8). Brome and timothy treatments contained comparable biomass. These differences were not present in the controlled portion (Figure 3.8).

Adult leafhopper numbers rose by July 30. The two alfalfa monoculture treatments had the highest adult numbers and both orchard mixtures had the lowest (Figure 3.5). Nymph numbers remained low (Figures 3.4). All fractions of the vegetation biomass survey were comparable (Table 3.6, Figure 3.8).

On August 7, both adults and nymph numbers rose, but with no differences between treatments (Figures 3.4 and 3.5). Alfalfa alone-high had the most adults while orchard-high again had the least. There was more alfalfa in the alfalfa-high monoculture on August 6 than in most of the treatments, except 7 (timothy-high), but this difference was not seen in the controlled portion (Figure 3.10, Table 3.6).

At the third cutting harvest date, August 13, nymph numbers in most of the treatments again declined, but adult numbers rose (Figures 3.4 and 3.5). There were nearly two times more leafhopper adults in the alfalfa high-rate monoculture than in the orchard-high treatment, but this difference was not significant (ANOVA p=0.357).

This second year alfalfa field had few weeds. On most dates, there were more weeds in the alfalfa alone treatments than in the intercropped treatments (significantly more on July 24).

Biomass generally did not differ significantly between the sprayed and unsprayed subplots. On two dates, however, contradictory differences were found. On July 30, there was more alfalfa in the sprayed subplot of the low rate monoculture than in the unsprayed (t-test p=0.037). On August 13, there was more alfalfa in the unsprayed subplot of the brome-high treatment (t-test p=0.018)

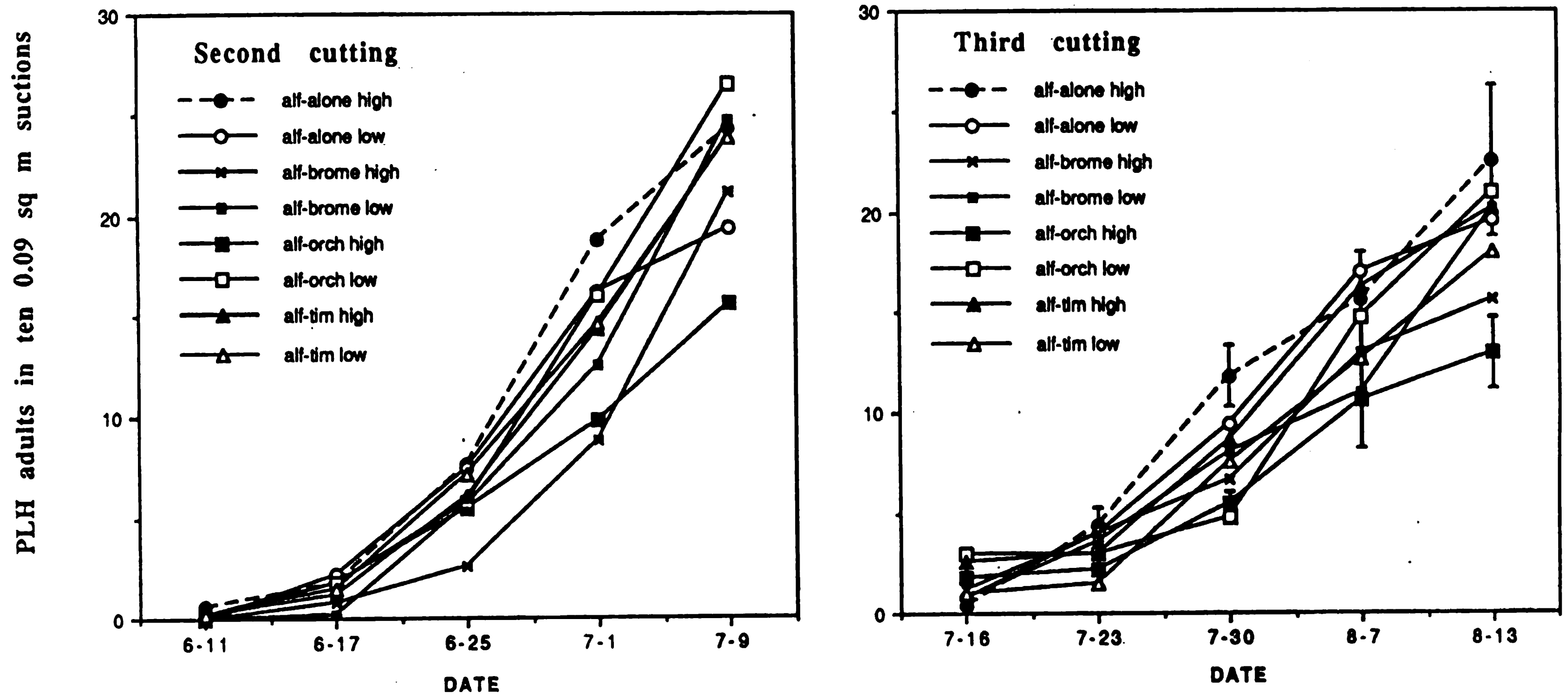


Figure 3.5. Adult potato leafhopper density in 1990 as determined by D-vac samples over two cuttings. Error bars, on the treatments with highest and lowest PLH numbers, (alfalfa alone high and alfalfa-orchard high, respectively) are standard error of the mean.

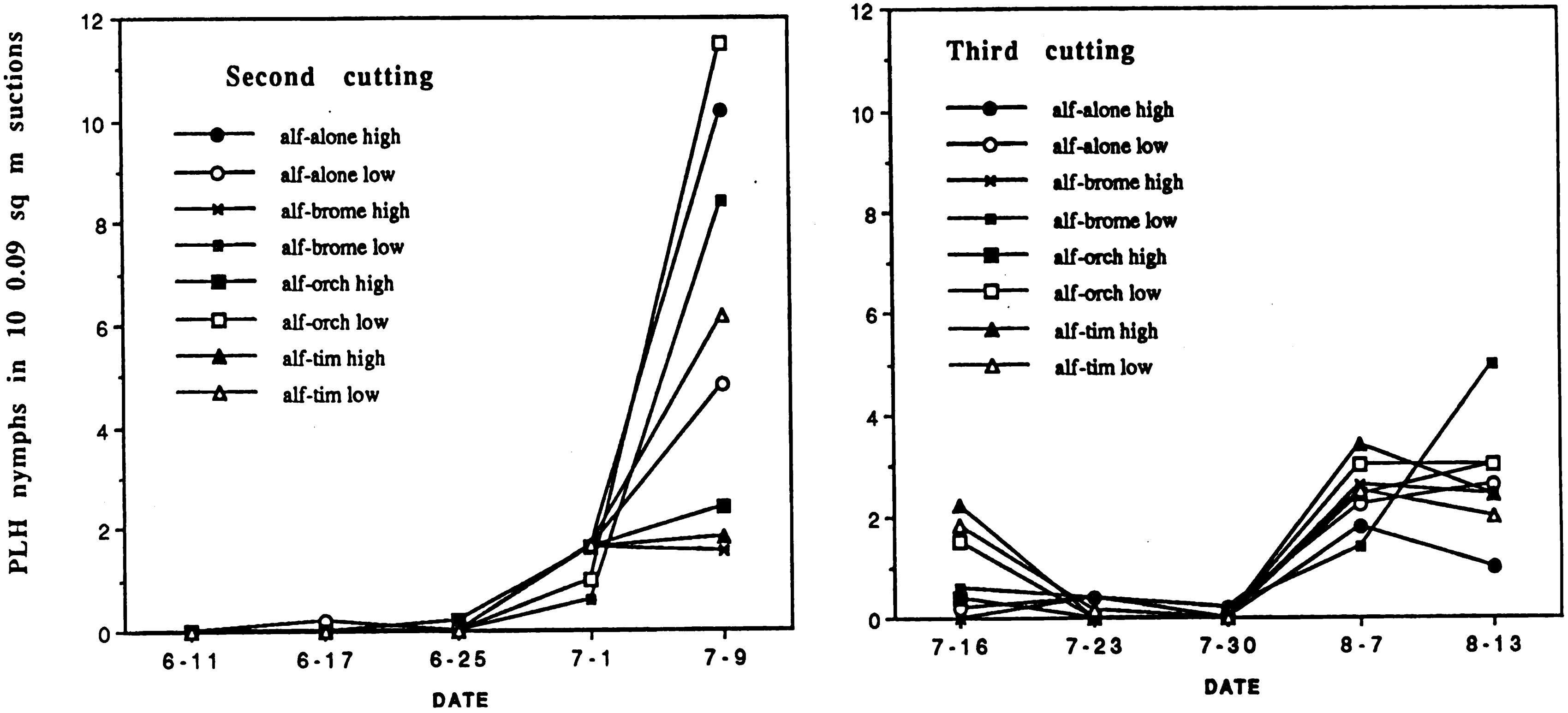
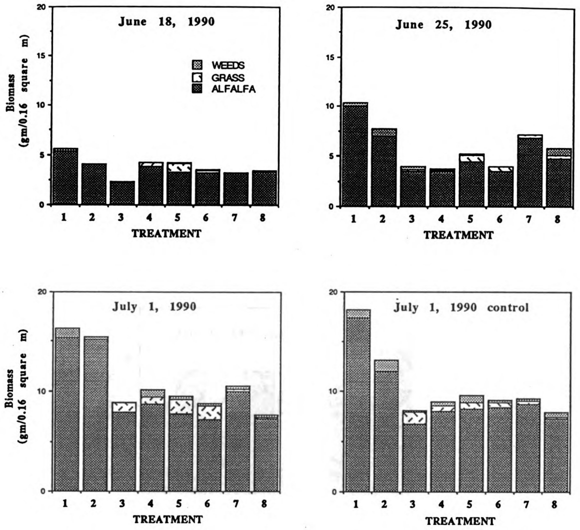


Figure 3.6. Potato leafhopper nymph density in 1990 as determined by D-vac samples over two cuttings. Only 7-9 and 7-16 contained significantly different treatments (ANOVA p=0.05 or less).

Table 3.5. Treatment contrasts of potato leafhopper numbers in pure and mixed forage stands. Dates span second and third cutting period.

	p Values		
ANOVAs and Contrasts	adult 7-1-90	nymph 7-9-90	nymph 7-16-90
ANOVA treatment effect	0.032	0.006[1]	0.002
Alfalfa high vs. alfalfa low	ns	ns	ns
Alfalfa alone (both) vs. all mixtures	0.011	ns	0.020
Alfalfa low vs. Brome high	0.011	ns	ns
Alfalfa low vs. Brome low	ns	ns	ns
Alfalfa low v. Orchard high	0.038	ns	ns
Alfalfa low v. Orchard low	ns	ns	0.041
Alfalfa low v. Timothy high	ns	ns	0.002
Alfalfa low v. Timothy low	ns	ns	0.002

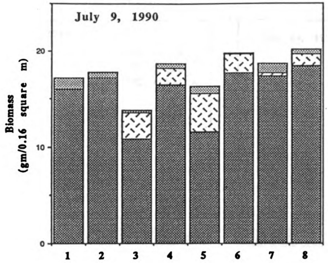
[1]For the brome and orchard mixtures, the high rate contained significantly lower numbers of potato leafhopper nymphs compared to the low rate.

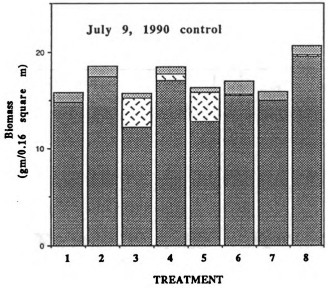


Treatment legend

1=alfalfa alone high   2=alfalfa alone low
3=alfalfa-brome high   4=alfalfa-brome low
5=alfalfa-orchard high   6=alfalfa-orchard low
7=alfalfa-timothy high   8=alfalfa-timothy low

Figure 3.7. Vegetation biomass from first three survey dates in second cutting period, 1990. Biomass equals vegetation within one 1/16th meter quadrat per replication, averaged over replications.

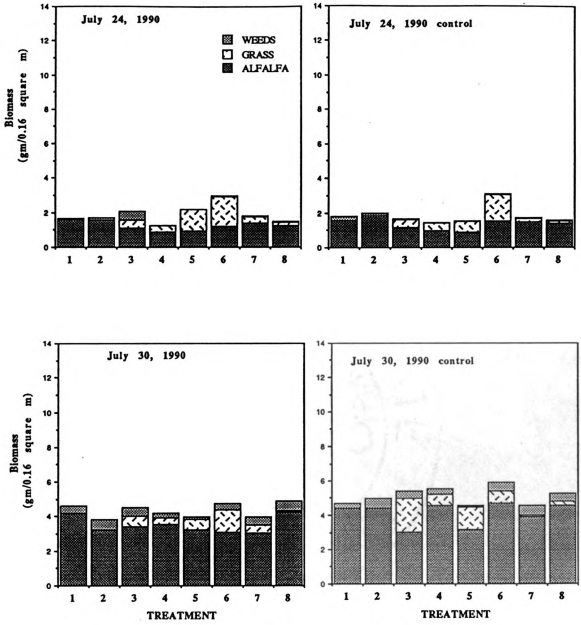




Treatment legend

1=alfalfa alone high	2=alfalfa alone low
3=alfalfa-brome high	4=alfalfa-brome low
5=alfalfa-orchard high	6=alfalfa-orchard low
7=alfalfa-timothy high	8=alfalfa-timothy low

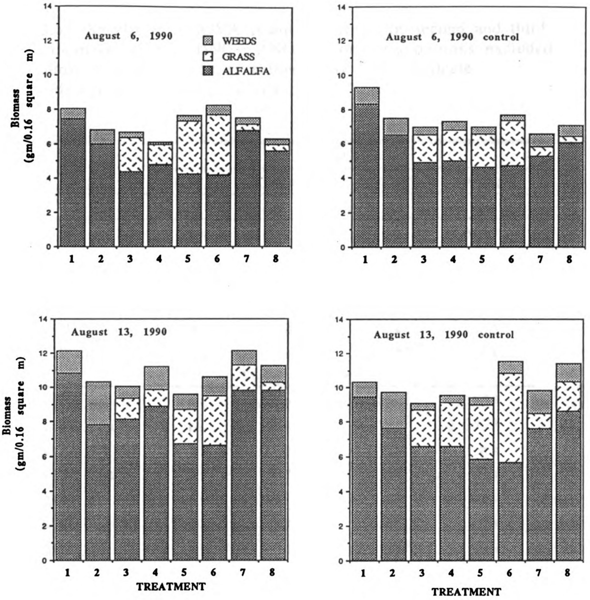
Figure 3.8. Vegetation biomass from last survey in second cutting period, 1990. Biomass equals vegetation within one 1/16th meter quadrat per replication, averaged over replications.



Treatment legend

1=alfalfa alone high	2=alfalfa alone low
3=alfalfa-brome high	4=alfalfa-brome low
5=alfalfa-orchard high	6=alfalfa-orchard low
7=alfalfa-timothy high	8=alfalfa-timothy low

Figure 3.9. Vegetation biomass from first half of third cutting period, 1990. Biomass equals vegetation within one 1/16th meter quadrat per replication, averaged over replications.



Treatment legend

1=alfalfa alone high	2=alfalfa alone low
3=alfalfa-brome high	4=alfalfa-brome low
5=alfalfa-orchard high	6=alfalfa-orchard low
7=alfalfa-timothy high	8=alfalfa-timothy low

Figure 3.10. Vegetation biomass from second half of third cutting period, 1990. Biomass equals vegetation within one 1/16th meter quadrat per replication, averaged over replications.

Table 3.6. Results of ANOVA treatment effects of second and third cutting biomass surveys, 1990. ANOVA for grass portions excluded monoculture treatments. Dates followed by "con" indicate insecticide-sprayed subplot surveys.

	p values[a]			
DATE	TOTAL	ALFALFA	GRASS	WEEDS
	Second cutting			
June 18	ns	ns	ns	0.011*
June 25	0.042*	0.021*	ns	ns
July1	0.014*	0.003**	ns	----
July 1 con	0.001***	0.001***	ns	ns
July 9	ns	ns	ns	ns
July 9 con	ns	ns	0.009**	ns
	Third cutting			
July 24	ns	ns	0.005**	0.015*
July 24 con	ns	ns	ns	0.047*
July 30	ns	ns	ns	ns
July 30 con	ns	ns	ns	ns
August 6	ns	0.006**	ns	ns
August 6 con	ns	ns	ns	ns
August 13	ns	ns	ns	ns
August 13 con	ns	ns	ns	0.011*

[a]Treatments are considered significantly different by ANOVA and contrasts if $p \leq 0.05$.

*p value ≤ 0.05
**p value ≤ 0.01
***p value ≤ 0.001

Table 3.7. Treatment contrasts of second cutting biomass surveys.

Contrasts	p values[a]						
	June 18 weeds	June 25 alfalfa	June 25 total	July 1 alfalfa	July 1 total	July 1 con alfalfa	July 1 con total
Alfalfa high vs. rest	<0.001***	0.001***	0.003**	0.002**	0.003**	<0.001***	<0.001***
Monocultures vs. rest	0.003**	0.001***	0.002**	<0.001***	<0.001***	<0.001***	<0.001***
alfalfa low vs. all mixtures	ns	ns	ns	0.001***	0.003**	0.020*	0.022*
alfalfa low vs. brome high	ns	ns	ns	0.005**	0.016*	0.023*	0.034*
alfalfa low vs. brome low	ns	ns	ns	0.011*	0.047*	ns	ns
alfalfa low vs. orchard high	ns	ns	ns	0.004**	0.028*	ns	ns
alfalfa low vs. orchard low	ns	ns	ns	0.003**	0.019*	ns	ns
alfalfa low vs. timothy high	ns	ns	ns	0.037*	ns	ns	ns
alfalfa low vs. timothy low	ns	ns	ns	0.002**	0.004**	0.028*	0.019*

[a]Treatments are considered significantly different by ANOVA and contrasts if $p \leq 0.05$.

*p value $\leq$ 0.05
**p value $\leq$ 0.01
***p value $\leq$ 0.001

Table 3.8. Contrasts of grass portions of biomass surveys in second and third cutting.

Contrasts	p values[a]	
	July 9 con grass	July 24 grass
Brome high vs. other mixtures	0.009**	ns
Orchard high vs. other mixtures	0.009**	0.026*
Brome high vs. brome low	0.026*	ns
Orchard high vs. orchard low	0.011*	ns
Timothy high vs. timothy low	ns	ns
Both brome vs. both orchard	ns	0.001***
Both orchard vs. both timothy	ns	<0.001***
Both brome vs. both timothy	0.022*	ns

Table 3.9. Treatment contrasts of third cutting biomass surveys.

Contrasts	p values[a]			
	July 24 weeds[b]	July 24 con weeds	August 6 alfalfa	August 13 con weeds
Alfalfa high vs. rest	ns	0.001***	0.003**	ns
Monocultures vs. rest	ns	0.001***	0.004**	0.004**
alfalfa low vs. all mixtures	ns	ns	ns	<0.001***
alfalfa low vs. brome high	0.017*	ns	ns	0.001***
alfalfa low vs. brome low	ns	ns	ns	0.001***
alfalfa low vs. orchard high	ns	ns	ns	0.001***
alfalfa low vs. orchard low	ns	ns	ns	0.007**
alfalfa low vs. timothy high	ns	ns	ns	ns
alfalfa low vs. timothy low	ns	ns	ns	0.003**

[a]Treatments are considered significantly different by ANOVA and contrasts if $p \leq 0.05$.

[b]Brome high vs. all other treatments $p<0.001$

*p value ≤ 0.05
**p value ≤ 0.01
***p value ≤ 0.001

Forage quality

There were no significant differences found in percent crude protein in alfalfa portions between any treatments on any date, either within insect controlled or non-controlled areas, or between them (Figure 3.11). Percent protein in alfalfa varied from about 22 percent to 26 percent. Differences in grass quality, in terms of both fiber and protein, could not be detected for second and third cuttings as replications were combined for chemical analysis. Protein varied in the grass portions of vegetation biomass samples from about 9 percent crude protein to 14 percent. No particular grass tended to be of higher quality than another. On July 9, the alfalfa from the insect controlled areas had a greater percentage of protein (numerically) than that from the non-controlled areas (Figure 3.10). On August 13, the situation was reversed, except for treatment 1, the high rate alfalfa monoculture. Protein percentages of alfalfa in non-controlled treatment 8 of August 13 were close to significantly greater (t-test $p=0.055$) than the alfalfa in the controlled area.

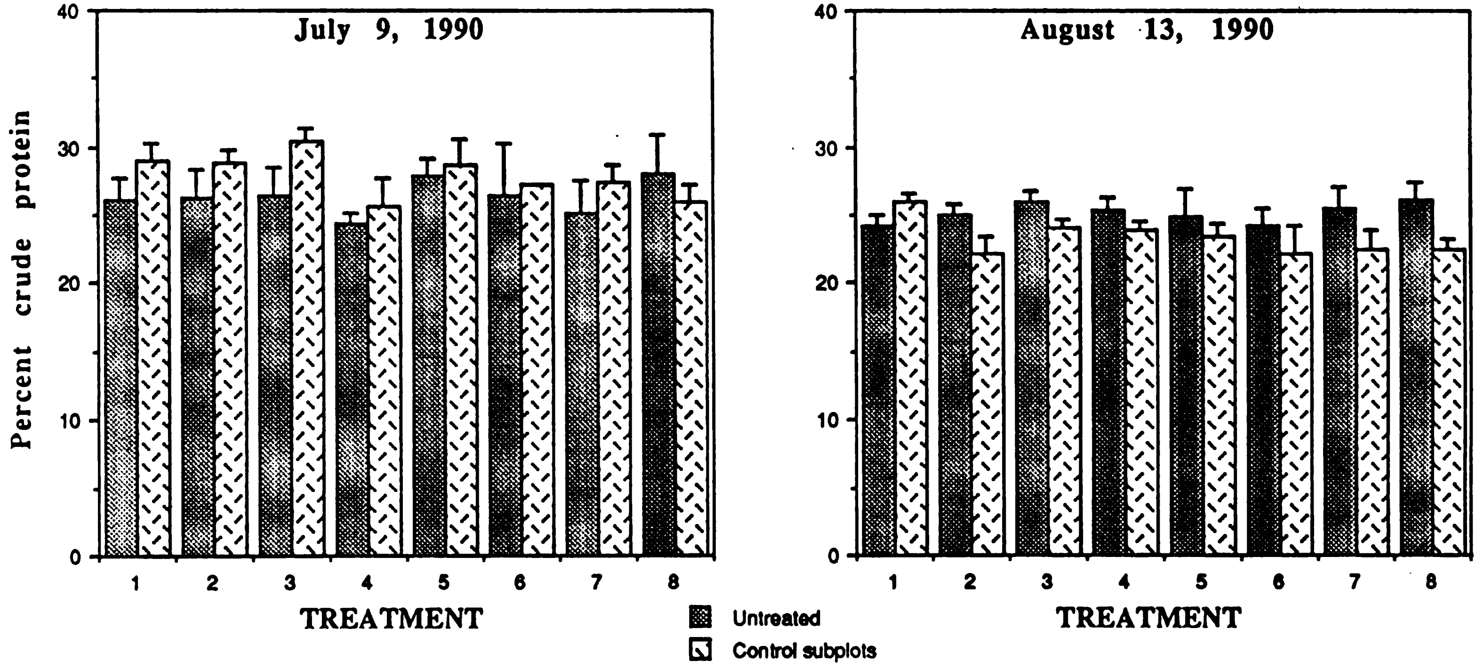
Alfalfa fiber percentages varied from 26 to 33 percent ADF and 33 to 41 percent NDF. Grass fiber percentages varied between 30 and 35 percent ADF and 54 to 64 percent NDF. Fiber percentage of alfalfa increased somewhat from second cutting to third for both sprayed and unsprayed subplots (Figure 3.12 and 3.13). There were no significant differences between any treatments on either date. On July 9, fiber percentages were not different between sprayed and unsprayed subplots also (Figure 3.12). On August 13, the ADF and NDF percentages of the alfalfa in the unsprayed subplots of treatment 1 (alfalfa-high monoculture) were significantly greater than in the sprayed subplots, t-test p=0.024 for ADF and 0.006 for NDF (Figure 3.13). This indicates lower quality in the unsprayed alfalfa, and is substantiated by slightly less protein in the unsprayed alfalfa, although here the difference is not significant (Figure 3.11).

Regression results

Regression lines for graphs of grass or biomass against leafhopper numbers showed no correlation (for July biomass dates, against July adults, r^2 tended to be below 0.2). The amount of grass or alfalfa cannot predict the number of leafhoppers in the same treatment.

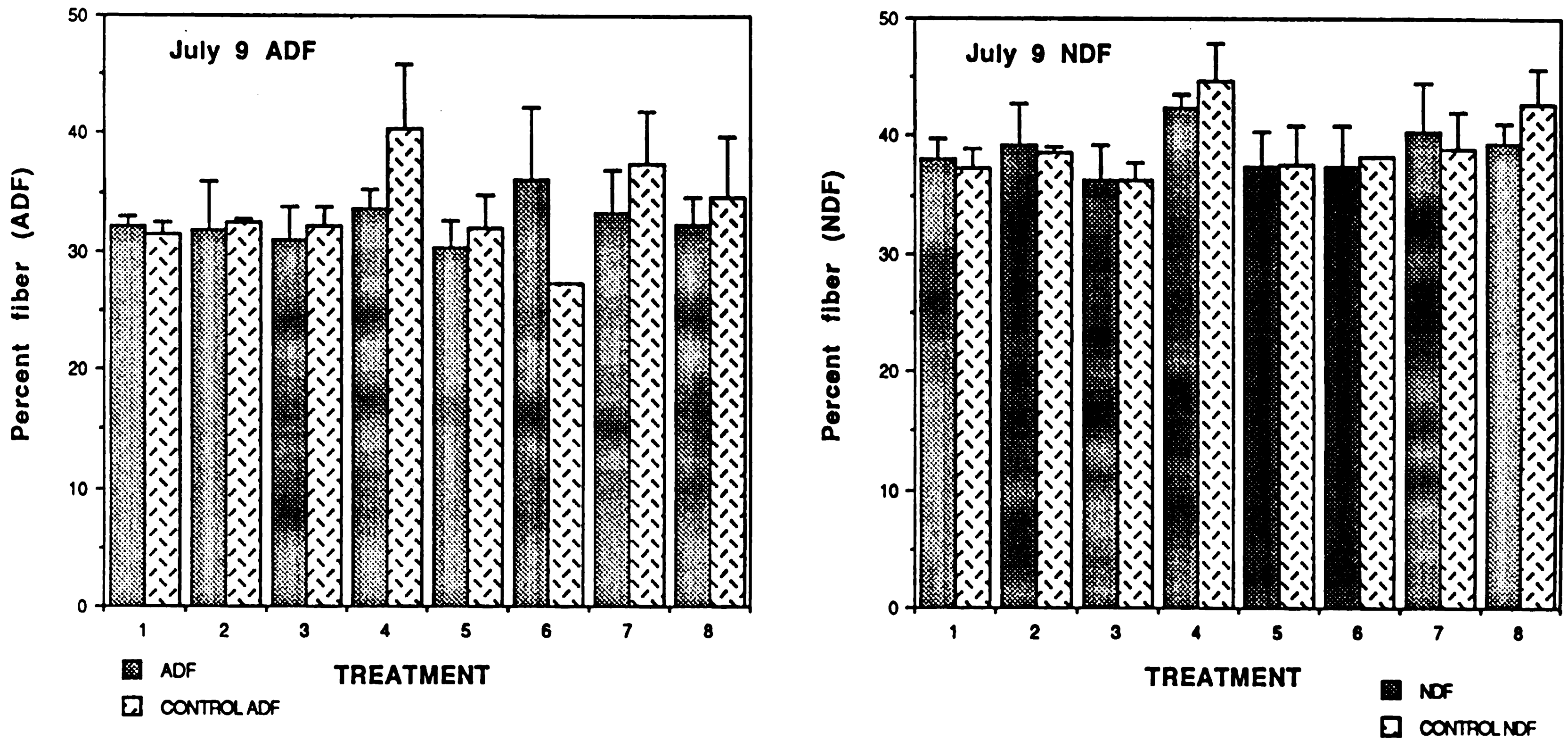
Table 3.10. Results of regressions comparing biomass against insect numbers for two sampling dates in July, 1990.

X vs. Y	r^2
7-30 PLH vs. 7-30 grass biomass	0.053
7-1 PLH vs. 7-1 grass biomass	0.006
7-30 PLH vs. 7-1 grass biomass	0.033
7-1 PLH vs. 7-1 alfalfa biomass	0.112
7-30 PLH vs. 7-1 alfalfa biomass	0.273



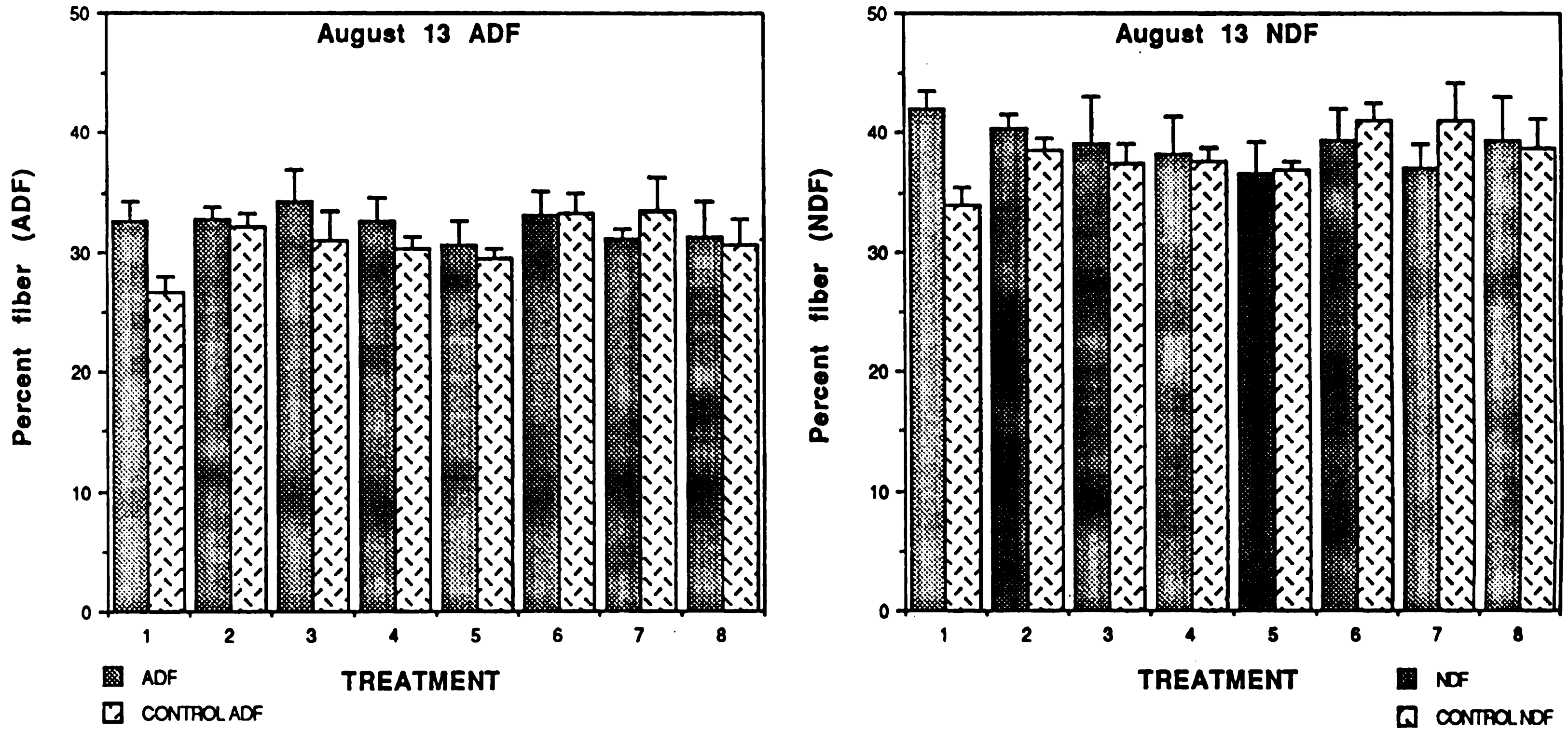
Treatment	legend
1=alfalfa alone high	2=alfalfa alone low
3=alfalfa-brome high	4=alfalfa-brome low
5=alfalfa-orchard high	6=alfalfa-orchard low
7=alfalfa-timothy high	8=alfalfa-timothy low

Figure 3.11. Crude protein of alfalfa fraction of forage at second and third cutting. Error bars are standard error of the mean.



Treatment	legend
1=alfalfa alone high	2=alfalfa alone low
3=alfalfa-brome high	4=alfalfa-brome low
5=alfalfa-orchard high	6=alfalfa-orchard low
7=alfalfa-timothy high	8=alfalfa-timothy low

Figure 3.12. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) of alfalfa fraction of forage at second cutting. Error bars are standard error of the mean.



Treatment legend

1=alfalfa alone high	2=alfalfa alone low
3=alfalfa-brome high	4=alfalfa-brome low
5=alfalfa-orchard high	6=alfalfa-orchard low
7=alfalfa-timothy high	8=alfalfa-timothy low

Figure 3.13. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) of alfalfa fraction of forage at third cutting. Error bars are standard error of the mean.

Laboratory studies

Reproduction and Survival on grass

In the preliminary experiment, potato leafhoppers were observed feeding on grass, both in monocultures and mixtures. Adults lived as long on all treatments, but no nymphs were seen in the pure grass or in the brome-alfalfa mixture (Table 3.11). One leafhopper lived on bare soil for two days, but the other leafhoppers on soil died within a day. Nymphs appeared 18 days after adult introduction in the alfalfa monoculture and orchard mixture, and two days later in the timothy mixture. Nymphs never appeared in the brome mixture or any grass monoculture.

The grass in each treatment in the preliminary test appeared fairly undamaged, except the timothy which was infested with thrips. The presence of potato leafhopper did correspond with differences in percent crude protein in the grasses between controls and treatment plants; the leafhopper-infested grass had higher percent crude protein than the uninfested (Table 3.13). There was no difference between alfalfa in mixtures or alone when in the presence of leafhoppers.

In the second test, nymphs appeared on alfalfa in 12 days, but never appeared on grass monocultures (Table 3.12). Ants and aphids infested each treatment, but even the most infested alfalfa had nymphs, and even the healthiest grass did not. Adults survived on the grass for the duration of the experiment. Because the adults were sexed before this second, replicated experiment, it verified that potato leafhoppers cannot reproduce on grass.

Table 3.11. Surviving potato leafhopper adults and emerged nymphs during three weeks of exposure of adults to alfalfa and forage grass monocultures and binary mixtures.

Treatment	Numbers		Date
	Surviving potato leafhopper	Nymphs	Nymphs first seen
alf alone	2 female, 1 male	34	1-7-90
alf & brome	0	0	
alf & timothy	2 female	14	1-10-90
alf & orchard	1 female (2nd gen)	19	1-7-90
brome	1	0	
timothy	0	0	
orchard	1	0	

Table 3.12. Replication of potato leafhopper reproduction experiment. Six adult leafhoppers, both male and female, were introduced into each of five replications of each treatment on September 11, 1990.

Treatment-all monocultures	Nymphs first seen
alfalfa	9-24-90
brome	never
orchardgrass	never

Table 3.13. Percent crude protein in plant matter after three weeks of potato leafhopper feeding (treatment) and in leafhopper-free controls.

TREATMENT	PERCENT CRUDE PROTEIN
alfalfa control	17.5
alfalfa treatment	13.8
alf-orchard grass control	10.0
alf-orchard grass treatment	10.1
alf-orchard alfalfa control	17.0
alf-orchard alfalfa treatment	13.8
alf-brome grass control	10.6
alf-brome grass treatment	11.1
alf-brome alfalfa control	16.4
alf-brome alfalfa treatment	13.7
alf-timothy grass control	14.1
alf-timothy grass treatment	8.4
alf-timothy alfalfa control	18.9
alf-timothy alfalfa treatment	12.8
orchard control	6.4
orchard treatment	8.6
brome control	8.7
brome treatment	10.4
timothy control	7.9
timothy treatment	9.7

Leaving assay

On the first replication of the leaving assay, many of the leafhoppers remaining in the cages were observed to be mating. Insects were mating in the alfalfa, and alfalfa-brome treatments, but not in soil, alfalfa-orchard, or either grass monoculture. To avoid pre-mated behavior, adults were kept in a separate cage for five days before using to assure all had been mated, and the data from the first replication were not used.

For the five remaining replications, significantly fewer leafhoppers left alfalfa monocultures than from any other treatment (contrast $p= 0.003$ when soil treatments were not included in the two way ANOVA, Figure 3.14). The numbers leaving soil tended to vary widely among replications (Table 3.14). Spiders were discovered in the cage of the soil treatment in the second replication, which had the lowest leaving rate. It is possible the leafhoppers were eaten or did not move to avoid being eaten in that replication. Averaged over all replications, more than half of the introduced leafhoppers left bare soil, often in the first minutes or hours of the test. To check how readily leafhoppers leave soil when not confined in a cage, five adults each were placed on four pots of bare soil. Leafhoppers left quickly, with only one out of twenty remaining an hour after placement.

Slightly more potato leafhoppers left orchardgrass than brome alone (Figure 3.14). More leafhoppers left orchardgrass in mixtures than brome alone and in mixtures, but these differences were not significant as determined by contrasts. Chi-square tests showed no difference in sex ratio of total "escaped" leafhoppers.

Table 3.14. Percent of total adult potato leafhoppers trapped in each treatment, by replication.

	percent					
Replication	soil	alfalfa	alf-brome	alf-orch	brome	orchard
1	70	16	33	52	32	43
2	13	20	40	53	33	66
3	60	25	15	20	55	25
4	75	25	45	50	35	40
5	45	15	50	35	60	50

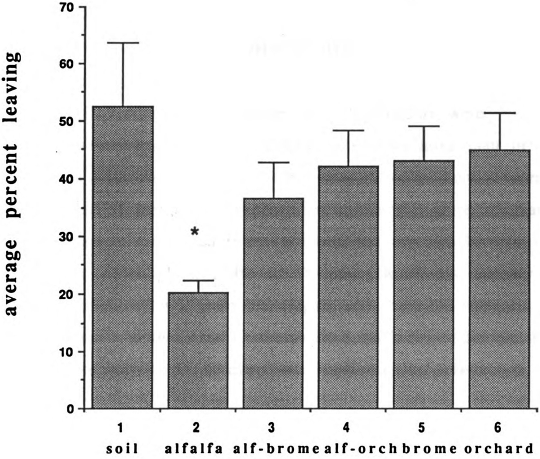


Figure 3.14. Percentage of "escaped" potato leafhoppers after three days, averaged over five replications. Error bars are standard error of the mean. Leafhoppers leaving alfalfa alone (*) were significantly less than all other treatments combined (contrasts p=0.003).

DISCUSSION

In 1989, prior to first cutting, adult leafhoppers were significantly more abundant in alfalfa-high monocultures than in all mixed plots with the exception of the orchard-low mixture (Figure 3.3). In second cutting, leafhoppers were generally more abundant in alfalfa monocultures than in mixed plots, but not significantly so (Figure 3.4). Throughout 1989, alfalfa monocultures and timothy mixtures tended to have more leafhoppers and the high rate of brome mixture had the least. Occasionally, orchard mixtures at the low rate contained about as many leafhoppers as the alfalfa monocultures.

These trends suggest fewer leafhoppers inhabit high brome and high orchard mixtures than alfalfa monocultures. Apparently, timothy and low rates of orchardgrass do not greatly affect leafhoppers. Orchardgrass grows in clumps, creating a heterogeneous plot of islands of grass in an alfalfa sea. Although grass biomass between the low and high orchardgrass rates was approximately the same throughout second and third cutting, leafhoppers tended to be more numerous in the low rate. Perhaps higher planting rates do not alter total grass biomass gathered from a plot, but creates numerous, smaller clumps. This could make the field nearly homogeneous, altering the visual or olfactory picture given by the field.

The lack of effect in timothy mixtures can also be explained by grass growth patterns. Timothy is a sod-forming grass, and grows best in cooler temperatures. It never grew above the alfalfa canopy, as did the other grasses in spring, and was essentially dormant during the summer when leafhoppers are active.

Differences in leafhopper numbers in establishing alfalfa fields may not be significant because of the vegetation allocation in these fields. Two biomass surveys were taken in 1989, but directly comparing them could be questioned as the first one (July 26) measured plant stems and the second (August 10) was a destructive sample. However, both detected greater alfalfa biomass in the monocultures than in the mixtures. Both showed far more weed biomass than either alfalfa or grass, and more alfalfa biomass than grass biomass in mixtures. Weeds could have confounded the leafhoppers' usual responses to grass.

The trends in potato leafhopper numbers continued in 1990. Again, adult potato leafhopper numbers increased as a group, with differences seen only on July 1 (Figure 3.5). The population crashed with cutting, as expected. The nymph population fluctuated differently. Nymph numbers were quite low until July 9, when they peaked (Figure 3.6). Adult populations began to grow on June 25, and eggs laid then probably hatched around July 9. Nymphs cannot fly and their location is determined mostly by oviposition site. On the one day adult numbers differed, July 1, the numbers of adults was greatest in the high-rate alfalfa monoculture, and more adults were in the low-rate alfalfa monoculture than in the high rates of brome and orchard (Table 3.5). This trend continued throughout the summer. On August 13, as an example, mean numbers of adults in the alfalfa-high monoculture were nearly two times that of the high orchardgrass treatment, but variances were too high to see significant differences between the treatments (Figure 3.6). Both timothy treatments and the low orchardgrass treatment generally had numbers of leafhoppers comparable to the low alfalfa monoculture.

The frequent biomass surveys of 1990 supported trends first seen in 1989. Alfalfa monocultures had more alfalfa than the mixtures on four dates, two of which also had significantly greater total biomass than in mixtures (Tables 3.7 and 3.9). On June 18 and July 24, monocultures were more weedy than most mixtures. Replications of weed biomass data for July 1 was inadvertently combined, making statistics impossible. In general, alfalfa monocultures produced more alfalfa than mixtures, but this did not always result in more total biomass in monocultures. Monocultures tended to have more weeds than mixtures.

Overall, the high rates of brome and orchardgrass contained more grass than the other mixtures. Brome tended to be more abundant in earlier dates, with orchardgrass producing more later. These two grasses at high rates seem to affect leafhoppers the most, as adult numbers in these treatments were generally lower than in others. The same grasses at low rates often contained the greatest amounts of leafhoppers for that date, however. Timothy treatments contained the least amount of grass and often frequently as many leafhoppers as the alfalfa monocultures.

One possible explanation for lower leafhopper numbers in high rates of brome and orchard could be the lower amounts of alfalfa in those plots. Brome at the high rate often had the least alfalfa of the mixtures. However, leafhopper numbers were not correlated with biomass of alfalfa, grass, and total biomass for any treatment or any date which had insect number differences (Table 3.10). However, the combination of plants could have created different odor plumes than pure alfalfa, as detected by colonizing insects, and may have elicited a different response by the insect. Stanton (1983) described decreasing olfactory attractiveness of hypothetical intercropped patches. Smith (1987) determined potato leafhoppers move away from weed grass macerates, even when painted on alfalfa. If forage grass odors causes leafhoppers to leave (or to avoid) intercropped alfalfa fields, a masked or altered odor plume could result in insects failing to detect alfalfa and to leave "hidden" alfalfa fields.

Mixtures did tend to have fewer leafhoppers, but this was not translated into better quality of the forage in those mixtures. It was hypothesized that if insects were deterred from intercropped plots, the quality of the alfalfa in the intercrops would be greater than in alfalfa monocultures. The data from the field do not support this. There were no differences in crude protein of alfalfa fractions of forage between treatments at second or third cutting. The alfalfa in the sprayed, insect-controlled subplots contained slightly more protein than in the non-sprayed subplot on July 9, but this was reversed on August 13 (Figure 3.11). There only difference between the sprayed and unsprayed plots was found in fiber percentages of alfalfa from alfalfa monocultures at the high rate on August 13 (Figure 3.13). Here the controlled subplot alfalfa had less fiber than the alfalfa subjected to insects. The protein in this treatment was also greater in the controlled area, but not significantly. Crude protein percentages obtained here were somewhat higher than expected (National Research Council 1982), but as all treatments are consistent, it is valid to compare treatments. Quality parameters for grass were not available for statistical analysis as replications were combined for chemical analysis.

The presence of a possibly repellent, non-food plant type in alfalfa could account for the differences in insect numbers. The laboratory experiments showed that leafhoppers cannot reproduce in grass, and adults of both sexes will attempt to leave grass monocultures more readily than from alfalfa (Tables 3.11 and 3.12, Figure 3.14). Because grasses are flat, normal egg development may not be possible even if oviposition occurs, perhaps due to desiccation of the eggs. Smith (1987) found grass weeds or grass weed volatiles reduce oocytes and eggs laid per female in alfalfa. Lamp et al. (1984) found eggs laid in grass weeds are few and generally do not survive. As leafhoppers do not prefer to remain on or lay eggs in grasses, reproduction may be hindered in alfalfa-grass mixtures due to fewer or hidden oviposition sites. No nymphs were seen in the preliminary brome-alfalfa laboratory mixture. It is not certain if bromegrass deterred oviposition or development, or if a biased sex ratio was responsible, but bromegrass seemed to provide better "protection" from potato leafhopper compared to the other grasses in regard to appearance of nymphs.

Planting grass in mixtures did not seem to increase alfalfa protein levels, either in the field or laboratory but, ironically, caged grasses with leafhoppers on them had higher crude protein percentages than the controls (Table 3.8). This was the reverse of what was expected, but perhaps the feeding of the leafhoppers removed most of the water and sugar in the plant, leaving a comparatively high percentage of protein behind (Mike Allen, Animal Science, Michigan State University, personal communication). If eggs were laid in the grass and did not develop, it was possible this material contributed to the higher protein amounts also.

Grass was not eliminated as a deterrent to potato leafhopper although improvement of alfalfa quality in caged experiments was not seen. The insects had no choice but to feed on the plants in their cages, and they fed on the same plants for three weeks. Leafhoppers reproduced, fed, and damaged alfalfa in alfalfa-grass mixtures almost at the same rate as in alfalfa monocultures. Given a choice between intercropped and monocropped alfalfa, they might have left the mixture and moved into the monocrop. The grass gathered in the field tended to have more protein than that of the laboratory experiments, suggesting the leafhoppers did not feed on it.

Apparently, grass cultivated with alfalfa will not prevent potato leafhopper damage when insects cannot leave. The reproduction in grass experiment suggests grass presence may provide inhospitable oviposition sites. Alfalfa may be hidden by grasses, visually or chemically. Grassy alfalfa stands may cause lower tenure time, or inhibit cessation of flight by migrating potato leafhopper. If leafhoppers survey the area they land in and the presence of grass incites them to continue migrating, leafhoppers placed in alfalfa-grass mixtures may attempt to leave.

The leaving assay results support this hypothesis. More leafhoppers remained in alfalfa monocultures than in any other treatment, significantly so when the soil treatment is dropped from the ANOVA. Potato leafhoppers cannot survive more than about 24 hours on moist soil. Leafhoppers in soil replications with low leaving probably died before attempting to escape.

Intercropping forage grasses with alfalfa could encourage potato leafhopper migration from mixed fields, or inhibit cessation of flight by host-seeking leafhoppers, resulting in fewer leafhoppers. Intercropped plots generally yield more biomass than monocultures. The added grass in the forage could reduce bloat and other problems associated with feeding animals forage too rich in protein. In addition, adding grass to alfalfa stands may help outcompete weeds (Drolsom and Smith 1976), prevent erosion, and increase stand duration (Casler and Walgenbach 1990).

From these tests, orchardgrass and brome appear to be the best grasses for intercropping. Future experiments should not include orchardgrass at the low rate used here, or timothy at any rate, as these treatments did not alter numbers of potato leafhoppers. To better detect significant differences between the remaining treatments, should they exist, more replications should be used to lower the variance of the treatment means.

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APPENDIX

APPENDIX 1.1

Voucher Specimen Data

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Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
<u>Empoasca fabae</u>	Mich., Ingham Co. Botany Farms, MSU E.Lansing, T4N, R2W Sec 36 10 June 1991, M.L. Coggins, Coll.					7	1		
<u>Hypera postica</u>	Mich., Ingham Co. Botany Farms, MSU E.Lansing, T4n, R2W Sec 36 10 June 1991, M.L. Coggins,Coll.					1			
	Mich., Kalamazoo Co. Kellogg Bio. Stn. Ross, T1S, R9W Sec 9 13 May 1991, M.L. Coggins, Coll.					2	4		

(Use additional sheets if necessary)

Investigator's Name(s) (typed)
Margi L. Coggins

Date 11 July 1991

Voucher No. 1991-04
Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator Date 15 July 1991