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EFFECTS OF GROWING ALFALFA WITH PERENNIAL **GRASSES UPON POTATO LEAFHOPPERS** (HOMOPTERA: CICADELLIDAE) AND ALFALFA WEEVILS (COLEOPTERA: CURCULIONIDAE)

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# EFFECTS OF GROWING ALFALFA WITH PERENNIAL GRASSES UPON POTATO LEAFHOPPERS (HOMOPTERA: CICADELLIDAE) AND ALFALFA WEEVILS (COLEOPTERA: CURCULIONIDAE)

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### A THESIS

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### ABSTRACT

## EFFECTS OF GROWING ALFALFA WITH PERENNIAL GRASSES UPON POTATO LEAFHOPPERS (HOMOPTERA: CICADELLIDAE) AND ALFALFA WEEVILS (COLEOPTERA: CURCULIONIDAE)

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Potato leafhoppers, *Empoasca fabae* (Harris), and alfalfa weevils, *Hypera postica* (Gyllenhall) can reduce the yield and quality of alfalfa forage. Intercropping forage grasses with alfalfa may decrease populations and damage of these pests. In field bioassays, significantly more leafhoppers left treatments when orchardgrass plants were interspersed among the alfalfa compared to equal densities of orchardgrass planted in a discrete patch. Observations of potato leafhoppers on stems of bromegrass, orchardgrass and alfalfa revealed that individuals fed on all three species, although the frequency and duration of probing differed between plants. Leafhoppers placed on bromegrass or orchardgrass remained longer than those placed on alfalfa. In a laboratory behavioral bioassay, leafhoppers showed *ca*. 9 fold increase in emigration from pure grass treatments and a *ca*. 5 fold increase from mixtures. Grass volatiles alone did not elicit emigration, but stimuli obtained from contact with the grass did increase emigration.

In 1995 field studies, the number of alfalfa weevil larvae, number of damaged tips and intensity of weevil feeding were reduced in alfalfa-forage grass mixtures containing either bromegrass, orchardgrass, timothy or Kentucky bluegrass in the first but not second year after establishment. These experiments indicate the potential role of intercropping a forage grass with alfalfa for both potato leafhopper and alfalfa weevil management. Dedicated with love to the memory of my father Robert Roda who laid a path with friendship, encouragement and counsel that enabled me to smile and embrace each challenge and adventure in life.

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## KEY TO SYMBOLS AND ABBREVIATIONS

.

ac	acre
ANOVA	analysis of variance
cm	centimeters
cm <sup>2</sup>	centimeters squared
d	days
df	degrees of freedom
F	Fisher distribution
ft	feet
g	grams
h	hours
ha	hectares
HSD	honestly significant difference
in	inches
kg	kilograms
L.	Linnaeus
lb	pound
lbs	pounds
m	meters
ml	milliliters
mm	millimeters
n	treatment sample size
°C	degrees centigrade
oz	ounces

- *P* probability of a type I error
- r correlation coefficient
- s seconds
- SEM standard error of the mean
- spp. species
  - x fold increase

### CHAPTER 1

# Introduction to potato leafhopper and alfalfa weevil in alfalfa production systems

Alfalfa, *Medicago sativa* (L.) is the most important forage crop in the United States. It provides protein and nutrients for livestock (Conrad and Klopfenstein 1988), nitrogen for succeeding crops and contributes to the rebuilding of soil tilth (Carlson and Newell 1985, Tiagalingam et al. 1991). In Michigan, 1994 production exceeded 4 million tons at a worth of over \$283 million (Michigan Agriculture Statistics, 1995). Insect pest populations, if left unchecked, can significantly reduce crop value. Two primary pests, the alfalfa weevil, *Hypera postica* (Gyllenhall) (Coleoptera: Curculionidae), and the potato leafhopper, *Empoasca fabae* (Harris) (Homoptera: Cicadellidae), can substantially reduce the yield and quality of the alfalfa forage (Hower and Muka 1975, Flinn et al. 1990, Nielson et al. 1990). Both pests affect root total non-structural carbohydrates causing a reduction in storage reserves; this leads to slower regrowth in subsequent cuttings, and potentially greater likelihood of winterkill (Fick and Liu 1976, Bjork and Davis 1984, Wilson and Quisenberry 1986, Godfrey and Yeargan 1989, Hutchins et al. 1990).

### <u>Alfalfa Management</u>

Alfalfa provides protein, energy, fiber and other nutrients vital to a balanced ruminant ration (Conrad and Martz 1985, Conrad and Klopfenstein 1988). As

an economical food source for dairy cattle and other livestock operations, growing alfalfa allows producers to reduce their dependency on protein supplements and feed grains (Hodgson 1974). A high-yielding stand containing quality forage lends greater efficiency and profitability to the operation and better nutrition for the animal (Dornfield et al. 1983).

Good management practices are associated with high productivity and stand persistence (Tesar and Marble 1988, Beuselinck et al. 1994). The ideal field is well drained with a soil pH ranging from 6.6-7.1 (Woodrut 1967, Tesar 1984, Barnes and Schaffer 1985). Growers use soil tests to determine if applications of lime, potassium, phosphorus, and trace minerals are necessary to promote optimal growth (Tesar et al. 1954, Sheard et al. 1971, Lanyon et al. 1983, Lanyon and Smith 1985). A careful selection of a variety (winterhardiness, disease resistance, and yield goals) seeded into a properly prepared seedbed leads to good establishment and persistence (Triplet and Tesar 1960, Tesar and Marbel 1988, Understander 1990, Hesterman et al. 1994). In Michigan, typical recommendations for alfalfa establishment include planting of 15 lb/ac of alfalfa seed planted 1/2 inches deep usually followed by a cultipacker or press wheel for good seed/soil contact (Copeland et al. 1987). Productive stands can be obtained from either spring or summer seedings (Tesar 1977). An oat, Avena sativa, companion crop is often used in spring seeded alfalfa to provide a fast growing ground cover to control erosion, reduce weed competition and offer an additional feed source through grazing, silage or cutting (Kust 1968, Buxton and Wedin 1970, Tesar 1984). Producers use cultural, mechanical and chemical measures (Integrated Pest Management or IPM) to control insects (Manglitz and Ratcliffe 1988, Lamp et al. 1991), diseases (Hart and Clayton 1986) and weeds (Peters and Linscott 1988).

Forage yield, quality and stand persistence all affect the optimal harvest time (Sheaffer et al. 1988). Harvest should begin when the plants have reached first flower (10% bloom) (Smith 1972, Sheaffer et al. 1988, Understander et al. 1994). This stage typically offers the best combination of high yield and quality (both energy and protein) and sustains adequate root reserves to promote stand persistence (Kalu and Flick 1983, Weir et al. 1960, Robinson and Massengale 1968, Winch et al. 1970). Harvesting based on plant maturity dictates when the alfalfa will be cut and the number of cuttings possible during the season (Unders) ander et al. 1994). This management strategy allows for differences in varietal maturities, yearly environmental fluctuations and locations (Shaffer et al. 1988). In the north central region four cuttings are possible although most fields are cut three times (Understander et al. 1994). The last cutting, in a 2-3 cut system, typically occurs at least six weeks ahead of the average date for the first killing frost to allow the alfalfa to enter dormancy with high levels of carbohydrate stored in the root system (Tesar 1984).

Smooth bromegrass, Bromus inermis Leyss., orchardgrass, Dactulis glomerata L., and timothy, Phleum pratens L., are commonly grown with alfalfa (Tesar 1984, Van Keuren and Matches 1988, Moline et al. 1991). Alfalfa-grass intercrops can provide hay, silage, pasture and greenchop with the added benefits of weed control (Casler and Walgenbach 1990), improved palatability (Conrad and Kloppfenstein 1988) and reduction in livestock deaths from bloat (Howarth et al. 1978). Alfalfa-grass pastures and hay can support high milk production and daily gains by sheep and cattle without supplemental feed (Van Keuren and Matches 1988). Bromegrass is highly productive when mixed with alfalfa and, unlike many grasses, does not lose much of its feed

value after the seed has formed (Moline et al. 1991). A bromegrass-alfalfa intercrop can be used in rotation with other crops. As bromegrass roots decay, they improve the organic matter content and soil structure (Carlson and Newell 1985). Orchardgrass is a bunch grass that will persist under hay production systems and grows under a wide range of environmental conditions (Jung and Baker 1985). Timothy establishes well with alfalfa and does not compete for resources as intensely as bromegrass or orchardgrass (Childers and Hanson 1985). Recommended seeding rates for forage grasses in Michigan are 3-5 lbs of smooth bromegrass, 2 lbs orchardgrass or 2-4 lbs timothy in combination with 12-16 lbs of alfalfa (Tesar 1984, Copeland et al. 1988). Alfalfa-grass fields can be established in early spring until mid-August (Copeland et al. 1988). The management of grass-alfalfa stands requires fertilization and harvesting practices which promote the growth of the grass to a level which does not out compete the alfalfa (Barnes and Shaffer 1985).

#### Potato Leafhoppers

Potato leafhoppers, *Empoasca fabae* (Harris), (Homoptera: Cicadellidae) cause substantial damage to alfalfa, soybeans, *Glycine max*, and potatoes, *Solanum tuberosum* (Smith and Poos 1931, Peterson and Granovsky 1950, Manglitz and Ratcliffe 1988). In addition to agricultural crops, they are known to utilize over 200 species of plants as hosts including clovers, weeds, grasses, ornamental plants and trees (Poos et al 1943, Medler 1957, Lamp et al. 1984, Lamp et al. 1994). Visual symptoms of potato leafhopper feeding begins as yellow, typically "v" shaped wedges of chlorotic tissue, originating from midrib and extending to the tip to the leaflet (Johnson 1936, Byers et al. 1977, Faris et al. 1981). This condition is often referred to as "hopperburn" (Ball 1919).

Reductions in dry matter (Poos and Johnson 1936, Kouskolekas and Decker 1968, Flinn et al. 1990), plant height (Nielsen et al. 1990), crude protein (Kindler et al. 1973, Hower and Muka 1975, Wilson et al. 1979, Nielsen et al. 1990), carotene (Kindler et. al. 1973), calcium and phosphorus (Smith and Medler 1959) are among the documented responses to potato leafhopper feeding. Heavily infested plots mature more slowly than uninfested plots (Oloumi-Sadeghi et al. 1988, Hutchins and Pedigo 1990) leading to losses in yields of dry matter and crude protein (Hutchins and Pedigo 1990). Alfalfa at earlier stages of growth is more susceptible to injury than older plants with similar levels of infestation (Korskolekas and Decker 1968). Potato leafhopper feeding also reduces accumulation of root carbohydrate reserves (Oloumi-Sadeghi et al. 1989) and slows the rate of regrowth following harvest. Injured alfalfa enters dormancy in a weakened condition, increasing the chance of winter kill (Wilson et al. 1979).

In the midwest, damaging infestations of potato leafhopper in alfalfa usually occur in the second and third cuttings (Lamp et al. 1989, Flinn et al. 1990). Because they cannot survive winters in the northern part of their range, potato leafhopper numbers at the time of the first cutting are rarely high enough to do damage (Decker and Cunningham 1967, Decker and Maddox 1967). Populations of potato leafhoppers overwinter in the southern pine region, extending through Texas, Louisiana, Arkansas, Mississippi, Tennessee, Alabama, Florida, Georgia, South Carolina, North Carolina, and Virginia. This pest also overwinters on exotic herbaceous legumes near the Gulf Coast (Decker and Cunningham 1967, Taylor et al. 1993, Taylor and Shields 1995). Overwintering females are in reproductive diapause until mid to late February (Taylor and Shields 1995). They shift from evergreens to

deciduous trees and legumes and start reproducing in late February to early March (Taylor and Shields 1995). Subsequent generations disperse northward on wind currents created by low-pressure areas over the Great Plains and high-pressure system over eastern US (Medler 1957, Pienkowski and Medler 1964, Carlson et al 1992). Northward movement continues until leafhoppers leave the air current, or rainfall, cooler temperatures and downdrafts terminate further flight (Pienkowski and Medler 1964, Carlson et al. 1992). This spring migration can extend E. fabae population range 1000 km into the northern states (Medler 1957, Pienkowki and Medler 1968). Immigrant females often colonizes woody plants (elm, oak, maple, hackberry, hickory, cherry, and basswood) outside an alfalfa stand (Lamp et al. 1989). These plants frequently are the primary location for maturation of the first generation of nymphs, as those nymphs located in alfalfa frequently do not complete development prior to the first harvest (Lamp et al. 1989). Primarily females make the long-distant migration northward (Glick 1960) which is reflected in the sex ratios found in early season field samples (Flinn et al. 1990). The population shifts from 80% female to a near equal ratio later in the season (Medler et al. 1966, Flinn et al. 1990).

Upon finding hosts, females soon initiate oviposition. Three to ten days after mating, an average of 2-3 eggs (1 mm long) per day are thrust into the main veins, petioles or stems (Metcalf et al. 1993). Simonet and Pienkowski (1977) found that most females (97%) placed their eggs in the primary and lateral stems, with few (3%) located in the leaf petioles; none were found in the leaves or leaf midribs. Female leafhoppers preferred succulent plant tissue for oviposition, and seldom oviposited more than 17 cm from the growing tip (Simonet and Pienkowski 1977). As the primary stem lignifies, more eggs

are placed in lateral stems (Simonet and Pienkowski 1977). Kieckhefer and Medler (1964) documented that temperature (maximum at 75 F°), photoperiod and time of day (maximum at 2000 to 0000 h) influenced oviposition. Eggs hatch in about 6-10 d, and nymphs pass through five stages before becoming adults in about 2 weeks (DeLong 1928, Davidson et al. 1987, Metcalf et al. 1993). There may be several overlapping leafhopper generations per year (DeLong 1965). Because both immature and adult leafhopper feeding injures alfalfa, substantial loss in forage quality and yield can occur quickly when all stages are present continuously (Flinn and Hower 1984, Hower and Flinn 1986).

Leafhopper adults and nymphs usually occur on the undersides of leaflets or on stems and petioles (DeLong 1928). In laboratory experiments, tethered adults preferred to settle on stems and petioles and avoided the leaves (Backus and Hunter 1989). The nymphs run sideways over the edge of the leaf when disturbed, while the adults are more apt to jump or fly away when disturbed. Measurements of local adult flight activity indicated that most movement (90%) occurs during the dark hours (Dysart 1962). Activity peaks about 30 min after sunset and accounted for approximately 50% of an entire day's flights. Approximately 85% of the leafhoppers captured in local flights were males (Dysart 1962). Male potato leafhoppers fly to bare soil and appear to take moisture along with any nutrients in the soil (Adler 1982). Nymphs move very little between plants, usually growing to adults on the plant where they hatch (Lamp et al. 1994).

Potato leafhoppers will migrate short distances in response to environmental disturbance (Poston and Pedigo 1975, Lamp et al. 1989, Flanders and Radcliffe 1989). During the summer, leafhopper numbers fluctuate widely in any given habitat (Lamp et al. 1989). When host plants become unsuitable or are removed during cutting, adults migrate to other hosts including weeds, deciduous trees or other crop species (DeLong 1965, Decker and Cunningham 1967, Lamp et al. 1989, Flanders and Radcliffe 1989). After cutting an alfalfa field, leafhopper populations rise dramatically in areas immediately adjacent to the field (Pinenkoski and Medler 1966, Lamp et al. 1989, Flinn et al. 1990). Although adults can move to non-crop hosts, increases in nymphal populations have not been observed on these plants (Lamp et al. 1989). During the summer, higher densities of leafhoppers occur on the exterior edges of alfalfa stands, most likely due to the local migration from outside the field (Kieckhefer and Medler 1966, Flinn et al. 1990). As crops begin to mature and temperatures decline, potato leafhoppers abandon their preferred hosts and move to locations providing a more optimal food source and microclimate (Decker and Cunningam 1967). Leafhopper populations in alfalfa, soybean and clover fields decrease while populations in weedy hedgerows and woodlot borders increase (Decker and Cunningham 1967). Short day length delay or preclude reproductive maturity (Taylor et al. 1995). Taylor and Reling (1986) found high numbers of leafhoppers in aerial samples taken at 150 m during late summer. Climatic conditions producing northerly winds combined with the reproductive diapause suggest a southward migration to overwintering areas (Taylor and Reling 1986, Taylor et al. 1995).

Potato leafhoppers can feed as an adults on many different plant species (Poos and Wheeler 1943, Lamp et al. 1984b). Lamp et al. (1995) found that 220 species, in 100 genera and 26 families are suitable hosts for reproduction and development; the majority of species are in the family Fabaceae. Monocotyledons, such as grasses and sedges, will not sustain the development of nymphs (Lamp et al. 1994). The development time of nymphs varies among hosts. Peterson et al. (1992) found that *E. fabae* survival was not significantly different on alfalfa, birdsfoot trefoil, *Lotus corniculatus*, and red clover, *Trifolium pratense*, however developmental time was fastest on alfalfa, intermediate on trefoil and slowest on red clover. They found leafhopper adults in field trials more often associated with hosts allowing the fastest developmental rates.

In studies on alfalfa resistance, leafhoppers exhibited clone-dependent feeding and ovipositional nonpreference (Jarvis and Kehr 1966) as well as different nymphal developmental times and survival (Newton and Barnes 1965, Elden and Elgin 1992). Anatomical features of the plant can contribute to potato leafhopper resistance. In alfalfa, clones having glandular hairs, stems with small cross-sectional areas, and highly lignified tissues were the most resistant (Brewer et al. 1986, Elden and Elgin 1992). Early lignification of tissues appeared to contribute to potato leafhopper resistance either by mechanically or by chemically deterring or preventing feeding and oviposition (Brewer et al. 1986). In studies of *Empoasca*, several resistance mechanism(s) have been postulated to influence selection of host plants including: changes in microclimate (Lamp 1981), production of volatiles (Altieri et al. 1977, van Schoonhoven et al. 1981, Smith et al. 1992) or nutritional differences (Sexena and Sexena 1974).

The process by which potato leafhopper feed and type of plant tissues they select are both involved in causing the associated quality and yield losses (Backus and Hunter 1989, Johnson 1934, Hunter and Backus 1989, Medler 1941, Nielson et al. 1990). Leafhoppers possess piercing-sucking mouth parts and obtain nutrients from extracted plant fluids (Borres et al. Backus 1985, Backus and Hunter 1989, Hutchins et al. 1990, Nielson et al. 1990). Potato leafhoppers insert their stylets into the plant (probe) and secrete a watery saliva into the host (Kabrick and Backus 1990). The saliva serves as a medium for digestive enzymes that liquefy plant cell contents and walls (Miles 1972, Hunter and Backus 1989). The saliva also can solidify, forming a semi rigid structure around the stylets and termed a stylet sheath (Smith and Poos 1931, Medler 1941, Nielson et al. 1990, Kabrick and Backus 1990). Sheath material has been observed between cells, in intercellular spaces and appears to terminate in the phloem (Smith and Poos 1931, Medler 1941, Kabrick and Backus 1990). Smith and Poos (1943) interpreted stained sections through the petioles and stems as showing that phloem was the tissue in which sustained feeding was most common. Phloem cells were found to be disorganized by physical tearing during probing and by the presence of residual inter and intracellular sheath material (Smith and Poos 1943, Karbrick and Backus 1990). These observations have lead some researchers to concluded that potato leafhoppers are predominately phloem-feeders (Johnson 1941, Smith and Poos 1943).

Hunter and Backus (1989) proposed that leafhoppers were not primarily sheath-producing phloem-feeders as described by earlier researchers. They proposed that potato leafhoppers "lacerate-and-flush" multiple cells from the mesophyll. While observing potato leafhoppers feeding on an artificial

media and semi transparent leaves, these investigators noticed a rapid movement of the stylets, whose action appeared to dissolve the medium. Using AC electronic monitoring system, wave patterns recorded from the artificial media were correlated to similar wave patterns of leafhoppers feeding on alfalfa leaves. Wave forms representing phloem ingestion were not as frequent as the "lacerate-and-flush" wave pattern.

Obstruction of the phloem appears to be the cause hopperburn (Medler 1941, Kabrick and Backus 1990, Nielson et al. 1990). Chlorosis results from an interruption of the translocation process by hypertrophied cells (Medler 1941, Nielson et al. 1990). Kabrick and Backus (1990) found that enlarged cambial cells "appeared to crush" the cells within the phloem. Potato leafhopper feeding disrupts upward movement of photosynthates in the plant and produces an accumulation of assimilates in the lower stem because of the phloem blockage (Nielson et al. 1990).

Interruption of the translocation process, leads to reduced photosynthesis as well as transpiration (Womack 1984). Fewer probes accessing the plants' vascular tissues would conceivably reduce the extent of hopperburn and subsequent losses of yield and quality.

Potato leafhoppers have several significant natural enemies. The egg parasite, *Anagrus epos* (Hymenoptera: Mymaridae), and generalist predators such as lacewing nymphs, nabids and spiders are know to feed upon adults and nymphs (Metcalf et al. 1993). Under cool, moist conditions, a naturally occurring fungal pathogen, *Zoophthora radicans* (Brefeld) (Zygomycetes: Entomophthorales), drastically reduces potato leafhopper populations

(McGuire et al. 1987). Although these biological controls can be found in Michigan alfalfa stands, potato leafhopper populations are more often controlled by early cuttings or with insecticide applications (Landis and Haas 1994).

The time of major buildup or influx of leafhoppers varies considerably from year to year (Pienkowski and Medler 1962). Growers must regularly scout fields in order to assess the need for control measures (Lamp et al. 1985). Techniques for sampling leafhopper populations and assessing economic damage are available to aid producers in making control decisions (Cherry et al. 1977, Simonet et al. 1979, Fleischer et al. 1982, Curperus et al. 1983, Onstad et al. 1984, Shields and Speckert 1989, Taylor and Shields 1995). Because leafhopper damage can be more severe on early growth stages (Koleouskolekas and Decker 1968), thresholds for potato leafhopper are often based on both leafhopper populations and height of alfalfa (Wilson 1979). In Michigan the first cutting, typically harvested in late May or early June, receives little leafhopper damage. However, new seedings and the second and third cuttings of established stands frequently sustain serious damage (Landis and Haas 1994). Potato leafhoppers should be controlled when their injury to the crop causes economic loss equal to or exceeding the cost of control (economic injury level). Harvesting the alfalfa effectively reduces potato leafhopper populations (Cuperus et al. 1986, Pienkowski and Medler 1962). With the food resources removed, the adults will abandon the field, leaving the wingless nymphs that quickly die without foliage (Simonet and Pienkowski 1979). Insecticides are applied when leafhopper populations have exceeded thresholds and the crop is not mature enough to harvest (Cuperus et al. 1986). Because the threat of leafhopper infestation remains throughout the season, fields must be monitored continuously until the final cutting.

#### Alfalfa Weevils

The alfalfa weevil, *Hypera postica* (Gryllenhall) (Coleoptera: Curculionidae), feeds only on legumes but shows a high preference for alfalfa (Titus 1910). Infested fields can experience substantial yield reductions (Liu and Flick 1975, Hinz et al. 1976, Barberet and McNew 1986) and lower forage quality (Wilson et al. 1979). Damage usually occurs during the first cutting cycle in north central United States (Manglitz and Ratcliffe 1988). Alfalfa weevil feeding on regrowth can reduce subsequent yields (Bjork and Davis 1984, Buntin and Pedigo 1986, Wilson and Quisenberry 1986) and lower alfalfa's ability to compete with weeds (Buntin 1989).

Of European or Eurasian origin, this pest was first detected in Utah in 1904 (Titus 1910). New invasions of the weevil spread from Maryland into all alfalfa-growing areas of Eastern North America (Dysart et al. 1976). In 1966, alfalfa weevil populations were found in Michigan (Dowdy 1966).

Adult weevils overwinter primarily outside of alfalfa fields (Casagrande 1971) then return to alfalfa fields in mid-April when new growth begins. After feeding for about 2 weeks, oviposition begins and continues through the spring (Manglitz and Ratcliffe 1988). Fall oviposition and winter survival of these eggs decreases from southern to northern United States. Because of the lack of fall oviposition in Michigan, there is no early spring larval feeding damage (Casagrande et al. 1973).

In 1957, a classical biological control program was initiated against the alfalfa weevil (Dysart et al. 1976). Hymenopterous parasitoids continue to reduce adult and larval weevil populations (Kinsley et al. 1993). *Microctonus aethiopoiedes* (Loan) (Hymenoptera: Braconidae), a parasite of adults, has two generations per year and overwinters as a 1st stage larvae inside the weevil (Stehr et al. 1971). *Microctonus aethiopoides* can kill from 70-90% of the overwintering weevils (Dysart et al. 1976) and renders those not killed sterile, making their control impact even more consequential (Neal et al. 1971). *Bathyplectes curcuionis* (Thomson) (Hymenoptera: Ichneumonidae) and *Bathyplectes anurus* (Thomson) (Hymenoptera: Ichneumonidae) are both endoparasites of alfalfa weevil larvae (Davidson et al. 1987). Females of these species oviposit inside the weevil larvae preferring the early instars. Areas with well-established parasitoid populations usually do not need chemical control (Kinsley et al. 1993).

A fungal pathogen, Zoophthora phytonomi (Arthur) (Zygomycetes: Entomopthoraceae) can also reduce weevil populations below economic thresholds (Puttler et al. 1978, Goh et al. 1989, Harcourt et al. 1990). With favorable moist weather, fungal epizootics often occur after first-cutting alfalfa matures when weevil populations have peaked (Goh et al. 1989).

Cutting alfalfa has been known to have a profound effect on alfalfa weevil and parasite populations (Hamilin et al. 1949). In Michigan, Casagrande et al. (1973) found cutting at 507 degree days (base 48 degree F) caused a 79% reduction in the number of alfalfa weevils produced and a 57% reduction of *B. curculionis*, while almost eliminating crop damage.

Advantages in Diversifying Agricultural Systems

Conventional agriculture contrasts drastically with natural ecosystems. Low biotic diversity is often maintained by chemical methods to insure consistent high production (Pesek et al. 1989). At times, these simple systems are susceptible to outbreaks of insect herbivores, whose populations remain low in diverse, natural communities (Pimentel 1961). The reduction of faunal richness and floral simplification in monocultures has been widely held responsible for pest problems in agriculture (Goldsmith et al. 1972).

Diversifying cropping systems, whether with two or more crops, or a single crop grown with weed species, can alter population sizes of pest communities (Risch et al. 1983, Vandermeer 1989). In many agricultural situations, plants growing in monocultures receive more injury than those growing intermingled with other plant species (Pimentel 1961). The "resource concentration hypothesis" (Root 1973) predicts that richer plant associations will cause changes in the behavior of herbivorous insects. This model predicts that an insect's ability to find a host plant and its probability of remaining in a habitat depend on: (1) the number of host species present and the herbivore's preference for each, (2) the density and spatial arrangement of each host species, and (3) interference or repellent effects from non-host plants. Lower herbivore populations may result from changes in orientation, inter-habitat movement, dispersion and reproduction (Stanton 1983). Some insects have been found to move more often in polycultures that contain non-preferred hosts than in monocultures (Risch 1980, Stanton 1983). Herbivores may tend to emigrate sooner, farther or straighter after repeated

sampling of a non-host plant (Altieri et al. 1977, Risch 1980). Reproductive behavior may also be affected, e.g. if herbivores tend to lay fewer eggs on host plants in an environment of lower resource concentration (Risch 1981)

Potato leafhopper populations are reduced in the presence of grasses within legume crops such as soybeans (Kretzschamar 1948, Hammond and Stinner 1987, Hammond and Jetters 1990), dry beans, *Phaseolus* spp., (van Schoonhoven et al. 1981, Tingey and Lamont 1988) and alfalfa (Lamp 1991, Lamp et al. 1984a, Kingsley et al 1986, Oloumi-Sadeghi et al. 1987, 1989). Lamp et al. (1984) found that herbicides used to control grasses raised leafhopper densities by 59% in first-year, spring established stands. Oloumi-Sadeghi et al. (1987, 1989) showed that alfalfa stands containing grasses reduced leafhopper densities by 40%. Companion cropping oats with alfalfa resulted in a 80% decrease in leafhopper numbers relative to pure-seeded alfalfa (Lamp 1991). Coggins (1991) found that alfalfa-bromegrass and alfalfa-orchardgrass intercrops had lower populations of leafhoppers compared to alfalfa monocultures. Lower populations have been correlated with reduced leafhopper damage (Oloumi-Sadeghi et al. 1989, Lamp 1991).

The presence of the grass in the stand may change the host plant condition, habitat structure and microenvironment or produce repelling compounds leading to the decrease in leafhopper numbers (Lamp 1991, Smith et al. 1992, 1994). Grasses can increase movement and decrease reproduction of potato leafhoppers. Greater densities of crabgrass, *Digitaria sanguinalis* (L.), per cage resulted in a corresponding decrease in oviposition and number of primary oocytes per female (Smith et al. 1992). Movement increased as crabgrass concentration increased compared to equivalent alfalfa densities (Smith et al.

1994). An oat-alfalfa intercrop had reduced nymph and adult densities which may have resulted from the reduced alfalfa biomass, stem length or the increased shading from the oats relative to alfalfa monoculture (Lamp 1991)

Several studies suggest fields containing grass weeds or intercrops suffer less alfalfa weevil damage. Fields in Summit County, Utah that contained a "considerable" amount of timothy were reported to suffer less damage (Titus 1910). Field plots heavily infested with cheat, *Bromus secalinus* (L.) and downy bromegrass, *Bromus tectorum* (L.) tended to have fewer alfalfa weevil eggs (Dowdy et al. 1992) and significantly lower populations of larvae (Berberet et al. 1987). Coggins (1991) found significantly fewer weevil larvae and less tip damage in alfalfa-grass mixtures containing orchardgrass, smooth bromegrass or timothy compared to pure-seeded alfalfa.

### Intercropping Forage Grasses with Alfalfa

Intercropping forage grasses as an integrated pest management strategy could offer a means to prevent several pest problems in an alfalfa forage production system. Previous studies indicate grassy weeds and forage grasses reduce alfalfa weevil and potato leafhopper numbers within the stand (Titus 1910, Lamp et al. 1984a, Kingsley et al. 1986, Berberet et al. 1987, Oloumi-Sadeghi et al. 1987, Oloumi-Sadeghi et al. 1989, Coggins 1991, Lamp 1991, Dowdy et al. 1992). Forage grasses also decrease weed invasion (Drolsom et al 1976, Triplett et al. 1977, Casler et al 1990) and provide comparable yields (Spandl et al. 1992). By appropriate selection and management of perennial forage grasses, the benefits may increase due to improved forage quality (Cords 1973) and palatability (Miller 1984, Heath et al. 1985) by reducing weeds and reduction in bloat (Howarth et al. 1978). Intercropping may potentially reduce the need for early cuttings and pesticides to control both weed and insect pests. This management practice could have practical application to may forage and animal production systems.

To determine the mechanisms causing the observed insect population decreases in grass-alfalfa intercrops, a series of laboratory and field bioassays were conducted on mixtures of alfalfa with smooth bromegrass, orchardgrass and timothy. Field bioassays were conducted to evaluate the influence of different stem density and spatial arrangements of a known non-preferred forage grass had on increasing movement from an alfalfa-grass mixture. Behavioral bioassays were conducted to evaluate the effectiveness of different species of forage grass in causing leafhoppers to leave a alfalfa-grass mixture and the role contact and non-contact stimuli obtained from the grasses had in eliciting emigration. Observations of individual female potato leafhoppers were made to ascertain the importance of feeding and activity patterns in explaining the reductions in populations and damage occurring in a mixed stand. Studies of established alfalfa-forage grass fields were made to determine whether the effect of grass on the number of alfalfa weevil larvae found in the field as well as the amount and intensity of weevil feeding. An understanding of pest insect behavior in alfalfa-grass intercrops may lead to appropriate varietal selection a grass that will offer long term preventative management of potato leafhoppers and alfalfa weevil.

### **CHAPTER 2**

# Forage grasses elicit adult potato leafhopper (Homoptera: Cicadellidae) emigration from alfalfa-grass mixtures

### Introduction

The potato leafhopper, *Empoasca fabae* (Harris) (Homoptera: Cicadellidae), is a serious pest of alfalfa, *Medicago sativa* L., in the midwestern U.S. (Barnes and Shaffer 1985). Infested fields may experience substantial yield loss (Faris et al. 1981, Nielson et al. 1990) as well as a marked decline in crude protein (Hower & Muka 1975), carotene (Kindler et al. 1973), digestible dry matter (Flinn et al. 1990), calcium, and phosphorus (Smith & Medler 1959). Injured plants exhibit reduced photosynthetic and transpiration rates (Womack 1984) and delayed maturation (Hutchings & Pedigo 1990). Potato leafhopper injury also depletes the plant's root non-structural carbohydrate reserves, which slows regrowth following harvest (Oloumi-Sadeghi 1988, Wilson et al. 1989) and can result in an increased incidence of winter kill (Wilson et al. 1979).

Potato leafhoppers migrate north from overwintering areas each spring, arriving in the northeast and central midwest states during May or early June (Medler 1957, Carlson et al. 1991, Taylor et al. 1995). Damage to alfalfa usually occurs only in the second and third cuttings (Decker and Cunningham 1967, Manglitz and Ratcliffe 1988). Following initial migration, adults move between habitats to locate hosts or as a consequence of habitat disruption (Lamp et al. 1989, Flinn et al. 1990).

As adults, potato leafhoppers are able to feed on over 220 plant species (Lamp et al. 1994). However, monocotyledonous plants, such as grasses and sedges, do not sustain the development of nymphs (Lamp et al. 1994). Both immature and adult leafhopper feeding injures alfalfa (Kouskolekas & Decker 1968, Hutchins et al. 1989) and populations frequently grow undetected until economic thresholds are exceeded. Producers must then bear both the cost of control, and the reductions in forage yield and quality.

The presence of grasses within legume crops such as soybeans, *Glycine max* (L.), (Kretzschamar 1948, Hammond & Stinner 1987, Hammond and Jetters 1990), dry beans (*Phaseolus* spp.) (van Schoonhoven et al. 1981, Tingey and Lamont 1988) and alfalfa (Lamp et al. 1984a, Kingsley et al. 1986, Oloumi-Sadeghi et al. 1987, 1989) have been shown to reduce potato leafhopper populations. Lamp et al. (1984) found that herbicides used to control grass weeds in alfalfa increased leafhopper densities by 59% in first-year, spring established stands. Oloumi-Sadeghi et al. (1987, 1989) recorded a 40% reduction in leafhopper densities in alfalfa stands containing grass weeds. Companion cropping oats, *Avena sativa* L., with alfalfa resulted in a 80% decrease in leafhopper numbers compared to pure-seeded alfalfa (Lamp 1991). Lower leafhopper populations have been correlated with reduced damage (Oloumi-Sadeghi et al. 1989).

The mechanism(s) causing reductions in leafhopper populations in alfalfagrass stands have not been fully explored. Grasses may be affecting potato leafhopper behavior by: changing the micro-climate (Lamp 1991), influencing the production of volatiles (Smith et al. 1992), or increasing non-host encounters.

Alfalfa and forage grass intercrops have been investigated for their potential as an IPM strategy to reduce insect damage while retaining desirable agronomic characteristics (Coggins 1991). The objective of our was to determine if the forage grasses; smooth bromegrass, *Bromus inermis Leyss.*, orchardgrass, *Dactylis glomerata* L.; and timothy, *Phleum pratense* L., reduced leafhopper populations when intercropped with alfalfa. We explored grass density and spatial arrangement as 2 factors which may affect the behavior of potato leafhoppers in mixed stands. Understanding how forage grasses affect potato leafhopper behavior will aid in the design of a preventive pest management system to reduce their damage.

### Materials and Methods

Orchardgrass density The study site was established spring 1993 on the Michigan State University Crops & Soil Science Farm (East Lansing, MI). The overall site was a large field containing 8 treatments consisting of pure alfalfa and alfalfa-grass intercrops in a randomized complete block design For this experiment, only plots containing pure-seeded alfalfa ('Apollo Supreme' at 17.78 kg/ha) and alfalfa (17.78 kg/ha) orchardgrass ('Potomac' at 1.11 kg/ha) mixtures were utilized. A representative section of each plot was selected and plants within a 0.6x0.6 m area were thinned to 180 stems in 4 ratios: 100% alfalfa (180 stems), 3:1 (120 alfalfa stems to 60 orchardgrass stems), 1:3 (60
alfalfa stems to 120 orchardgrass stems), and 100% orchardgrass (180 stems). As each stem was selected, it was carefully inspected for the presence of wild potato leafhoppers which were removed. Any weeds or other grass species occurring in the plot were also hand-removed.

A 0.6x0.6x1.2 m cage constructed of 0.8x0.4 cm pine frame overlain with lumite screen (32x32 mesh, Lumite Division of Synthetic Industries, Gainesville, GA) was placed over the prepared area (Figure 1). The top of the cage was covered with a 0.6x0.6 m piece of Plexiglas, the lower surface of which was coated with approximately 2 mm of Tangletrap<sup>®</sup> insect trap coating (The Tanglefoot Company, Grand Rapids, MI). Hardware cloth (0.5 cm<sup>2</sup> mesh) was attached approximately 8 cm below the Plexiglas, to serve as a partial barrier and resting area to reduce trivial movement of leafhoppers into the Tangletrap<sup>®</sup>. All edges were sealed with duct tape and the base surrounded with soil to prevent leafhopper escape. The enclosed plants were left undisturbed for *ca*. 8 h before initiation of the experiment.

Adult potato leafhoppers were collected from soybeans in 1993 and reared in a laboratory colony 8 generations on fava beans, *Vicia fava* L. Adults were removed from rearing cages daily and held in a mixed-sex cage containing fava bean plants for 4 d to insure mating prior to use in experiments. Ten mated leafhoppers were aspirated from mating cages and held in 1 dram screw cap vials. In early evening (1900-2100) 10 vials (100 leafhoppers) were placed in each cage on a stand 15 cm above the soil surface. Vials were oriented horizontally with each opening facing plant stems. The following morning, vials and stands were inspected for injured/dead leafhoppers which



Figure 1. Field bioassay depicting alfalfa and orchardgrass treatment.

were counted and removed. The sex ratio was determined by randomly selecting 5 vials before the trial and sexing the leafhoppers for both the 1st and 4th replication.

Cages remained in the fields for 72 h. Each morning (0700-0900), afternoon (1200-1300) and evening (1900-2000) leafhoppers entangled on the inner surface of the cage top were identified by placing a mark on the top surface of Plexiglas using a permanent marker. A different color was used for each day to facilitate counting. At the end of 72 h the Plexiglas lid was removed and trapped leafhoppers sorted by day, sexed and counted.

To become entrapped, leafhoppers had to move approximately 0.5 m above the canopy before reaching the inner surface of the top panel. We defined this movement as emigration behavior. A sample of 20 plants (10 of each type) was removed from each plot for measurement of leaf surface area (cm<sup>2</sup>, LI-COR Model LI-3000, Lambda Instruments Corporation) and biomass (dry weight).

The experiment was conducted on 4 occasions (blocked in time) starting on 23-June, 27-June, 30-June, and 5-July respectively with each block containing 2 replications of each treatment. Data were analyzed as a randomized complete block design using analysis of variance (ANOVA). Means were further separated using Tukey's HSD test with a  $P \le 0.05$  significance level. Natural logarithmic transformations of count data were made to satisfy analysis of variance assumptions. The proportion of male and female leafhoppers emigrating was determined by adjusting the number of each sex trapped in each replication by the initial sex ratio. A post-hoc comparison was made

between males and females using ANOVA on the arc sin transformed proportions. To determine if leaf area and biomass influenced leafhopper emigration, a series of post-hoc comparisons was conducted. In each case, replicate means for the parameters were used to compare leaf area and biomass within treatments by simple linear correlation or each of these to leafhopper emigration by simple linear regression (Zar 1984).

Orchardgrass spatial arrangement. Alfalfa and orchardgrass were planted in 14 cm clay pots and grown under greenhouse conditions for 4 weeks. They were then transplanted into 0.5 cm<sup>2</sup> hardware cloth flats (60x60x10 cm) lined with paper towel and grown under greenhouse conditions for an additional 3 w. Plants were fertilized weekly using 10g of 20-20-20 (Peters Professional, Milfpitas, CA) dissolved in one gallon of tap water.

Each flat contained 64 plants arranged in an 8x8 grid pattern, 4 cm apart. Three treatments were established by altering the spatial arrangement of orchardgrass (height of *ca*. 30 cm) within alfalfa (height of *ca*. 27 cm). An "alfalfa" treatment contained 64 alfalfa plants. A "distributed" treatment consisted of 48 alfalfa plants with 16 orchardgrass plants arranged as alternating plant species in every other row. A "clumped" treatment contained 48 alfalfa plants with 16 orchardgrass plants arranged in a 4x4 grid in one corner of the flat.

Flats of plants were placed into pure seeded alfalfa fields established spring 1995 at Michigan State University Crops and Soils Farm, East Lansing, MI. A clear plastic bag was laid on top of the alfalfa followed by the container of plants and the 0.6x0.6x1.2 m cages previously described (Figure 1). One

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hundred colony-reared, mated potato leafhoppers were introduced to the cages as before. The experiment was run for 72 h with the number of leafhoppers trapped recorded on the first and final day. Leafhoppers were separated by day, sexed and counted as before. Leaf-surface area was estimated using a LI-COR Model LI-3000 by sampling 5 plants of each species. Biomass of each treatment was measured as dry weight of all the separated plant species.

The experiment was arranged in a randomized block design with 3 replications blocked by location in the field. The total numbers of leafhoppers and the proportion of male and female leafhoppers emigrating were analyzed by ANOVA using Tukey's HSD means separation test with a  $P \le 0.05$  significance level. Differences in male and female emigration was determined by adjusting the number of each sex trapped in each replication by the initial ratio then comparing the arc sin transformed proportions using ANOVA.

#### Results

**Orchardgrass density.** The total number of potato leafhoppers trapped over 72 h varied significantly between different treatments (n=4, F=30.24, P=0.0001). More leafhoppers tended to emigrate as the number of orchardgrass stems increased (Figure 2). However, only the treatment with 100% orchardgrass had significantly greater levels of emigration. Sex ratios of leafhoppers placed in the experiments were female biased 67:33 for the first replication and 68:32 for the fourth replication. When adjusted for initial sex ratio, there was a significant effect of sex with more female leafhoppers than male leafhoppers emigrating (n=32, F=134.68, P=0.001).



Figure 2. Mean number of potato leafhoppers (± SEM) trapped at different ratios of alfalfa and orchardgrass in field established in 1994 at Michigan State University, East Lansing, MI.

Stem density did not reflect actual leaf area biomass because the structural complexity of alfalfa caused these ratios to be alfalfa-biased. Alfalfa-grass stem ratios of 3:1 produced a leaf area ratio of only 2:1.1 and a 2:0.5 biomass ratio. For stem ratios of 1:3, the actual leaf area ratio was 1:2 and 1:1.7 for biomass. Thus, these treatments failed to create evenly graded treatments in respect to these parameters.

Within each plant species, leaf area and biomass were positively correlated; alfalfa (F=24.2, P<0.00, r=0.78), orchardgrass (F=80.18, P<0.00, r=0.91). Between treatments, as alfalfa leaf area or biomass increased, leafhopper emigration decreased (F=26.91, P=0.00,  $r^2=0.47$ ; F=14.67, P=0.00,  $r^2=0.40$ ). The opposite was true for orchardgrass. As orchardgrass leaf area or biomass increased, leafhopper emigration also increased (F=11.93, P=0.02,  $r^2=0.28$ ; F=6.88, P=0.02,  $r^2=0.24$ ).

**Spatial arrangement of orchardgrass.** The spatial arrangement of orchardgrass significantly affected the number of potato leafhoppers leaving the treatment (n=3, F=44.48, P=0.00, Figure 3). Significantly more leafhoppers emigrated from the distributed orchardgrass plantings (67%) than either the clumped planting (51%) or pure alfalfa (35%) plots. Orchardgrass leaf-surface area did not differ significantly between the distributed and clumped plantings (n=3, F=2.94, P=0.16) nor were there differences in orchardgrass biomass (n=3, F=0.621, P=0.5132) between the 2 treatments.

In mixed treatments, transplanted orchardgrass grew more vigorously than alfalfa. Even though the planted ratio was 3 alfalfa to 1 orchardgrass stem, leaf-surface area was 0.9:1 in clumped treatments and a 1:1 in distributed



Figure 3. Mean number of potato leafhoppers (± SEM) emigrating from different spatial arrangements of alfalfa and orchardgrass.

plantings. Sex ratios were again female biased 88:12. When adjusted for initial ratio, there was no difference between the mean proportion of females and males leaving the all alfalfa (females: 31%, males: 20%) and clumped treatments (females: 47%, males: 42%). However, a significantly greater proportion of the females (71%) left the distributed treatment compared to males (33%).

#### Discussion

The relative concentration of host plants found in a habitat influences the behavior of herbivorous insects (Root 1973). In field experiments, alfalfa intercropped with smooth bromegrass or orchardgrass at high planting densities contained consistently lower numbers of potato leafhopper adults, although other treatments were more variable (Coggins 1991). The variability appeared to coincide with the amount of grass present within the field. Coggins (1991) initial field results suggested that the amount of grass present within the field and perhaps its distribution, strongly influenced whether leafhopper populations increased or decreased relative to alfalfa-alone at low seeding density.

The decrease in leafhopper populations observed by Coggins (1991) could be attributed to either immigration or emigration. The presence of these grasses may have reduced potato leafhopper numbers by reducing immigration into stand. With increasing grass densities the distribution and relative abundance of alfalfa was decreased, possibly limiting the leafhoppers' ability to locate their preferred host within the field. However, the field bioassay suggested that emigration could also have caused the decreases. In plots containing 100% orchardgrass, 80% of the leafhoppers emigrated within 72 h. With increasing the stem density of orchardgrass, emigration rates were increased by 3% and 5% but they were not significantly greater than from the alfalfa monoculture probably because actual grass leaf area and biomass were not increased proportionally.

In these stands, the growth form of orchardgrass may also have influenced potato leafhopper emigration rates. Orchardgrass is a bunch grass and as a mature plant the stems originate from a single crown. In this experiment, the field distribution of orchardgrass crowns was left unmanipulated. Within the cages of some replications the orchardgrass stems were highly clumped while in others they were more evenly distributed. This could have led to the lack of a clear treatment affect in the stem density study.

In the spatial arrangement study, distributing the grass uniformly in the stand resulted in a 16% increased in emigration compared to an equal amount of grass in a single patch. Risch (1980) reported that in polycultures containing non-preferred hosts, some herbivores move more frequently than in monocultures of preferred hosts. Non-host encounters by highly mobile leafhopper adults may explain increases in emigration. If a leafhopper lands on plant structures at random while moving through the canopy, then the frequency of non-host encounters should be proportional to their surface area in the plant community. The time leafhoppers spend on forage grasses is significantly less than on alfalfa (Chapter 3), suggesting the amount of leafhopper activity in an alfalfa-forage grass may be greater than in a monoculture. Greater leafhopper activity may have lead to the greater emigration rates. Several encounters with non-preferred hosts (e.g. orchardgrass) may lead to an increased rate of searching at ever increasing distances (Risch 1980).

One or more mechanisms may be interacting to elicit emigration by adult leafhoppers including: nutritional, allelochemical or physical effects. Adult potato leafhoppers will feed on a wide variety of plants but fewer were suitable for oviposition and nymphal survival (Lamp et al. 1984). Potato leafhoppers are unable to produce offspring on monocots (Lamp et al. 1994). Morphological and chemical factors of the grasses may limit their host utilization for reproduction. Lamp et al. (1984b) suggest this may be a function of the relatively smaller vascular bundles or the inability of their nymphs to access them. Gustatory stimuli may also be important in a females selection of a host. Cues received while probing, may indicating insufficient nutrients, deterrent compounds or toxic substances and could cause an increase in female movement.

These field studies suggest that orchardgrass may exert a stronger influence on female than male behavior. The number of female leafhoppers emigrating from the orchardgrass stem density bioassays was greater than males. When adjusted for the highly female biased sex ratio, no differences were found between the mean proportion of males and females emigrating from pure alfalfa and the clumped orchardgrass-alfalfa planting arrangement. However, the proportion of females tended to be greater than the males. Significantly greater proportion of females left when orchardgrass was interspersed among

the alfalfa. It appears that the more frequent the contacts a female leafhopper has with orchardgrass the more likely she will leave the treatment. However, the skewed sex ratios limit the definitiveness of these conclusions.

The field studies indicated that intercropping a forage grass with alfalfa could lead to at-harvest reductions in leafhopper numbers as high as 48% compared alfalfa monocultures (Coggins 1991). Intercropping a forage grasses with alfalfa may prevent leafhopper populations from exceeding economic threshold. By appropriate selection of perennial forage grasses, the benefits may increase by reducing weed invasion (Casler and Walgenbach 1990) thereby improving forage quality (Cords 1973), stand duration (Wolf and Smith 1964) and palatability (Heath et al. 1985).

Further research is needed to elucidate the mechanisms that cause increased rates of potato leafhopper emigration. These may then be used to develop assays to determine the forage species most practical to application in commercial forage and animal production systems.

## **CHAPTER 3**

# Contact-induced emigration of potato leafhoppers (Homoptera: Cicadellidae) from alfalfa-forage grass mixtures

# Introduction

Potato leafhoppers, *Empoasca fabae* (Harris), are highly mobile, polyphagus insects that can inflict severe economic damage on alfalfa (Barnes and Shaeffer 1985). E. fabae migrates annually from southern states, and colonizes alfalfa prior to second cutting in the north central United States (Medler 1957, Carlson et. al. 1991, Taylor and Shields 1995). Due to their small size and mobility, potato leafhoppers are frequently not detected in alfalfa before visual signs of injury, termed "hopperburn," have become apparent (Ball 1919, Byers et al. 1977, Faris et al. 1981). Reductions in dry matter (Hower & Flinn 1986, Hutchins & Pedigo 1989), plant height (Nielsen et al. 1990), crude protein (Kindler et al. 1973, Nielsen 1990) and carotene (Kindler et al. 1973) occur in response to potato leafhopper feeding. The process by which potato leafhopper feed and the type of plant tissues they select have been implicated in causing hopperburn and the corresponding losses in yield and quality (Backus & Hunter 1989, Johnson 1934, Hunter and Backus 1989, Medler 1941, Nielson et al. 1990). Factors which may result in fewer probes made on alfalfa would conceivably reduce the extent of injury.

Leafhopper numbers can fluctuate widely in a particular habitat (Lamp et al. 1989). When host plants become unsuitable or are removed during cutting, adults migrate to other hosts, including weeds, deciduous trees and other crop species (DeLong 1965, Decker and Cunningham 1967, Lamp et al. 1989, Flanders and Radcliffe 1989). In studies of *Empoasca*, several resistance mechanisms have been proposed to influence selection of host plants including changes in microclimate (Lamp 1981), production of volatiles (Poos 1929, Alteri et al. 1977, van Schoonhoven et al. 1981, Smith et al. 1992), and physical barriers (Johnson and Hollwells 1935, Taylor 1956). Potato leafhopper adults can survive on over 200 different plant species (Poos and Wheeler 1943, Lamp et al. 1984). The development time of nymphs varies among these hosts (Newton and Barnes 1965, Elden and Elgin 1992, Paterson et al. 1992) with monocotyledons, such as grasses and sedges, inadequate for *E. fabae* development (Lamp et al. 1994).

The presence of grasses can reduce potato leafhopper populations when present within alfalfa stands (Lamp 1981, Lamp et al. 1984a, Oloumi-Sadeghi et al. 1987, 1989). Coggins (1991) examined the potential of intercropping forage grasses with alfalfa to reduce leafhopper damage while still providing a high quality forage. In subsequent field experiments, leafhopper emigration was greater from orchardgrass stands and alfalfa intercropped with orchardgrass than from alfalfa monocultures (Chapter 2).

A knowledge of leafhopper behavior on different forage grasses may aid development of alfalfa-grass intercrops as a preventative pest management strategy. The objective of the current study was to explore the mechanism(s) leading to increased leafhopper emigration from alfalfa-forage grass

intercrops. We evaluated the influence of alfalfa-grass mixtures and plant volatiles on leafhopper emigration in laboratory bioassays. Feeding behavior and residency time of individual leafhoppers placed on alfalfa or grasses were also recorded.

#### **Materials and Methods**

Adult potato leafhoppers were collected from soybeans and reared in a laboratory colony eight generations on fava beans, *Vicia fava* L. To insure mating, adults were removed from rearing cages daily and held in mixed-sex mating cages with fava beans for 4 d prior to use in experiments.

For all experiments, alfalfa ("Big Ten"), smooth bromegrass ("VNS"), orchardgrass ("Potomac") and timothy were planted into 14 cm clay pots at a density of 50 seeds per pot using a automatic seeder (Ames Power Count Co., Brookings, SD). An alfalfa-grass "mixture" received an application of 25 alfalfa seeds and, upon a 1 cm shift of the seed disposition head, a second application of 25 grass seeds. The potting media (Baccto, Michigan Peat Co., Houston, TX, pH 5.8-6.0) was adjusted to provide an optimal pH for alfalfa (pH 6.8-7.0) by incorporating 3 g of pulverized lime into the top 3-4 cm<sup>2</sup> of soil. Plants were watered as needed and fertilized weekly with 15 g of Peters Professional 20-20-20 (Milfpitas, CA) dissolved in 3.81 of tap water. Plants were grown in a greenhouse for 6 weeks. Plants were thinned to 40 plants per pot one week prior to use. For experiments requiring a single plant, all others were cut 0.5 cm below the soil with a razor blade and discarded. Test plants were selected based on height (18-20 cm), diameter (0.2 cm for alfalfa and smooth bromegrass and 0.3 cm for orchardgrass) and general vigor (absence of brown or chlorotic leaves).

Experiments were conducted in a chamber (2 m wide x 1 m high x 0.5 m deep) constructed of white cotton sheet secured over wire shelving which provided uniform diffuse lighting to the interior of the chamber. The base of the chamber was covered with opaque black cloth (cotton/polyester blend). Two florescent light bulbs (Philips cool white, F72T12/CW), suspended 0.75 m above the plant, illuminated the chamber. A second set of lights was located 1 m away. Daylight (12 h) was simulated by having both lights lit, "dusk" (2 h) and "dawn" (2 h) were simulated with only the distant set of lights on and "night" (8 h) had no lights on. The temperature ranged from 27-28 °C and relative humidity ranged from 20-45% both reflecting surrounding laboratory and colony conditions.

Effect of forage grasses on emigration. Eight treatments were evaluated: alfalfa alone or in combination with smooth bromegrass, orchardgrass or timothy; each grass species alone; and a control with no plants (soil). The experiment was conducted as a randomized complete block design with blocks conducted over time. For each block of the experiment all treatments (plant species or combinations) were represented each in one bioassay arena randomly assigned to a location within the chamber. Emigration behavior was measured using a bioassay arena modified from Coggins (1991) and constructed out of an inverted, colorless 3 l plastic bottle enclosing the test plants (Figure 4). Each arena had one 3 cm exit hole offset 3 cm from the top to which was attached a 1 oz colorless plastic diet cup (Fill-Rite Corporation, Newark, NJ) with its base removed. Leafhopper movement (walking and flying) resulted in individuals encountering the hole and leaving the arena where they were trapped on the inner surface of a second cup affixed over the first and coated on its inner surface with Tanglefoot<sup>®</sup> insect trap coating (The



**Figure 4.** Bioassay arena used to determine emigration of *E. fabae* from alfalfa, grass, and alfalfa-grass mixtures.

Tanglefoot Company, Grand Rapids, MI). Leafhoppers found entangled on the inner surface were defined as to have emigrated. In preliminary experiments, this design allowed nearly all leafhopper to leave the arena within 24 h when no host plant was present. Conversely, very few individuals left when a preferred host plant (alfalfa) was included.

Fifteen mated leafhoppers were gently aspirated into a glass tube inserted into a hole at the base of arena. Covering the transfer tube with a black paper sheath caused leafhoppers to move into the lighted arena where they fed, rested, oviposited or left through the exit hole. The outer 1 oz cup was replaced every 2 h with a new cup for the first 12 h of the assay, a final sample was taken at 24 h. The numbers and sex of the potato leafhoppers trapped at each time were recorded. The experiment was replicated 8 times. Data were analyzed by analysis of variance (ANOVA) using Tukey's HSD test with a  $P \le$ 0.05 significance level (SYSTAT, Inc., Evanston, IL).

Effect of non-contact stimuli on emigration. A second set of experiments were conducted to determine how non-contact stimuli (odors, sight, etc.) effected potato leafhopper emigration behavior. In these tests, a barrier, made of 1 mm<sup>2</sup> white tent cotton screen supported by 0.05 cm<sup>2</sup> wire mesh, bisected the 3 l bioassay arena. The barrier allowed the exchange of volatiles (confirmed visually using smoke) and did not conceal the visual cues from the adjacent plants. Plants for these experiments were grown as before except grass and alfalfa were planted on opposite halves to separate the plant species by the barrier.

Treatments were: a positive control (alfalfa on both sides), a negative control (soil on both sides), smooth bromegrass separated from alfalfa and orchardgrass separated from alfalfa. In the first experiment, 15 leafhoppers were introduced to the side containing alfalfa to determine if volatiles produced by the grass increased leafhopper emigration. In another experiment, fifteen leafhoppers were introduced to the side containing the grasses to test if volatiles or the visual presence of alfalfa retained leafhoppers on a forage grass. Each treatment was replicated 6 times. Leafhoppers emigrating were trapped and data recorded and analyzed as described previously except natural logarithmic transformations of count data were made to satisfy analysis of variance assumptions.

**Potato leafhopper behavior on alfalfa and forage grasses.** Differences in female potato leafhopper feeding behavior were determined by observing individuals placed on single alfalfa, smooth bromegrass, or orchardgrass plants. Individual, mated females were collected from the rearing cages into 4 ml glass tubes. The potato leafhopper was anesthetized by filling the test tube with CO<sub>2</sub> for 5 s. After an additional 10 s, insects were then placed onto a flat surface. A camel-hair brush was used to transfer the anesthetized leafhopper onto the main stem or blade *ca.* 4-5 cm from the base of the plant. Observations began after a 15 min recovery period. A Bausch & Lomb dissecting microscope (10x oculars, 10 cm working distance) mounted on a cantilever stand was used to observe deployment of mouth parts, production of excreta, preening and movement on the plant. The scope was carefully moved into position so as not to touch or disturb the plant or insect and adjusted to maintain focus as the insect moved. Behaviors were recorded continuously ("all-occurrences," as defined by Martin and Bateson 1993) for 90

min or until the insect left the plant. Treatments were single plants of: alfalfa, smooth bromegrass or orchardgrass. Upon completion of the observation, the first plant was removed and leafhopper behavior on the next species (treatment) observed.

The experiment was conducted in a randomized complete block design with each of the 11 blocks of the 3 treatments occurring on separate days. Females used for each replication were randomly selected from the holding cage and placed on a predetermined, random ordering of the treatments. Four types of behaviors were recorded: probing, preening, moving and non-activity. A leafhopper was defined to be "probing" when its mouth parts were positioned perpendicular to the plant surface and inserted into the plant. Probing was analyzed as probing duration (min) and frequency (probes/90 min) as well as total time probing (min/90 min). Proportions of activity (probing, preening, and moving) and non-activity were based on the total time an individual was on the plant to adjust for insects that did not remain for the entire 90 min period. Means were analyzed by ANOVA (Abacus Concept 1989) with log (x+1) or arc sin transformations made as necessary to meet the assumptions of ANOVA. Means were separated after a significant *F* value using Tukey's HSD means separation test with a  $P \leq 0.05$  significance level (Zar 1984).

**Residency Time.** A bioassay was conducted to measure the length of time potato leafhoppers remained on alfalfa, smooth bromegrass and orchardgrass. Female leafhoppers taken from the holding cages were anesthetized and placed on plants as before. Treatments consisted of single alfalfa, smooth bromegrass or orchardgrass plants, bare soil moistened with tap water, and a stem "mimic." The stem mimic was a 12 cm x 4 mm diameter hollow glass

rod coated with green oil pigments (Windsor and Newton Cadmium Yellow, Flake Everwhite No. 2, Windsor Green, and Lamp Black) and oven-dried for 3 weeks at 100 °C to remove paint odors (Harris and Miller 1991). Each treatment was enclosed by a 3 l colorless plastic bottle coated on the inside with Tanglefoot<sup>®</sup> insect trap coating. Leafhoppers leaving a plant, stem mimic or soil were caught on the inner surface of the bottle cage. Leafhopper locations (on the plant, soil or cage) were recorded every h for 12 h and once again at 24 h. There were 10 replications of each treatment; experimental design and analysis were identical to the prior experiment.

# **Results and Discussion**

Effect of forage grasses on emigration. Leafhopper emigration was significantly affected by treatment (F=22.29; df=7, P<0.00). An average of 97% of the leafhoppers left the soil-alone within 24 h (Figure 5). Mean numbers emigrating from pure-seeded bromegrass and orchardgrass were not significantly different than the soil-alone treatment, indicating unacceptability of these two grasses. Mixtures of alfalfa with orchardgrass or bromegrass resulted in intermediate numbers leaving (52-53%) as did timothy alone (61%). Alfalfa alone had the lowest mean emigration with only 1% leaving in 24 h. The alfalfa-timothy mixture had a mean of 29% emigration, this was not significantly different from the alfalfa monoculture.

Plant composition within a habitat is known to influence the behavior of herbivorous insects (Root 1973). Some herbivores move more frequently in polycultures that contain non-preferred hosts than in monocultures (Risch 1980). Intercropping these forage grass species with alfalfa increased the



Figure 5. Mean number of adult potato leafhoppers ( $\pm$  SEM) emigrating from soil alone, pure grass or alfalfa and 1:1 grass/alfalfa mixtures. Treatment effect significant by ANOVA (*F*=22.39; df=7; *P*<0.00); means separated by Tukey's HSD at P $\leq$ 0.05.

movement of leafhoppers in the bioassay arenas and resulted in increased leaving rates, reproducing results from field trials with alfalfa-grass intercrops.

Effect of non-contact stimuli on emigration. Volatiles produced by the grasses did not cause leafhopper emigration from smooth bromegrass and orchardgrass. In this test, the highest emigration (98%) occurred when leafhoppers were introduced to soil with no adjacent plants (Figure 6A). Potato leafhopper emigration from alfalfa was low regardless of adjacent plant specie. Emigration from orchardgrass (29%) and smooth bromegrass (31%) was significantly less than emigration from, the negative control, but not significantly more than from alfalfa (29%). Thus, grass volatiles alone did not induce leafhopper emigration. Our result varies from the suggestion of Smith et al. (1994) that olfactory cues from crabgrass lowered *E. fabae* residence time on alfalfa adjacent to grass. Although, the role of visual cues on potato leafhopper emigration behavior was not explicitly tested, our experiments suggest that the sight of the forage grasses did not increase movement off the alfalfa plants.

Similarly, whole plant volatiles produced by alfalfa or its visual presence did not arrest or prevent potato leafhopper movement from non-preferred hosts. Again, the negative control showed the highest level of emigration (99%) which was not significantly different than emigration from orchardgrass (88%) or bromegrass (85%) adjacent to alfalfa (Figure 6B). In contrast, leafhoppers placed on the positive control emigrated at significantly lower levels (22%) than those placed on either grass species or soil. In our experiments, physical contact with a grass induced emigration while odors



**Figure 6.** Mean number of adult leafhoppers ( $\pm$  SEM) emigrating from divided arenas: (A) leafhoppers placed on alfalfa (F= 37.5; df=3, P<0.00), (B) potato leafhoppers placed on grass (F= 29.3; df=3; P<0.00) means separated by Tukey's HSD at P≤0.05.

from close proximity to a grass species did not. Over 59% more potato leafhoppers left the arena when they physically encountered orchardgrass and over 54% more emigrated when in contact with bromegrass compared to leafhoppers placed on alfalfa adjacent to each grass. While olfactory stimuli from non-host plants can in some cases repel or deter specialist insects (Tahvanainen and Root 1972), such cues may have variable effects on polyphagus insects (Andow 1991).

Potato leafhopper behavior on alfalfa and forage grasses. Potato leafhoppers fed on each of the plants offered (alfalfa, smooth bromegrass and orchardgrass). Probing comprised over 91% of a female leafhoppers dynamic behaviors on a plant (Table 1). Other active behaviors of preening and walking constituted less than 9% of their activity. All leafhoppers observed probing plants produced excreta, suggesting ingestion. Although probing comprised the greatest proportion of activity, leafhoppers were motionless for most of their tenure on the plant (Table 1). Leafhoppers on smooth bromegrass were inactive 77% of the time, probing only 21% of their time on the plant. Potato leafhoppers placed on orchardgrass were inactive 53% of the time but probed 50% of their time on the plant. On alfalfa, leafhoppers were inactive 64% and probed 38% of the time.

Although the frequency of probes females made did not differ between plant species (F=1.83; df=2; P=0.18, Figure 7A), the total time spent probing (F=5.20; df=2; P=0.011, Figure 7B) and the duration of a single probe (F=6.34; df=2; P=0.005. Figure 7C) varied significantly across plants. Mean probing duration (total in 90 min) was longer on orchardgrass ( $42.1 \pm 5.6$  min) then on alfalfa ( $34.0 \pm 5.3$  min). On smooth bromegrass, leafhoppers probed on average only

Table 1: Mean proportion of time female potato leafhoppers spent probing, preening, moving or inactive from observations of individual leafhoppers on single plants.

	Prob	ing <sup>a</sup>	Preer	uing <sup>a</sup>	Walk	cing <sup>a</sup>	Inactive <sup>a</sup>
Treatment	Proportion total time <sup>b</sup>	% of active behavior <sup>c</sup>	Proportion total time <sup>b</sup>	% of active behavior <sup>c</sup>	Proportion total time <sup>b</sup>	% of active behavior <sup>c</sup>	Proportion total time <sup>b</sup>
Alfalfa	0.38ab	92.0	0.03a	7.3	0.003a	0.73	0.64ab
Bromegrass	<b>0.21a</b>	90.5	0.02 <b>a</b>	8.6	0.002a	0.86	0.77b
Orchardgrass	0.50b	95.6	0.02a	3.8	0.003a	0.57	0.53a

<sup>4</sup>Mean proportions followed by the same letter are not significantly different (P>0.05), Tukey's HSD. <sup>b</sup>Mean calculated by dividing total time of active behavior by total minutes on the plant.

<sup>c</sup>Proportion of time in particular behavior divided by total of (probing + preening + walking)x 100.



**Figure 7.** (A) The frequency of probes ( $\pm$  SEM) by female leafhoppers (F=1.83; df=2; P=0.18), (B) the total minutes ( $\pm$  SEM) females probed (F=5.20; df=2; P=0.011) and (C) the length of time ( $\pm$  SEM) females sustained a single probe on smooth bromegrass, orchardgrass and alfalfa (F=6.34; df=2; P=0.005) means separated by Tukey's HSD at  $P_{\leq}0.05$ .

17.8  $\pm$  5.4 min, a significantly shorter duration than those placed on orchardgrass or alfalfa. An individual probe on orchardgrass lasted 12.8  $\pm$  3.2 min. By contrast, probes on alfalfa and smooth bromegrass grass lasted only 4.2  $\pm$  1.0 and 3.5  $\pm$  1.0 min respectively. This combined with the observed production of excreta suggest the grasses served as a food or water source or both.

**Residency Time**. The average length of time potato leafhoppers remained on a plant varied significantly (F=6.57; df=4, P<0.000). They remained the longest on alfalfa (x =10.7 ± 0.9 h, Figure 8) followed by bromegrass (x = 7.5 ± 1.4 h) and orchardgrass (x = 5.9 ± 1.3 h). Residency time on the two grasses did not differ statistically, however time on orchardgrass was significantly less than on alfalfa. Leafhoppers were observed actively probing the forage grasses; patterns of probing, walking and preening were similar as described above.

Leafhoppers also remained for appreciable periods on bare soil ( $x = 3.0 \pm 0.8$  h) and the stem mimic ( $x = 5.3 \pm 1.0$  h) despite the lack of food resources (Figure 5). On both of these treatments, leafhoppers probed the soil. Those placed on the mimic occasionally walked off, probed the soil then returned. With the added feature of a vertical, green "stem" leafhoppers remained 2.3 h longer than in its absence. Indiscriminate probing of host and non-hosts has previously been documented with other *Empoasca* species. In laboratory experiments, Sexena and Sexena (1974) found that *E. devastans* Distant probed a variety of surfaces regardless (e.g. glass, muslin, parfilm), suggesting that a chemical stimulus was not necessary to induce a probe. Sexena et al. (1974) found that *E. devastans* and *E. kerri motti* Phuthi probed host and non-host



Figure 8. Residency time of female leafhoppers ( $\pm$  SEM) placed on single alfalfa, smooth brome and orchardgrass plants, a stem mimic and soil(F=6.57; df=4, P<0.000) means separated by Tukey's HSD at P $\leq$ 0.05.

plants with equal frequency. In their study, these insects did not discriminate or show preferences for any of the test plants. However, they ingested different quantities of food from each of the species.

In alfalfa-grass intercrops, long residency times and probing on forage grasses may divert a substantial portion to the leafhoppers feeding from alfalfa. Studies of potato leafhopper behavior on crabgrass, Digtaria sanguinalis (L.), showed a similar residency pattern (Smith et al. 1992, 1994). Leafhoppers were observed "resting" on the grass before increases in movement (measured by flights per minute) occurred (Smith et al. 1994). Roltsch and Gage (1991) found that potato leafhoppers spent long periods on tomato plants resulted in less feeding (measured by amounts of honeydew collected) in cages containing bean and tomato leaves compared with those containing only bean leaves. Although leafhoppers will probe a forage grass for extended periods, they left the grasses sooner than alfalfa. In other studies with *Empoasca*, increased activity occurred in the presence of preferred hosts as densities of nonpreferred hosts increase (Alteri et al. 1977, Smith 1992, 1994, Roltsch and Gage 1991). Smith et al. (1994) found that potato leafhopper activity in the presence of crabgrass was 2-4x greater than alfalfa alone under equivalent vegetation density. Measures of activity increased as crabgrass concentration increased compared to equivalent alfalfa densities.

Increased movement off of forage grasses may occur due to a female's ability to discriminate hosts that can best support nymphal development. While adult potato leafhoppers can survive on monocots, they are not known to produce nymphs thereon (Lamp et al. 1994). Information about nutritional quality of a host, obtained when feeding on a monocot, may contribute to female leafhoppers acceptance of hosts for oviposition. In laboratory experiments with *E. devastans* and *E. kerri motti*, plants varied in nutritive values and suitability for the growth, survival, egg-production and oviposition (Sexena and Sexena 1974). Studies of potato leafhopper development on birdsfoot trefoil (*Lotus corniculatus*) and red clover (*Trifolium pratense*) suggest that potato leafhoppers discriminated against plants that support lower rates of nymphal development (Peterson et al. 1992). Resistant plant varieties yielded poor nymphal development and survival, are less acceptable for feeding and oviposition (Jarvis and Kehr 1966, Newton and Barnes 1965, Tingey 1985, Elden and Elgin 1992). In studies of Smith et al. (1992) potato leafhoppers preferred to oviposit on and reside on pure alfalfa over alfalfa mixed with crabgrass.

Inability to access acceptable plant tissues may also have caused potato leafhoppers to leave the forage grasses. Trichomes present on smooth bromegrass in our study appeared to hinder potato leafhopper probing. Pubescence is the best known characteristic associated with plant resistance to leafhoppers (Poos 1929, Johnson and Hollwell 1935, Taylor 1956). It is believed to create a mechanical barrier, limiting access to preferred feeding sites as well as hindering locomotion and attachment to the host plant (Tingey 1985). Taylor (1956) observed that the amount of hopperburn on alfalfa was inversely correlated with density of trichomes.

The morphology of the grass blade may also be a factor reducing the time leafhoppers spent on grasses. Smith and Poos (1931) reported potato leafhoppers made "exploratory" punctures in the vicinity of the vascular bundles of the leaflet's main and lateral veins and sometimes pierced the

epidermis on the opposite side of the leaf. We observed probes lasting 3-5 min in which the leafhoppers stylets completely penetrated a grass blade and emerged on the other side. Leafhoppers making several such probes on blades without ingestion may depart.

Our studies indicate that leafhopper feeding behavior and residence time are different on a grass versus alfalfa. While leafhoppers probed for long periods on the grasses they also left the grasses earlier than did individuals on alfalfa. Combined with prior laboratory bioassays and field experiments (Coggins 1991), we propose a model explaining observed reductions in potato leafhopper density and damage in alfalfa-grass intercrops. As leafhoppers alight within a stand, contact with a given plant is contingent upon its leaf surface area and distribution. Leafhoppers contact a given plant then probe to determine suitability. Contact with a forage grass results in several hours of feeding followed by movement to another plant. We propose that repeated encounters with a grass not only diverts feeding from alfalfa but also leads to increased movement within the stand, some proportion of which results in emigration from the field. Thus, increasing the frequency of contact with a forage grass leads to lower levels of feeding on alfalfa and higher levels of emigration, reducing potato leafhopper feeding injury to alfalfa and recruitment.

Knowledge of these behaviors can have practical application to forage production systems. Management of potato leafhoppers in alfalfa production depends primarily on the therapeutic practices of cutting and pesticide application (Pienkowski and Medler 1962, Manglitz and Ratcliffe 1988). Routine inspection and early detection are essential to determine need for

intervention (Curperus et al. 1983, Lamp et al. 1985). However, leafhopper populations often grow undetected until economic thresholds are surpassed. Intercropping orchardgrass or smooth bromegrass with alfalfa may reduce the need for chemical or mechanical control of *E. fabae*. However, further research is needed into how repeated contacts with a forage grass affects movement within the field and whether increased activity leads to overall emigration from the field. Field studies are still needed to determine which grass species cause the greatest reduction in potato leafhopper numbers and injury.

#### **CHAPTER 4**

# Forage grasses decrease alfalfa weevil (Coleoptera: Curculionidae) damage and larval numbers in alfalfa-grass intercrops.<sup>1</sup>

## Introduction

Alfalfa weevil, *Hypera postica* (Gyllenhal) is a serious early-season pest of alfalfa, *Medicago sativa* L., throughout most of the United States east of the Rocky Mountains. Heavily infested fields can experience substantial yield reductions (Liu and Flick 1975, Hintz et al. 1976, Berberet and McNew 1986) and lower forage quality (Wilson et al. 1979). Alfalfa weevil feeding on regrowth can reduce subsequent yields (Bjork and Davis 1984, Buntin and Pedigo 1986, Wilson and Quisenberry 1986) and hinders alfalfa's ability to compete with weeds (Buntin 1989).

Integrated pest management (IPM) techniques including biological, cultural and chemical controls are typically recommended to manage alfalfa weevil populations (Landis and Haas 1990). Natural enemies of both adults and

<sup>&</sup>lt;sup>1</sup>1990 data collected by Margi Coggins reported in Potato Leafhopper (*Empoasca fabae*) and Alfalfa Weevil (*Hypera postica*) Density and Damage in Binary Mixtures of Alfalfa and Forage Grasses. 1991. Michigan State University Masters Thesis. 1995 data collected by A. Roda funded by a USDA NCS-3 IPM grant to O. Hesterman, D. Landis and J. Kells. This chapter was accepted for publication to Journal of Economic Entomology 10-95 with authorship as follows: A.L. Roda, D.A. Landis, M.L. Coggins, E.S. Spandl and O.B. Hesterman.

larvae (Kinsley et al. 1993) in combination with a timely cutting schedule (Hamlin et al. 1949, Casagrande and Stehr 1973, Onstad and Shoemaker 1984, Harper et al. 1990) can minimize damage and the need for insecticides. Intercropping forage grasses with alfalfa may offer growers other IPM options for effective and economical control of alfalfa weevils (Coggins 1991).

Alfalfa weevil feeds only on legumes but prefers alfalfa (Titus 1910). Mixed stands may not provide a suitable or preferred environment. Titus (1910) noticed that alfalfa and timothy, *Phleum pratense* (L.) intercrop fields in Summit County, Utah had less alfalfa weevil damage than in pure alfalfa fields. Increases in numbers of alfalfa weevil larvae and eggs occurred in alfalfa stands where broadleaf weeds (Wolfson and Yeargan 1983) and grass weeds (Norris et al. 1984, Berberet et al. 1987, Dowdy et al 1992) were controlled with herbicides. Dowdy et al. (1992) found that stands treated with herbicides tended to have more alfalfa weevil eggs than those containing grass weeds, predominantly cheat, Bromus secalinus (L.) and downy bromegrass, Bromus tectorum (L.) weeds. They stated that these two grass species were not suitable for oviposition. Norris et al. (1984) showed that herbicidal removal of winter annual weeds typically increased the number of larvae of the Egyptian alfalfa weevil, Hypera brunneipenis (Bohman) larval population by a factor of about 1.2 - 1.5 in California alfalfa fields. Berberet et al. (1987) also found that unsprayed plots heavily infested with grassy weeds (cheat and downy bromegrass) had significantly lower populations of alfalfa weevil larvae. These studies indicate that alfalfa weevil damage is lower in fields with grass weeds, but the effects of intercropped forage grasses on this pest had not been rigorously tested.

After feeding on alfalfa tips and leaves in the early spring, full-grown larvae spin cocoons, pupate and emerge as adult in two to three weeks (Titus 1910). In North Central Region of the United States, most of these adults migrate to sheltered areas to overwinter (Metcalf and Metcalf 1993). In spring, they reach sexual maturity, returning to the alfalfa fields to oviposit. Intercropping grasses may interrupt the life cycle of the weevil at four important junctions: feeding, recolonization, oviposition, and larval development. Forage grasses within the stand might alter the number of eggs, larvae or recolonizing adults found in the stand, and change the amount or intensity of feeding damage.

In this study, the effects of inter-seeding alfalfa with four common, coolseason forage grasses, smooth bromegrass, *Bromus inermis* (Leyss.), orchardgrass, *Dactylis glomerata* (L.), timothy and Kentucky bluegrass, *Poa pratensis* (L.) on alfalfa weevil larval density and damage were investigated in 1990 and 1995. If intercropping these grasses reduces alfalfa weevil larval populations and damage, this technique could reduce pesticide usage and the need for premature cuttings thereby lowering the cost of producing forage. Intercropping a forage grass may serve as a long term preventative strategy to manage pest populations, offering an alternative to conventional reactive measures.

#### Materials and Methods

Field studies were initiated in 1990 (Coggins 1991). Due to low weevil populations from 1991-1994 this study was not repeated. However, in 1995 an unusually large weevil population was found in experimental plots
established to investigate the impacts of alfalfa-forage grass mixtures on weed infestations. Similar tests were conducted in these fields to substantiate the results of the 1990 study.

**1990 Field Study.** Alfalfa (cultivar 'Big Ten'), alone or with orchardgrass (cultivar 'Potomac'), timothy, or smooth bromegrass ('VNS') were established spring 1989 at Michigan State University's Kellogg Biological Station, Hickory Corners, MI. The following treatments were arranged in a randomized complete block design with five replications: alfalfa 18 kg/ha(high density), alfalfa 14.6 (low density), alfalfa 14.6 kg/ha + smooth bromegrass 5.6 kg/ha(high density), alfalfa 14.6 kg/ha + smooth bromegrass 2.8 kg/ha (low density), alfalfa 14.6 kg/ha + orchardgrass 1.1 kg/ha(high density), alfalfa 14.6 kg/ha + orchardgrass 0.6 kg/ha (low density), alfalfa 14.6 kg/ha + timothy 4.5 kg/ha(high density) and timothy 2.2 kg/ha (low density). Individual plot size was 9.88 m by 12.16 m. The two alfalfa seeding rates were selected following recommendations given for conventional pure-seedings (18 kg/ha) and for alfalfa-grass intercrops (14.6 kg/ha) (Copeland et al. 1992). Outer field edges and areas between blocks were planted to 6.1 m strips of alfalfa (14.6 kg/ha). Post-emergence herbicides were used as needed to control broadleaf weeds (2,4-D) in all treatments and to remove weed grasses (sethoxydin) from alfalfa monocultures. The field was managed in a threecut system.

From 2 May 1990 until 6 June 1990, alfalfa weevil density was estimated using sweep sampling (10 sweeps per plot). Sweeps were taken using the "pendulum" sweep technique with a 37 cm diameter net. Both adults and larvae were counted, however adult numbers were too low to analyze

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statistically. Alfalfa weevil larval damage was assessed on 6 June 1990, the day prior to cutting. Twenty alfalfa tips were randomly selected in each plot, examined for signs of weevil feeding damage, and designated damaged or undamaged. The tips in each category were totaled and percent damage in each plot was calculated. Damage and larval density were analyzed using two-way ANOVA (Systat, Evanston, IL). Significant treatment effects ( $P \le$ 0.05) were further explored using planned contrast comparisons (Zar 1984).

Stand composition was determined on 6 June 1990 by removing plant samples from a 1/16 meter quadrate randomly selected from each treatment replication. Plants were sorted into groups consisting of alfalfa, planted grasses and weeds then dried at 60 °C for 72 h. Percent composition of each plant group was calculated based on the mean dry matter (g) for each treatment.

**1995 Field Study.** Two experimental fields were planted in spring of 1993 and another two in spring 1994 at Michigan State University's Agronomy Farm, East Lansing, MI. Plots within each of the fields were established with an oat (*Avena sativa* L., cultivar 'Newdak') companion crop (53.35 kg/ha). Individual plot sizes were 3.7 X 6.4 m with the following treatments arranged in a randomized complete block with four replications in each of the four fields: alfalfa (cultivar 'Apollo Supreme') 17.78 kg/ha, alfalfa 17.78 kg/ha + smooth bromegrass (cultivar unnamed) 3.33 kg/ha, alfalfa 17.78 kg/ha + orchardgrass (cultivar unnamed) 1.11 kg/ha, alfalfa 17.78 kg/ha + timothy (cultivar unnamed) 4.45 kg/ha, and alfalfa 17.78 kg/ha + Kentucky bluegrass 6.67 kg/ha. Each field contained two pure seeded alfalfa treatments. Herbicide 4-(2,4-DB) amine and hexazinone were applied to one treatment (hereafter called alfalfa-weed free) to control broadleaf weeds; any remaining weeds were removed by hand. The second pure seeding of alfalfa received no weed control measures. Insecticides dimethoate, chlorpyrifos and permethrin were applied in 1993 and 1994 for weevil and potato leafhopper control. The fields were all managed in a four-cut system.

Alfalfa weevil damage and larval populations were assessed on 23-25 May 1995, just prior to first cutting. Twenty alfalfa tips were randomly selected from each plot. The tips were examined and assigned either damaged or undamaged as before. Additionally, a 10 point damage rating scale was devised to evaluate the intensity of feeding damage that occurred to the 20 tips collected. The linear scale was similar to that of Berberet and McNew (1986), but was altered by assigning a value of 0 as undamaged and 9 as 100% defoliated.

We estimated larval densities by shaking the tips vigorously against the inside of a 19 l plastic bucket to dislodge larvae (Legg et al. 1985). Larvae were placed in 70% EtOH and stored for later counting. Larval densities, number of damaged tips and feeding intensity were analyzed using analysis of variance (Super ANOVA, Abacus Concepts, Inc. 1989). Natural logarithmic transformations of count data were made to satisfy analysis of variance assumptions. Significant treatment effects ( $P \le 0.05$ ) were further explored using planned comparisons (contrasts).

## Results

**1990 Field Study**. Low numbers of alfalfa weevil larvae were found in all treatments on 18 May 1990 (Figure 9). On 23 May larval populations began to increase; the high-rate alfalfa monoculture had the most larvae and the high-rate bromegrass and orchardgrass mixtures had the fewest. However, differences were not significant. By 30 May the numbers of larvae in the alfalfa monocultures (both seeding rates) were significantly greater than in any of the mixtures (Figure 9, Table 2). The number of weevils in mixtures rose uniformly; numbers in the high rates of bromegrass and orchardgrass remaining the lowest.

On 6 June the day before cutting, alfalfa comprised 87.2 to 95.3% of the stand dry weight and weeds 4.7 to 12.8% (Table 3) in pure seedings. When intercropped with a grass, alfalfa composition was much lower, ranging from 42.6-65%, with grass (32.3 - 55.9%) and weeds (1.5 - 8.1%) comprising the remainder of the stand dry weight. Greater numbers of alfalfa weevil larvae were found in the alfalfa monocultures which contained 22-42% more alfalfa compared with the intercrops (Table 3). The numbers of weevils in the two monoculture treatments were not significantly different, but weevils were significantly less numerous in all six mixtures than in alfalfa monocropped at the low rate (Table 2).

The damage estimate made on 6 June 1990 reflected the weevil larval numbers for the same date (Table 3). The alfalfa monocultures together were significantly more damaged than the mixtures as a whole (Table 2), but alfalfa intercropped with bromegrass and orchardgrass at the low rates sustained damage comparable to alfalfa alone (low rate). The least damaged treatment

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Figure 9. Alfalfa weevil larval density  $\pm$  SEM in alfalfa and alfalfa-forage grass intercrops on four sampling dates; mean of ten sweeps per replication, five replications per treatment taken from Kellogg Biological Station, Hickory Corners, MI

Table 2. Treatment contrasts of alfalfa weevil larvae numbers and percentalfalfa weevil damage in pure and mixed forage stands in 1990 at MichiganState University Kellogg Biological Station, Hickory Corners, MI.

		P values	8
	No. of	larvae	% damage
Contrasts	May 30 <sup>b</sup>	June 6	June 6
ANOVA treatment effect	<0.001	0.001	0.006
Alfalfa high density vs. alfalfa low	NS	NS	NS
Alfalfa alone (both) vs. all mixtures	<0.001	<0.001	<0.001
Alfalfa low density vs. bromegrass high	<0.001	<0.001	0.019
Alfalfa low density vs. bromegrass low	0.008	0.007	NS
Alfalfa low density vs. orchardgrass high	<0.001	0.002	0.037
Alfalfa low density vs. orchardgrass low	0.026	0.002	NS
Alfalfa low density vs. timothy high	0.005	0.004	0.005
Alfalfa low density vs. timothy low	0.003	0.002	0.001

<sup>*a*</sup> Numbers of alfalfa weevil larvae in different treatments are considered significantly different by ANOVA and contrasts where  $P \leq 0.05$ .

<sup>b</sup> No significant differences between treatments were found on the first four sampling dates, 2 May, 9 May, 18 May, and 23 May.

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Treatment	Stan (%	d compos dry weigh	ition ht) <sup>e</sup>	Mean no. of	% Tin damage
	Alfalfa	Grass	Weeds	larvae ± SEM <sup>b</sup>	± SEM <sup>6</sup>
Alfalfa high density	95.3	0	4.7	$47.0 \pm 10.4$	73 <u>+</u> 1.8
Alfalfa low density	87.2	0	12.8	<b>41.4</b> ± 9.9	70 <u>±</u> 1.1
Alfalfa/bromegrass high density	42.6	55.9	1.5	7.8 <u>+</u> 3.2	$46 \pm 1.0$
Alfalfa/bromegrass low density	64.0	32.3	3.7	24.4 ± 8.8	53 ± 0.9
Alfalfa/orchardgrass high density	65.0	26.9	8.1	18.8 ± 4.4	49 <u>+</u> 1.4
Alfalfa/orchardgrass low density	59.3	33.6	7.1	17.5 ± 2.9	59 ± 0.9
Alfalfa/timothy high density	47.0	46.0	7.0	21.4 ± 4.8	41 ± 1.3
Alfalfa/timothy low density	44.8	37.2	7.6	20.0 ± 4.7	36 <u>+</u> 1.6

Percent composition of each plant group was calculated based on the mean dry matter (g) for each treatment. <sup>b</sup>Mean number of larvae obtained from 10 sweeps per treatment in 4 replications using a 37 cm sweep net. <sup>c</sup>Percent damage based on 20 tips examined per treatment in 4 replications. was alfalfa with the low seeding density of timothy (Table 3). The percentage of larvae in each instar for this date was not significantly different among treatments, suggesting that grass presence did not alter development rates and/or sampling effectiveness.

**1995 Field Study.** The percent stand composition of pure seedings of alfalfa paralleled the 1990 study (Table 4). However in contrast to the 1990 study, there was not as great as reduction in percent alfalfa composition (63.9-98.3%) in the alfalfa-grass intercrops. Weeds composed a relatively small proportion (range 0.1-5.3% dry weight) and had little impact on alfalfa weevils. All four fields showed virtually no differences in weevil populations and damage between alfalfa treatments with and without weeds (Table 4). Percent weed composition tended to be lower in all intercrop plots, with alfalfa-orchardgrass tending to have the lowest percentage of weed dry matter.

Fields 1 and 2, which were sampled 2 years after establishment, did not any show significant differences between number of larvae, percent damaged or intensity of feeding damage (Table 4). Although, in Field 2, plots containing a forage grass had a 7-30% reduction in number larvae and a 10-30% reduction in number of tips damaged compared to the alfalfa-weed free treatment. For Fields 3 and 4, sampled 1 year after establishment, treatments containing a forage grass tended to have lower populations of larvae, lower percent tip damage, and a decrease in feeding intensity compared to plots containing only alfalfa (Table 4). In Fields 3 and 4 alfalfa monocultures consistently had more larvae than alfalfa-grass intercrops, although this trend was significant only in Field 3. In this field, smooth bromegrass contained approximately one-half 

 Table 4. Stand composition, mean number of alfalfa weevil larvae, percent tip

 damage tips, and tip damage rating for alfalfa and alfalfa-forage grass intercrops one

 and two years post-establishment, 23-25 May 1995, MSU, East Lansing, MI

Treatment	Stan (%	d compos dry weigl	ht) <sup>4</sup>	Mean no. of	Mean no. of tips	Tip damage
	Alfalfa	Grass	Weeds	larvae ± SEM <sup>®</sup>	damaged ± SEM <sup>e</sup>	rating ± SEM
Field 1 established 1993						
Alfalfa - Weed Free	100	0	0	7.3 <u>+</u> 1.9	37 <u>+</u> 0.1	1.7 <u>+</u> 0.8
Alfalfa	<b>9</b> 5.5	0	4.5	7.5 <u>+</u> 2.3	37 <u>+</u> 0.1	1.5 <u>+</u> 0.1
Bromegrass/alfalfa	<b>95.6</b>	1	3.4	6.0 <u>+</u> 1.2	35 <u>+</u> 0.9	1.6 <u>+</u> 0.1
Orchardgrass/alfalfa	<del>69</del> .5	30	0.5	6.8 <u>+</u> 2.0	30 <u>+</u> 0.5	1.7 <u>+</u> 0.5
Timothy/alfalfa	98.3	0.2	1.5	3.8 <u>+</u> 1.5	29 <u>+</u> 0.9	1.3 ± 0.3
Bluegrass/alfalfa	86.6	13	0.4	6.5 <u>+</u> 2.1	40 <u>+</u> 0.6	1.3 <u>+</u> 0.2
ANOVA value <sup>4</sup>				F=0.18; P=0.96	F=0.73; P=0.61	F=0.78; P=0.98
Field 2 established 1993						
Alfalfa - weed free	100	0	0	27.0 <u>+</u> 5.8	72 <u>+</u> 0.9	2.8 <u>+</u> 0.2
Alfalfa	96.8	0	3.2	22.8 <u>+</u> 2.5	67 <u>+</u> 0.7	2.2 <u>+</u> 0.1
Bromegrass/alfalfa	<b>95.2</b>	2	2.8	<b>25</b> .0 <u>+</u> <b>2.0</b>	58 <u>+</u> 0.5	<b>2.1</b> <u>+</u> 0.1
Orchardgrass/alfalfa	63.9	36	0.1	21.5 <u>+</u> 4.1	55 <u>+</u> 0.7	2.6 <u>+</u> 0.3
Timothy/alfalfa	93.7	4	2.3	22.0 <u>+</u> 6.0	62 <u>+</u> 0.7	2.1 <u>+</u> 0.2
Bluegrass/alfalfa	88.2	11	0.8	19.0 <u>+</u> 2.9	40 <u>+</u> 0.4	2.2 <u>+</u> 0.2
ANOVA value <sup>4</sup>				F=0.50; P=0.77	F=1.49; P=0.25	F=2.43; P=0.08
Field 3 established 1994						
Alfalfa - weed free	100	0	0	14.0 <u>+</u> 2.5	75 <u>+</u> 0.6	2.2 <u>+</u> 0.1
Alfalfa	<b>98.0</b>	0	2.0	14.3 <u>+</u> 2.6	78 <u>+</u> 0.5	1.8 ± 0.2
Bromegrass/alfalfa	79.8	19	1.2	6.3 <u>+</u> 0.8	50 <u>+</u> 0.6	1.6 <u>+</u> 0.2
Orchardgrass/alfalfa	67.0	32	1.0	11.3 <u>+</u> 1.9	62 <u>+</u> 0.3	1.7 <u>+</u> 0.2
Timothy/alfalfa	82.7	16	1.3	8.3 <u>+</u> 2.0	60 <u>+</u> 0.5	1.6 <u>+</u> 0.2
Bluegrass/alfalfa	92.4	7	0.6	9.0 <u>+</u> 1.4	64 <u>+</u> 0.3	1.8 <u>+</u> 0.1
ANOVA value <sup>4</sup>				F=3.50; P=0.027	F=5.56; P=0.004	F=1.51; P=0.24
Field 4 established 1994						
Alfalfa - weed free	100	0	0	40.8 <u>+</u> 9.3	92 <u>+</u> 0.3	3.7 <u>+</u> 0.2
Alfalfa	94.7	0	5.3	35.8 <u>+</u> 7.3	85 <u>+</u> 0.5	3.1 <u>+</u> 0.4
Bromegrass/alfalfa	83.3	15	1.7	28.0 <u>+</u> 4.7	<b>79 <u>+</u> 0.3</b>	2.7 <u>+</u> 0.2
Orchardgrass/alfalfa	79.5	20	0.5	28.8 <u>+</u> 5.5	65 <u>+</u> 0.6	2.9 <u>+</u> 0.4
Timothy/alfalfa	81.0	19	0	8.3 <u>+</u> 3.5	75 <u>+</u> 0.3	2.7 <u>+</u> 0.3
Bluegrass/alfalfa	90.2	9	0.8	33.0 <u>+</u> 8.3	72 <u>+</u> 0.3	2.7 <u>+</u> 0.3
ANOVA value				F=1.50; P=0.25	F=6.70; P=0.002	F=3.43; P=0.03

<sup>4</sup>Percent composition of each plant group was calculated based on the mean dry matter (g) for each treatment.

<sup>b</sup>Mean number of larvae obtained from 20 tips per treatment in 4 replications using a shake bucket sample method.

<sup>4</sup> Analysis based on mean number of damaged tips from 20 tips examined per treatment in 4 replications.

Tip damage rating based on avg score (0 no damage - 9 greatest damage) of 20 tips taken from each treatment in 4 replications. df=5, ANOVA treatment effect significant at  $P \le 0.05$ . the number of larvae found in alfalfa monocultures (Table 4). Paired contrasts showed that the smooth bromegrass and timothy mixtures had significantly fewer larvae than the weed-free alfalfa monoculture (Table 5). Significant treatment effects for percent tip damage occurred in both fields established in 1994 (Table 4). Planned comparisons between weed-free alfalfa and each forage grass mixture revealed significant reductions in percent tip damage for smooth bromegrass, orchardgrass, and timothy mixtures (Table 5). Kentucky bluegrass mixtures contained significantly lower percentage of damaged tips only in Field 4, although the same trend occurred in Field 3.

Tip damage ratings were consistently lower in treatments containing forage grasses in 1994 established fields although only significant in Field 4 (Table 4). Contrasts of forage grass mixtures to weed-free alfalfa showed significant reduction in damage ratings for all grass mixtures in Field 4 (Table 5).

## Discussion

Intercropping forage grasses with alfalfa consistently reduced alfalfa weevil larval numbers and damage in the year following establishment. For example, in Field 3 and 4 established alfalfa-grass fields was reduced by 10-25% and larval numbers were reduced 19-80% compared to the weed free alfalfa plots. Reductions of this magnitude could, depending on overall infestation level, keep larval numbers and damage levels below economic thresholds, preventing or delaying the need for early cutting or insecticide applications.

In the 1995 study, Fields 1 and 2 did not show significant reductions in weevil infestation or damage. Although in Field 2, the number of larvae were reduced by 7-30% and number of tips damaged was reduced 10-31%. We

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Table 4. Treatment contrasts of alfalfa weevil larvae numbers, percent tip damage and tip damage rating in alfalfa and alfalfa-forage grass intercrops one year post-establishment, 23-25 May 1995, Michigan State University, East Lansing, MI

		Ρ	values <sup>a</sup>	
	Fie	ld 3 <sup>6</sup>	Fie	eld 4 <sup>b</sup>
Contrasts	No.	% tip	% tip	Tip damage
•	larvae	damage	damage	rating
Alfalfa weed free vs. all forage grass/alfalfa mix	0.020	0.004	0.000	0.029
Alfalfa weed free vs. bromegrass mix	0.009	0.001	0.029	0.005
Alfalfa weed free vs. orchardgrass mix	NS	0.040	0.000	0.022
Alfalfa weed free vs. timothy grass mix	0.042	0.027	0.007	0.005
Alfalfa weed free vs. Kentucky bluegrass mix	NS	NS	0.0015	0.005
Number of larvae number of tine damaged and da	w niter orem	re mreidered ei	imificantly differ	rant where

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alaliw Number of larvae, number of tips damaged and damage rating were considered significantly different

 $P \le 0.05$ . Analysis of percent tip damage based on mean number of damaged tips/20.

<sup>b</sup>Field 3 and 4 established in 1994.

originally suspected that this was a direct result of the four-cut management system imposed on the fields. Due to the more frequent cutting, smooth bromegrass and timothy biomass declined in the second year following establishment when compared to the same harvest period of the previous year (Spandl unpublished data). To test the hypothesis that grass biomass and alfalfa weevil numbers were related, we performed a simple regression analysis on the biomass (dry weight) of each species of grass found within in each plot to the number of larvae collected. When all intercropped grass treatments were combined, no relationship was found between biomass and number of larvae (P=0.623,  $r^2 = 0.004$ ). Individual regression analysis of grass species biomass on larval numbers showed that orchardgrass had a significant inverse relationship between number of larvae and grass biomass (P=0.034) although the amount of variation explained was small ( $r^2 = 0.282$ ). For other grasses there was no significant relationship between number of larvae and grass biomass. Lack of consistent relationship between alfalfa weevil larval numbers and grass biomass indicates that grass biomass is not the sole factor responsible for the observed reduction in damage.

The species of forage grass and the seeding density had variable effects on weevil numbers and damage. In the 1990 study, smooth bromegrass and orchardgrass appeared to reduce weevil numbers more than timothy but the opposite was true for percent damaged tips. In the 1995 study, there was also no clear advantage of one species over the others.

How the intercropped grass exerts its influence on alfalfa weevils remains unknown. Limited sampling during the 1990 study (Coggins unpublished data) indicated that natural enemy populations were not different among treatments, however, the "natural enemies hypothesis" (Root 1973) has not been effectively tested in these studies. The grasses may affect weevil host finding and oviposition behavior. Golik and Pienkowski (1969) showed alfalfa leaf odor more than doubled turning rates of hungry adult alfalfa weevils in an arena. Meyer and Raffensperger (1973) found alfalfa weevils were primarily attracted by visual properties of their host plants. The forage grasses may be interrupting cues used by the weevils to locate and remain within the stand. Alfalfa weevils must also taste alfalfa to oviposit (Byrne 1969). Repeated tasting of forage grass or contact without tasting could trigger an emigration response or impair oviposition. This study contrasts Waldrep et al. (1969) who showed more alfalfa weevil feeding damage in weedy stands containing a suitable species for oviposition. However, very few grass species have been found to serve as oviposition hosts (Ben Saad and Bishop 1969, Dowdy et al. 1992).

The benefits of forage grass and alfalfa intercrops may not be limited to alfalfa weevil suppression. Recent studies have shown that alfalfa and grass intercrops may have fewer weeds compared to alfalfa monocultures (Spandl unpublished data). Decreasing weeds may improve the palatability (Heath et al. 1985) and quality (Cords 1973) of the forage as well as increase the longevity of the stand. A forage grass may also out compete weed species such as dandelions, *Taraxacum officinale*, (Weber) that increase drying time of hay (Doll 1984).

A potential disadvantage of alfalfa-grass intercrops is a reduction in forage quality, especially as the proportion of grass increases (Marten et al. 1988, Sheaffer et al. 1990). However, reductions in quality may be confined only to

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certain cuttings. Studies with bromegrass or timothy binary mixtures showed lower crude protein and neutral detergent fiber relative to pure alfalfa stands only at the first spring harvest, but not in subsequent regrowths (Spandl and Hesterman unpublished data). Forage quality was higher for alfalfa grown with grass than for pure seeded alfalfa at the second harvest. Grass-alfalfa intercrops could provide pest (both weed and insect) management benefits without significantly reducing the value of the forage.

Additional advantage of an alfalfa-grass mixture, include a more consistent forage yield across a wide range of environments (Haynes 1980, Willey 1979). In stands grown for grazing, an alfalfa-grass mixture could be a particularly good option for reducing weevil damage while also lowering the potential for bloat (Howarth et al. 1978).

Intercropping forage grasses as a integrated pest management strategy could offer an additional means of managing alfalfa weevil. These studies indicate that reductions in number of larvae up to 80% and tip damage up to 25% are possible when forage grass species are grown with alfalfa. This suppression of alfalfa weevil numbers and damage combined with lower weed biomass may allow growers to produce greater quantities of forage for more seasons than monoculture alfalfa. Even when weevil populations are low the mechanism(s) responsible for reducing weevil populations and damage in a grass-alfalfa stand should still impact their behavior, further decreasing their numbers and likelihood of surpassing established thresholds in succeeding years. Additional studies are needed to examine the interaction between grass species, establishment density and cutting management in order to balance

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the benefits of pest management with the potential reductions in forage quality. Elucidation of the mechanism(s) of the intercrop effect on alfalfa weevils is also needed to direct these agronomic studies.

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APPENDIX

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## APPENDIX 1

Record of Deposition of Voucher Specimens\*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 1995-06

Title of thesis or dissertation (or other research projects):

Effects of growing alfalfa with perennial grasses upon potato leafhoppers (Homoptera: Cicadellidae) and alfalfa weevils (Coleoptera: Curculionidae)

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name (s) (typed) Amy L. Roda

Date 1 May 1996

\*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America. Bull. Entomol. Soc. Amer. 24:141-42.

Deposit as follows:

Original:	Include as Appendix 1 in ribbon copy of thesis or dissertation.
Copies:	Included as Appendix 1 in copies of thesis or dissertation. Museum(s) files. Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

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Species or other taxon	Label data for specimens collected or used and deposited	Eggs		Numpha	Adults ¥	Adults of	Other	Museum where depos- ited
<u>Empoasca fabae</u> (Harris)	MI: Ingham Co.							
(Homoptera: Cicadellidae)	Botany Farms, MSU							
	E. Lansing, T4N, R2W, Sec. 36							
	6 June 1995				<u>ں</u>	2		MSU
Hypera postica (Gyllenhal)	MI: Ingham Co.							
(Coleoptera: Curculionidae)	Botany Farms, MSU							
	E. Lansing, T4N, R2W, Sec. 36							
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APPENDIX 1.1 Voucher Specimen Data

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