INTEGRATED PEST MANAGEMENT FOR THE POTATO LEAFHOPPER (*EMPOASCA FABAE*) IN ALFALFA

By

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Abstract

Alfalfa, *Medicago sativa* L., is one of the best quality dairy forages and as the principal forage legume in the U.S., is grown on roughly 10 million ha. Potato leafhopper, *Empoasca fabae* Harris, is its most economically damaging insect in the Midwest and northeast United States. Integrated pest management (IPM) programs for *E. fabae* in alfalfa consist of sampling and monitoring throughout the season, foliar insecticide treatments when economic thresholds are reached, host plant resistance and the cultural control of early harvest when economic thresholds are reached within a week of a planned harvest. The work presented here gives a thorough review of *E. fabae* ecology and biology, migration patterns and injury to host plants. A thorough review of available pest management strategies for *E. fabae* in alfalfa is discussed (chapter 1). An IPM system incorporating host plant resistance and orchardgrass intercroppings as a cultural control is studied with regards to affects on *E. fabae* abundance and alfalfa yield and forage quality (chapter 2). Alfalfa yield loss response to *E. fabae* feeding is assessed in order to validate the current economic injury level and economic threshold models for both susceptible and resistant alfalfa varieties (chapter 3). Lastly, soil fertility treatments are considered as a potential cultural control tactic for managing *E. fabae* in response to farmer observations (chapter 4).

Keywords: IPM, alfalfa, *E. fabae*, host plant resistance, economic injury level

Table of Contents

Chapter 1: Potato Leafhopper (Hemiptera: Cicadellidae) Ecology and Integrated Pest Management Focused on Alfalfa

Abstract

This paper summarizes the knowledge to date on the biology of the potato leafhopper, *Empoasca fabae* Harris including its distribution, development, migration, agricultural host plants, and the mechanism of injury to host plants. Damage to alfalfa, potatoes, soybeans and snap beans, as well as treatment guidelines, are summarized. Particular attention is given to integrated pest management options in alfalfa, the host plant most frequently incurring economically damaging populations of potato leafhopper. Alfalfa scouting and economic thresholds are discussed along with cultural controls and host plant resistance.

Key Words: PLH, IPM, migration, host plant resistance, economic threshold

Native to North America, the potato leafhopper, *Empoasca fabae* Harris (Hemiptera: Cicadellidae) migrates northward from the Gulf States each summer, to the Midwest and eastern United States where it is a key agricultural pest in many crops. Populations remaining in the southern US overwintering habitats can contribute to crop damage, but it is typically less severe (Fick et al. 2003). The geographical range of the potato leafhopper extends from the eastern seaboard of the US westward to the Rocky Mountains (Delong 1931a) and northward into the bordering Canadian provinces (Fick et al. 2003). In addition, presence of potato leafhopper in California cropping systems has recently been confirmed from late-summer sweep net samples of uncut alfalfa in Parlier (Fresno Co.), CA, in the mid-Central Valley[1]. Nonetheless, some previous records from California may be based on misidentification of the closely related species, *E. mexara* Ross and Moore, which also occurs on alfalfa in California (C.D., *unpub. data*). It remains unknown whether California *E. fabae* overwinter in the Central Valley, or migrate in from southern locations; attempts to collect *E. fabae* in Imperial Co. (southern California) in early summer were unsuccessful.

The potato leafhopper's diverse host plant list of over 200 plant species includes alfalfa (*Medicago sativa* L.), soybean (*Glycine max* L.), and potato (*Solanum tuberosum* L.), as well as roadside, weedy and forest plants (Lamp et al. 1994). As the key economic pest of alfalfa in the North Central and Northeast US, yield losses have been documented up to $66/ha ($27/acre) (Lamp et al. 1991).

The first records of *E. fabae* date back to 1841 when it was detected in Massachusetts as a pest on fava bean (*Vicia faba* L.) (Harris 1841). By 1931, it was considered an economically important pest in many cultivated crops (Delong 1931b). Unfortunately, because *Empoasca* is a

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[1] Specimens examined for this study were identified by the Chris Dietrich (Center for Biodiversity, University of Illinois-Urbana Champaign) and are deposited in the insect collection of the Illinois Natural History Survey.

large and complex genus of leafhoppers with hundreds of described species, many of which are nearly identical in external appearance, other species of *Empoasca* have often been misidentified as *E. fabae.* DeLong's (1931b) initial studies of the male genitalia of North American *Empoasca* revealed features that distinguish *E. fabae* from other common *Empoasca* species. However, later studies (Ross 1959a, b; Ross and Moore 1957) revealed that "*E. fabae*" of various authors is a complex of at least 27 closely related species. Thus, positive identification of species belonging to this complex requires examination not only of the male terminalia, but also the internal apodemes of the first two male abdominal segments. Fortunately, nearly all of the currently recognized species of the complex appear to be restricted to the tropics and only four have so far been recorded from the continental USA: *E. fabae* (widespread), *E. hastosa* Ross and Moore (Florida), *E. kraemeri* Ross and Moore, and *E. mexara* (Arizona, California) (Ross 1959a and C. D. *unpub. data*).

As well as early identification errors, the relationship between the potato leafhopper and crop damage was originally not well understood. Although the effect of potato leafhopper on alfalfa was noted as early as 1907, plant damage symptoms known as alfalfa “yellows” were initially attributed to abiotic factors such as weather and soil nutrient deficiency. Greenhouse experiments at the University of Wisconsin Agricultural Research Station confirmed that alfalfa “yellows” was caused by the potato leafhopper (Granovsky 1928). In potatoes, farmers and researchers originally believed that potato leafhoppers were the vector of a pathogen leading to the characteristic yellowing of the leaves (Dudley 1920). Although closely related to some known insect vectors of phytoplasma to agriculturally important plants (Galetto et al. 2011), there are no known records of pathogen transmission to plants by potato leafhopper.

The current pest management strategy in alfalfa for the potato leafhopper is to monitor the pest throughout the season with a sweep net and treat with foliar insecticide when economic threshold populations are reached (Degooyer et al. 1998, Cullen et al. 2012). A fully developed integrated pest management (IPM) program is comprised of multiple strategies for a given pest or pest complex in a cropping system incorporating host plant resistance, biological, cultural and physical controls when available and chemical control when necessary (Pedigo 1999). Several integrated management strategies have been developed for the potato leafhopper in alfalfa. For example, alfalfa cultivars bred for resistance to the potato leafhopper were first available to farmers in 1997 (Miller 1998). Despite advances in pest management for potato leafhopper in alfalfa, it continues to be considered the most important economic pest of alfalfa through much of its range. With near doubling value of the alfalfa hay market over the last decade (Gould 2012), economic loss potential from potato leafhopper has also increased. Thus, a more thorough understanding of potato leafhopper biology and IPM is a timely subject. In this pest profile, we summarize knowledge of potato leafhopper life history, ecology, scouting procedures and management options in alfalfa.

Description of Life Stages and Life History

Egg. Eggs are oviposited into the stems of host plants (Delong 1938). In order to examine the eggs, the stem either must be dissected or stained using McBride's stain (Backus et al. 1988). They are cylindrical, translucent pale green, and about 0.8 mm x 0.25 mm (Hutchins 1987). Once oviposited, time to eclosion ranges from 7-14 days, with warmer temperatures promoting faster development (Hogg 1985).

Nymph. Potato leafhoppers have 5 nymphal instars (Fig. 1). Instars can be distinguished by color, size, and presence of external wing pads. The first instar is pale white with red eyes, and extremely small. Subsequent instars gain more of the vibrant yellow-green color typical of the adults. Wing pads (Fig. 2) begin developing in the third instar. Sizes of the instars range from 1 mm for 1st instars to 3 mm long for 5th instars (Hutchins 1987). Developmental time is more rapid in warmer temperatures and ranges from 9-18 days to complete all 5 instars (Hogg 1985). All nymphal stages resemble the adult body shape in that the head segment is wider than the abdomen, which gives the body a wedge-shaped appearance. Potato leafhopper nymphal movement is distinct from adults in that nymphs scuttle sideways. However, both nymphs and adults are able to utilize specialized hind legs (Fig. 2) for jumping.

Adult. The presence of fully developed wings and ability to fly makes adults morphologically distinct from the nymphs. Adults are approximately 3 mm long by 0.5-1 mm wide. They are bright yellow-green colored with 6 white spots behind the eyes on top of the head (Fig. 3). Mating can take place as soon as 48 hours after adult emergence (Delong 1938). Once females have mated, they oviposit 2 to 5 fertile eggs, individually, each day for the remainder of their lives (Delong 1938, Decker et al. 1971). Optimal temperatures for egg laying are 70-75 ºF (Kieckhefer and Medler 1964). The average lifespan of an adult in the field is 30 days, though in the laboratory adults can live up to 3 months (Delong 1938).

Migratory Patterns. Potato leafhoppers overwinter as adults in reproductive diapause (females are unmated) throughout the Gulf Coast States (Louisiana, Mississippi, Alabama, and parts of Florida and Texas) (Decker and Cunningham 1968) and the Southern Pines region, including eastern Arkansas, Tennessee, South Carolina, North Carolina and Virginia (Taylor and Shields 1995). In the overwintering habitats, reproductive diapause ends and mating begins in late February as populations shift from pines to legumes (Taylor and Shields 1995). Populations migrate to the northern and eastern US with the occurrence of warm, long distance southerly winds (Carlson et al. 1992). However, the timing of this event is not an indicator of pest pressure or severity of crop damage (Maredia et al. 1998).

The first potato leafhopper populations arriving in the north are largely female-biased (Medler and Pienkowski 1966) and begin arriving sometime in May (Maredia et al. 1998). Arriving females are typically mated already (Medler and Pienkowski 1966) and will oviposit for the duration of their lives (Delong 1938, Decker et al. 1971). Field studies indicate a female-biased sex ratio near 4:1 through most of the season until it approaches 1:1 towards the end of the growing season (Medler and Pienkowski 1966, Decker et al. 1971, Flinn et al. 1990, Emmen et al. 2004). Development from egg to adult can occur in as little as just over 2 weeks or can take more than 4 weeks depending on temperatures, which gives rise to 3 to 5 overlapping generations during summer months in the northern U.S. (Delong 1938, Hogg and Hoffman 1989).

In late summer, potato leafhoppers begin abandoning crop hosts for wild host plants along crop borders and woodlots, enter reproductive diapause, and then return to their overwintering habitat via northerly winds on a southward migration (Taylor 1989, Taylor and Shields 1995). About two months after the first frost, they are completely absent from the northern habitats (Decker and Cunningham 1968), due both to their southward migration and their inability to survive low temperatures in the northern winters (Specker et al. 1990).

Injury from Feeding

Hopperburn is the term used to describe symptoms of potato leafhopper feeding injury to host plants. Hopperburn symptoms (Figs. 4 and 5) always include stunted plant growth. In addition, various leaf symptoms include tip-wilting and chlorosis in alfalfa, but leaf curling and marginal necrosis in other host plants, ultimately leading to premature leaf-drop (Backus et al.

2005). Theories regarding toxins in saliva have been proposed since the earliest years of potato leafhopper research. However, more recent research has shown that feeding injury is actually caused by varying plant responses to the complicated feeding behaviors of the potato leafhopper (as well as its relatives in the *E. fabae* complex).

The potato leafhopper feeds by inserting its piercing-sucking mouth parts (stylets) (Fig. 6) into plant tissues, rupturing and ingesting nutrients from all types of mesophyll, parenchyma and phloem cells, depending upon the host plant (Backus et al. 2005). Unlike other leafhoppers, potato leafhoppers do not produce a true salivary sheath that encases the stylets during feeding. Instead, the naked stylets repeatedly probe plant tissues, mechanically lacerating cells and simultaneously injecting watery saliva into the tissues. The watery saliva is composed of digestive, hydrolyzing, and cell wall-degrading enzymes, and to date has not been found to contain any non-enzymatic "toxin." Instead, hopperburn is caused by a combination of mechanical and salivary mechanisms (Ecale and Backus 1995a), so it is termed a "saliva-enhanced wound response." Unique to potato leafhopper, the symptoms of feeding injury on different host plants are related to three different tactics of stylet probing (Backus et al. 2005).

On alfalfa, adult potato leafhoppers use the *lacerate-and-sip* tactic, which is also thought to be the most injurious, mostly on stems and petioles. Adults insert their stylets perpendicular to the stem and proceed to arc the stylets back and forth, essentially cutting multiple channels through the vascular bundle (all types of phloem cells) for 1 – 2 min before removing the stylets, taking a couple of steps forward and repeating the action. The wounded but still living vascular cells then undergo saliva-enhanced wound responses over the next several days that result in temporary blockage of nutrient movement up the phloem (Nielson et al. 1990) that is ultimately healed, but permanent blockage of xylem cells (Ecale and Backus 1995b). Both types of blockage cause systemic decreases in photosynthesis and decreased transport of sugars to growing areas of the plant, leading to both leaf chlorosis and plant stunting in all host plants (Backus et al. 2005). On alfalfa, potato leafhopper nymphs feed on both stems and leaves; on leaves, their feeding is similar to adult feeding on non-alfalfa host plants (below).

On most host plants (e.g., snap and fava bean, soybean and potato) that show leaf shriveling, curling and necrosis, potato leafhopper adults prefer to feed on leaves, not stems, and add two more tactics to their feeding style, *lacerate-and-flush* and *lance-and-ingest*. In lacerate-and-flush, the stylets are inserted into individual mesophyll/parenchyma cells between veins on leaves, and the cells are partially to wholly emptied. When large numbers of such cells are emptied, the leaf surface collapses. If the feeding has occurred mostly on the lower surface, cell collapse causes leaf curling (e.g., in snap bean); if both surfaces, collapse causes leaf shriveling (e.g., fava bean). In lance-and-ingest, phloem sieve element cells are lanced during stylet laceration and fluid contents are briefly sucked up. When combined with lacerate-and-sip performed on leaf veins, the three tactics together lead to leaf curling, chlorosis, then ultimately necrosis and leaf drop (Backus et al. 2005).

Interestingly, the three feeding tactics of *E. fabae*-complex species are mixed-and-matched on different host plants in relation to the degree of susceptibility or resistance of the plant. Some genotypes of resistant snap bean cause less of the most-damaging tactics of feeding (lacerate-and-flush and lacerate-and-flush) to be performed, while more of the less-damaging tactic, lance-and-ingest (Serrano et al. 2000, Backus et al. 2005). In addition to genetic mechanisms of resistance or susceptibility, drought or desiccation can enhance hopperburn symptoms because water and carbon transport are impaired after potato leafhopper feeding.

Hosts

Potato leafhoppers have an extensive host plant range including 220 plant species in 26 families; both cultivated crops and non-cultivated or weed plant species, the majority of which (62%) are in the legume family, Fabaceae (Lamp et al. 1994). Most host plants are non-native species, herbaceous and in human modified landscapes (Lamp et al. 1994). Host plants of economic importance include cultivated plants from the legume family (Fabaceae) such as alfalfa, soybean, and other bean plants, as well as apples (*Malus domestica* Burkh), potatoes, eggplant (*Solaum melongena* L.), cotton (*Gossypium spp.*), rhubarb (*Rheum rhabarbarum* L.), and ornamentals such as dahlias (*Dahlia spp.*) (Delong 1938). Economic thresholds have been established for alfalfa (Cuperus et al. 1983), soybean (Ogunlana and Pedigo 1974), and potato (Cancelado and Radcliffe 1979).

Alfalfa. Potato leafhopper is the most economically damaging pest of alfalfa in the North Central and Northeast US. The characteristic hopperburn feeding damage symptom is expressed as triangular v-shaped yellowing at the leaflet tips (Figs. 4 and 5). The first cutting of a season typically escapes potato leafhopper damage due to the timing of spring migration (first cutting is harvested before significant potato leafhopper population buildup), but mid- and late-season cuttings are at risk of intensive potato leafhopper pressure and damage. Damage to alfalfa is most severe for young plants, either in the seeding year or just after a harvest during initial regrowth (Kouskolekas and Decker 1968, Cuperus et al. 1983, Hower 1989). Severe feeding can decrease the yield in the following cutting or year (Hower 1989, Vough et al. 1992) due to disruption in photoassimilate translocation to the roots and crown tissues (Lamp et al. 2001). Potato leafhopper feeding reduces stem height, decreasing alfalfa yield, and also reduces crude protein content (Lamp et al. 1985). However, Wilson et al. (1989) discuss alfalfa's ability to better tolerate leafhopper populations when harvests are appropriately timed and the stand is not otherwise stressed by factors such as disease or drought.

Soybean. Heavy potato leafhopper infestations on soybean can lead to plant stunting, smaller seed size and decreased yield (Yeargan et al. 1994). These negative impacts are more severe on seedling soybeans while larger plants can better tolerate potato leafhopper feeding (Hunt et al. 2000). Yield loss from potato leafhopper damage is more severe when the plant is under moisture stress (Yeargan et al. 1994). However, heavy infestations are not common on soybeans (Ogunlana and Pedigo 1974) except when nearby alfalfa fields are harvested (Poston and Pedigo 1975). Economic thresholds for soybeans vary by plant age: early vegetative stages can be treated when there are 2 leafhoppers per plant, flowering fields can be treated when there is 1 leafhopper per trifoliate leaf, and while pods are developing, fields should be treated when there are 2 leafhoppers per trifoliate leaf (Krupke et al. 2013).

Potato. Potato leafhoppers are a member of the key pest complex of potatoes. Feeding causes a reduction in photosynthesis and may result in leaf necrosis. Nymphs are more damaging than adults but can be efficiently sampled to implement effective management strategies. Damage on potatoes can be predicted not only by the intensity of feeding, but also on which leaves the feeding is occurring and the age of those leaves (Johnson and Radcliffe, 1991). Economic thresholds are based on both adult and nymph populations for which there are separate scouting methods. Adults are scouted with a sweep net and nymphs are scouted by examining leaves from the mid-canopy. When 1 adult per sweep or 2.5 nymphs per 25 leaves are found, insecticide treatment is recommended (Sexson et al. 2005).

Snap Bean. Snap beans (*Phaseolus vulgaris* L.) are regularly infested by potato leafhopper, and under intense feeding this can result in complete leaf drop while at moderate pressure, plant stunting and yield loss may occur (Gonzalez and Wyman 1991). Duration and timing of infestation are important when making management decisions; infestation on younger plants causes more significant yield loss than the equivalent pressure on older plants (Gonzalez and Wyman 1991). Economic thresholds for green beans vary by plant age: for seedlings the threshold is set at 0.5 potato leafhoppers per sweep and for the third trifoliate to bud stage the threshold is set at one per sweep. For dry beans the thresholds are 0.5 potato leafhoppers per plant at the unifoliate stage and one potato leafhopper per trifoliate leaf once the plants have reached trifoliate stage (Flood and Wyman, 2005). Neonicotinoid seed treatments are also commercially available and have been largely successful at controlling a suite of snap bean pests including potato leafhoppers, especially for roughly the first 30 days of plant growth (Nault et al. 2004). However, when potato leafhopper populations are exceptionally high, growers should still be mindful of scouting for economic threshold populations later in the summer.

Ecology

Abiotic Factors. Throughout the summer, population growth is regulated by abiotic factors such as precipitation and temperature. On moisture-stressed alfalfa, development time of potato leafhopper eggs, nymphs and adults slows, mortality increases and fecundity decreases (Hoffman et al. 1990, 1991). However, hopperburn seems to appear more frequently during summer droughts (Hoffman et al. 1991). This is likely due to an additive effect of leafhopper feeding and drought stress on alfalfa’s physiological response (Schroeder et al. 1988). Moreover, drought stress interspersed with bouts of rain throughout the summer may increase potato leafhopper performance, which has been proposed as a theory that could explain discrepancies between field observations and laboratory studies (Huberty and Denno 2004).

As with other cold-blooded organisms, potato leafhopper development is dependent on environmental temperatures. Potato leafhopper development stops when temperatures drop below a lower developmental threshold of 45°F (7.6°C) and the rate begins to decline when temperatures consistently exceed an upper developmental threshold of 86°F (30°C) (Hogg 1985).

Natural Enemies. Under no-choice laboratory conditions various generalist predators will feed on potato leafhopper nymphs and adults. These predators include the minute piratebug (*Orius insidious* Say), damsel bug (*Nabis americoferus* Carayon), lacewings (*Chrysopa spp.*) and various lady beetles (Coccinellidae) (Martinez and Pienkowski 1982, Erlandson and Obrycki, 2010). Flinn et al. (1985) demonstrated through choice experiments with potato leafhopper nymphs and the pea aphid (*Acyrthosiphon pisum* Harris) that damsel bugs exhibit a strong preference for the pea aphid. Preference for alternative, less mobile prey could be one reason that none of the generalist predators abundant in alfalfa and other cropping systems play a crucial role in suppressing potato leafhopper populations. There are a few egg parasitoids that have been collected and reared from potato leafhopper eggs (*Anagrus* sp. and *Aphelopus* sp.) (McGuire 1989). A naturally occurring entomopathogenic fungus detected in Wisconsin, *Erynia radicans*, has had some success at suppressing potato leafhopper outbreaks in Illinois (McGuire et al. 1987a). However, this method of control has not been effective because temperatures exceeding 86°F prohibit successful establishment of this fungus (McGuire et al. 1987b). Regardless of the presence of potential natural enemies, the efficacy of biological control agents at suppressing potato leafhopper populations in alfalfa and other cultivated crops remains limited.

Scouting and Management Options in Alfalfa

Scouting. Scouting for potato leafhoppers in alfalfa is standardized through the use of a 15-inch diameter sweep net. University extension recommendations are to monitor alfalfa fields weekly beginning mid-June or when potato leafhopper migrants are known to have arrived in the area by taking five sets of 20 sweeps at various locations in a W-shaped pattern throughout the alfalfa field (University of Wisconsin-Extension 2010). Adult potato leafhoppers may be found at the bottom of the sweep net while nymphs can be found along the rim of the sweep net as well as throughout the net (University of Wisconsin-Extension 2013). Insecticide recommendations are based on the average potato leafhopper number per sweep calculated from total samples taken across the field, including nymphs and adults (Cullen et al. 2012). Because taller alfalfa can tolerate more potato leafhopper feeding, established economic thresholds depend on the average height of the alfalfa stand. When scouting for potato leafhoppers, it is important to avoid taking sweep net samples at field edges, as potato leafhopper populations are typically higher along field margins and this is not representative of population density throughout the field (Emmen et al. 2004). It is also important to avoid taking sweep samples while it is raining or when dew is present on the plants, and if possible, avoid sweeping when winds are greater than 10 miles per hour as this reduces the sweep net sample efficiency (Cherry et al. 1977).

Management: Economic Thresholds and Foliar Insecticides. Established economic thresholds are based on research done by Cuperus et al. (1983). They concluded that treatment was economic when 0.15 potato leafhoppers per sweep were present on 2-inch alfalfa and when 0.42 potato leafhoppers per sweep were present on 7-inch alfalfa. These conclusions have been adapted to current university recommendations of roughly one-tenth of a leafhopper per sweep per inch height of alfalfa growth (Table 1) (Fick et al. 2003, Townsend 2002, University of Wisconsin-Extension 2013). Some university extension recommendations suggest a dynamic economic threshold based on varying costs of insecticide treatment (Rice et al. 1999). Under this threshold model, as the treatment cost increases, a greater density of potato leafhoppers is required to cause economic yield loss equivalent to the treatment cost, and therefore the threshold is increased.

Foliar insecticides registered for potato leafhopper control on alfalfa are effective against nymphs and adults. Pyrethroids are the most commonly recommended and used insecticides for control of potato leafhopper. There are a limited number of insecticide active ingredients in the organophospate chemical class registered for potato leafhopper control in alfalfa. In addition, insecticide premix products are registered that combine two insecticide classes (e.g., organophosphate + pyrethroid; chlorantraniliprole + pyrethroid; neonicotinoid + pyrethroid). Because potato leafhopper populations vary from year to year, and field to field, populations within a given year cannot be predicted, and fields must be monitored weekly to accurately determine damage potential before insecticides are applied. Other pests and beneficial insects in the alfalfa field should also be considered before application of these broad-spectrum insecticides. For example, insecticides that control potato leafhopper at economic thresholds can also kill beneficial insects such as honeybees. To reduce hazards to honeybees in alfalfa, applicators can notify beekeepers before using insecticides, apply between 4 p.m. and nightfall when bees are least likely to be foraging, and refrain from spraying alfalfa when in bloom (Cullen et al. 2012).

Management: Cultural Control.

Harvest Timing. If economic thresholds are reached within 7 days of a planned harvest, early harvest is advised, rather than an insecticide spray (Undersander et al. 2004). Early harvest helps alfalfa stands to avoid further potato leafhopper feeding damage. Addtionally, potato leafhopper population dynamics can be influenced by the harvest (Pienkowski and Medler 1962, Simonet and Pienkowski 1979, Cuperus et al. 1986). Cuperus et al. (1986) showed that greater populations of both nymph and adult populations were correlated with taller stubble or lodged growth left behind after harvest. Cuttings at stubble height of 2-5 cm (1-2 inches) with no remaining leaves or succulent stems can reduce populations up to 95% in the next growth cycle (Simonet and Pienkowski 1979). These effects are due to high nymph and egg mortality from their lack of mobility and exposure to hot, drying conditions (Simonet and Pienkowski 1979) and adult dispersal post harvest to neighboring fields (Poston and Pedigo 1975).

Grass Intercrop. Lamp (1991) showed that alfalfa-oat mixtures have fewer potato leafhopper adults, both per area as well as per alfalfa stem. Several forage grass-alfalfa mixtures have had similar effects on potato leafhopper density. Alfalfa stands containing 9% forage grass, either smooth bromegrass (*Bromus inermis*) (Leyss) or orchardgrass (*Dactylis glomerata*) (L.), had 4-37% reduction in potato leafhopper densities compared to alfalfa monocultures (Roda et al. 1997). Degooyer et al. (1999) similarly observed significantly fewer leafhoppers in alfalfa stands intercropped with smooth bromegrass or orchardgrass compared to alfalfa monocultures. These patterns may be due to higher leafhopper emigration out of plots containing grass (Roda et al. 1997), as well as potato leafhopper inability to reproduce on monocots such as grass (Lamp et al. 1994).

The results of the studies performed by Roda et al. (1997) and Degooyer et al. (1999) show that potato leafhoppers are typically reduced in alfalfa-grass stands but that is not the case for every harvest. Moreover, even when the population is reduced it is not always reduced below economic threshold so it is still important to monitor the population and use other management strategies when necessary. There is a great deal of variability in the response of the potato leafhopper to the presence of grass in the alfalfa stands, in part due to the relative proportion of grass to alfalfa as well as the spatial arrangement of the grass in the alfalfa stand (Roda et al. 1997).

Management: Host Plant Resistance. Observations regarding alfalfa host plant resistance to potato leafhopper date back to 1928, when Granovsky (1928) noted that "hairier" *Medicago spp.* demonstrated greater tolerance to leafhoppers before exhibiting hopperburn. Laboratory studies have shown greater potato leafhopper mortality and reduced reproduction on glandular-haired *Medicago spp.* as well as leafhopper preference for smooth stem alfalfa varieties in choice tests (Shade et al. 1979, Brewer et al. 1986, Ranger and Hower 2002). Both physical and chemical traits associated with the glandular hairs have been reported as resistance mechanisms: 1) entrapment of the first instars in trichome exudates (Ranger and Hower 2001) and 2) adult settling deterred by a) fatty acid amid compounds in the exudate (Ranger et al. 2004, Ranger et al. 2005a) as well as b) reduced production of attractant volatiles in resistant foliage (Ranger et al. 2005b).

The wild glandular-haired *Medicago spp.* were integrated into breeding programs that eventually led to the first line of commercially available alfalfa cultivars with host plant resistance in 1997 (Miller 1998). However, in the field, glandular-haired alfalfa cultivars have had varying levels of success. Lefko et al. (2000) found that established stands of resistant alfalfa could tolerate greater than twice the potato leafhopper pressure as established susceptible stands, but this was not the case for the first cutting of seeding-year stands. Established resistant stands also had greater yield (Sulc et al. 2001) as well as higher forage quality over susceptible cultivars when leafhopper pressure was high (Sulc et al. 2004). However, under low potato leafhopper pressure, resistant alfalfa stands have no yield benefit and sometimes express a yield drag (Hogg et al. 1998, Hansen et al. 2002).

Glandular haired alfalfa varieties with >50% resistance offer a valuable trait to potato leafhopper IPM programs. A highly resistant variety may only require one insecticide treatment in the seeding year. The first cutting of seeding-year stands of glandular haired varieties should be treated for potato leafhopper using the same economic thresholds established for susceptible alfalfa (Lefko et al. 2000). Alfalfa varieties bred for resistance to the potato leafhopper no longer demonstrate visual hopperburn (especially yellowing), but this does not necessarily indicate that there is no yield or quality damage to the alfalfa because some stunting can still occur (Kindler et al. 1973, Shockley et al. 2002). Under moderate to heavy potato leafhopper infestations, glandular-haired varieties may still benefit from timely scouting and insecticide treatment when leafhopper populations have exceeded thresholds established for susceptible alfalfa varieties.

Conclusions. Though the potato leafhopper is highly polyphagous and its host range includes several agricultural crops, it is most economically damaging to alfalfa in the Midwestern and eastern U.S. This is why there are many pest management options for potato leafhopper in alfalfa. The diversity of non-chemical options provide proactive management with the potential to maintain populations below those that warrant chemical control, however this is rarely attained especially in years and locations where populations are considerable. The following three chapters are intended to further the progress of integrated pest management programs for potato leafhopper in alfalfa. Chapter 2 looks at the effect of combining orchardgrass intercroppings with host plant resistance on potato leafhopper populations and alfalfa yield. Chapter 3 revisits the economic thresholds that were established over 30 years ago in order to determine if decreasing the thresholds is warranted by higher alfalfa market values. Chapter 4 examines the potential cultural control of applying liquid dairy manure on alfalfa stands to suppress potato leafhopper populations.

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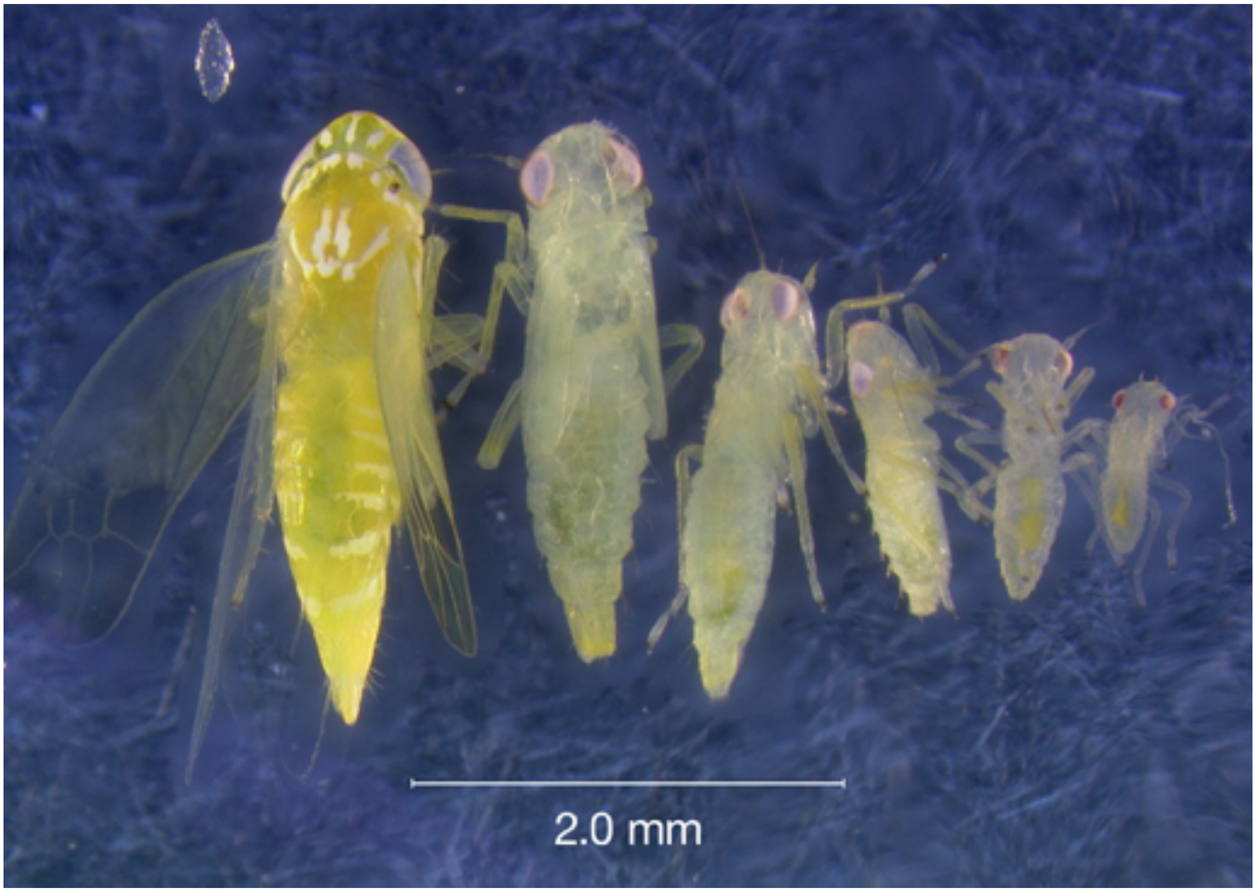

Figure 1. All five potato leafhopper nymphal instars and adult, left.

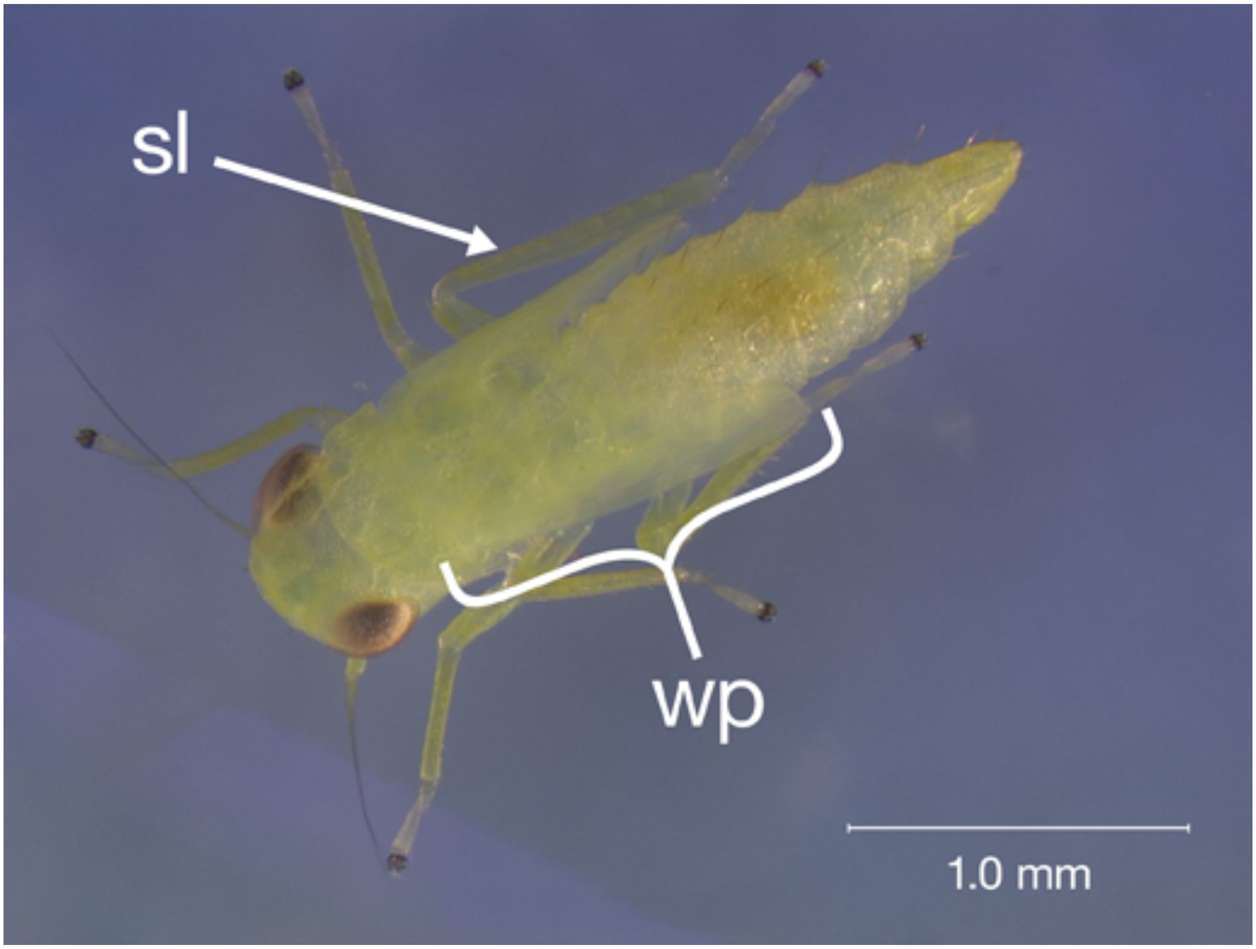

Figure 2. Late instar with wing pads (wp) and specialized legs (sl) used for jumping.

Figure 3. Adult potato leafhopper; note the 6 white spots located on top of the head.

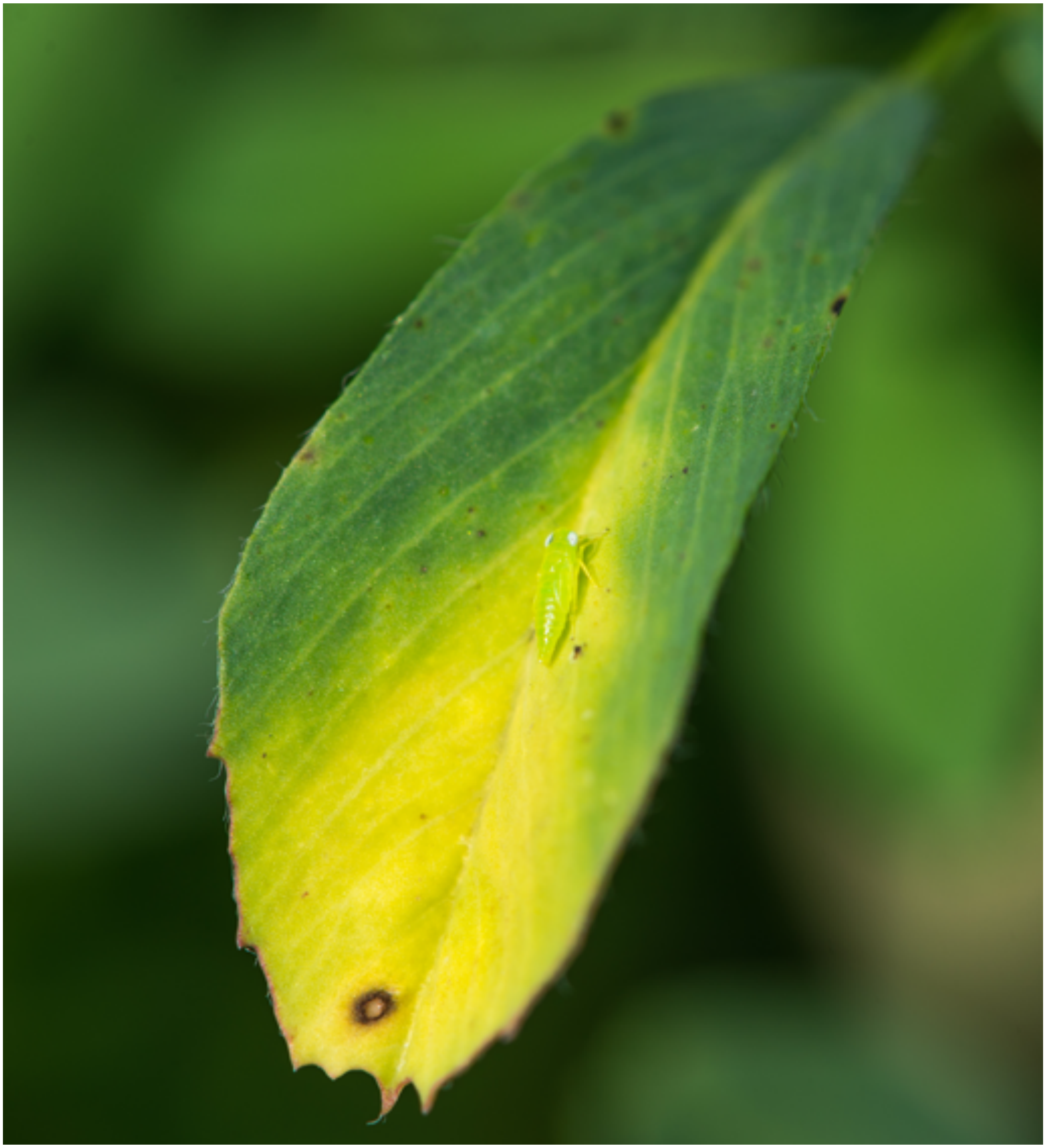

Figure 4. Characteristic triangular, v-shaped yellowing hopperburn damage to alfalfa, including nymph.

Figure 5. Characteristic triangular, v-shaped yellowing hopperburn damage to alfalfa. Older damage turns brown.

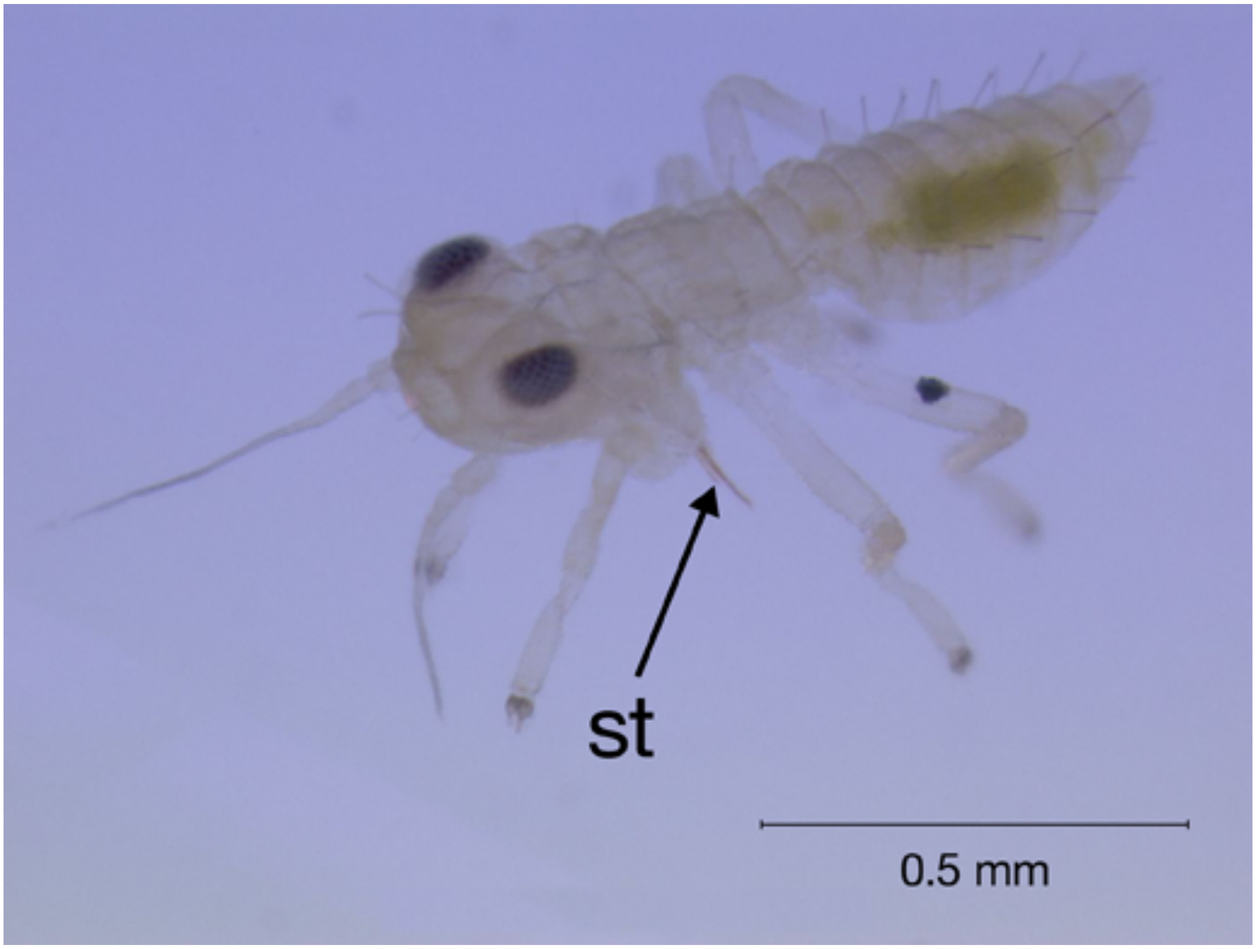

Figure 6. Early instar with piercing-sucking mouthpart, or stylet (st).

Table 1. Economic thresholds for potato leafhoppers in alfalfa (adapted from Cullen et al. 2013).

Alfalfa Height	PLH sweep^{-1}
0-4 inches	0.2
4-8 inches	0.5
8-11 inches	1.0
12+ inches	2.0

Chapter 2: Afalfa Host Plant Resistance and Orchardgrass Intercrop Effects on Potato Leafhopper Populations, Yield and Forage Quality

Abstract

Glandular haired alfalfa varieties resistant to the potato leafhopper, *Empoasca fabae* have been available in the market since 1997 but success for management is inconsistent. Regarding cultural control, several studies have observed potential for potato leafhopper management in alfalfa-grass stand mixtures. Neither host plant resistance nor alfalfa-grass mixtures alone have been successful at suppressing potato leafhopper populations below economic threshold populations. In this study, potato leafhopper response to resistant alfalfa and orchardgrass intercroppings was monitored in a factorial experiment established at two research stations in WI, one site from 2010-2012 and one site for 2012. Alfalfa yield and forage quality were assessed in relation to potato leafhopper densities and whole plot factorial treatments at one site. At the same site, split plots design consisting of insecticide treatment when potato leafhopper populations reached economic threshold and half-economic threshold was employed to determine if reducing the current economic threshold will increase alfalfa yields. Potato leafhopper populations only reached economic threshold once, indicating that populations were low throughout most of this study. Potato leafhoppers were suppressed by resistant alfalfa more consistently in seeding years and the effect of orchardgrass on potato leafhoppers was minimal and inconsistent. Consistent with past research, resistant alfalfa expressed a small yield drag under low potato leafhopper populations. Resistant alfalfa consistently had greater crude protein content than susceptible alfalfa. Orchardgrass improved alfalfa yield at the first crops of the season but plots with more orchardgrass had lower yields at the last crops of each season. Orchardgrass presence consistently increased neutral detergent fiber content.

Keywords: IPM, host plant resistance, cultural control, economic threshold, potato leafhopper

Alfalfa, *Medicago sativa* L., is one of the best quality dairy forages. Since being introduced into the Americas by the Spanish in the 1500s, alfalfa has become the principal forage legume in the U.S., grown on roughly 10 million ha. Over 50% of this area is found in Wisconsin, Minnesota and North Dakota (Sheaffer and Evers 2007). Alfalfa hay and haylage provide an important source of energy, protein and fiber for dairy cows (Jennings 2006). Lactating cows in Wisconsin consume an average 25% of their diet as alfalfa, equaling 12- 15 lbs/day (R. Shaver, pers. comm.). Alfalfa is the second largest Wisconsin crop by harvested acreage, over 2 million acres in 2012 (USDA NASS 2013a). The production of alfalfa hay dry matter (roughly half of the acreage of alfalfa production) in WI in 2010 was valued at over $3.5 million (USDA NASS 2013a). Wisconsin is the nation's second largest dairy producer for export, bringing $630 million into the state (USDA ERS 2013). Within the state, dairy products are the highest grossing agricultural commodities; in 2011 dairy sales brought in $5.2 billion (USDA ERS 2013). Wisconsin alfalfa growers play a large part in supporting this strong dairy economy.

Alfalfa, a perennial, nitrogen-fixing crop, is typically grown for 3 to 4 years and each growing season consists of 3 or 4 harvests also referred to as cuttings or crops. It can be planted in either the spring or the late summer. The first growing season of spring planted alfalfa, or seeding year, is comparatively low yielding and more susceptible to pest damage than the following production years, while the first growing season of a late summer planted crop yields higher and is less susceptible to weeds and pests (Rankin 2001).

Alfalfa growth intervals signify distinct stages of development correlated with plant physiological processes. The outcomes of these physiological processes explain how different harvest practices lead to tradeoffs between forage yield and quality. During the vegetative, or prebud stage, there is rapid translocation of carbohydrate energy upward within the first 10 days after a harvest (Lamp et al. 2001). This is when root carbohydrate reserves are depleted as this energy is sent to the growing shoots and root nitrogen fixation declines significantly. During this prebloom time, energy and protein are maximized in plant stem and leaf tissues (Undersander 2001). When plants bloom, the direction of carbohydrate movement is reversed, sending the energy back down to root and crown tissues (Lamp et al. 2001). As alfalfa continues to flower, the stem lignifies and fiber increases. Carbohydrates continue to accumulate in the root reserves, which increases stand longevity. Therefore, alfalfa harvested later in the growth cycle will have higher yield and stand longevity but lower forage quality, demonstrated by increased fiber content and decreased energy and protein (Ball et al. 2001).

Along with its valuable role as livestock feed, alfalfa provides ecological services. The perennial alfalfa taproot penetrating 7 to 9 m helps stabilize soil and prevent erosion (Sheaffer and Evers 2007). As a legume, alfalfa forms root nodules in a symbiotic relationship with *Rhizobium* nitrogen fixing soil bacteria. Nitrogen added to the soil from this relationship is available to the whichever crop is seeded following the termination of the alfalfa stand; on medium and fine textured soils it is still available for the second year following the alfalfa crop. Nitrogen credits are subtracted from the recommended nitrogen inputs for the following crop ranging from 45-213 kg N ha^{-1} (40-190 lbs N acre^{-1}) depending on stand density, amount of regrowth and soil type (Laboski and Peters 2012). Additionally, alfalfa fields support biodiversity by providing habitat for up to 1,000 arthropod species including pollinators and natural enemies (Flanders and Radcliffe 2013).

However, not all arthropods inhabiting alfalfa fields are beneficial insects. In particular, the potato leafhopper, *Empoasca fabae* Harris (Hemiptera: Cicadellidae) is the most economically damaging pest of alfalfa in the North Central and northeast US. Its diverse host plant list of over 200 species includes the cultivated crops alfalfa (*Medicago sativa* L.), soybean (*Glycine max* L.) and potato (*Solanum tuberosum* L.), as well as roadside, weedy and forest plants (Lamp et al. 1994).

Adults are bright green, approximately 3 mm long by 0.5-1 mm wide with the head segment wider than the abdomen, and 6 white spots on the pronotum. Eggs are oviposited into host plant stems (Delong 1938). Time to eclosion ranges from seven to 14 days with warmer temperatures promoting faster development (Hogg 1985). *E. fabae* has five nymphal instars, which take nine to 18 days to complete development (Hogg 1985). Nymphal stages resemble the adult, with a wider torpedo shape at the head, tapering to the anterior. Development from egg to adult can occur in as little as two weeks or take more than four weeks depending on temperatures. *E. fabae* has three to five overlapping generations during summer in the northern U.S. (Delong 1938, Hogg and Hoffman 1989).

Native to the United States, *E. fabae* annually migrate north from overwintering sites in the Gulf Coast States (Louisiana, Mississippi, Alabama, parts of Florida and Texas) (Decker and Cunningham 1968) and Southern Pines region (eastern Arkansas, Tennessee, South Carolina, North Carolina, and Virginia) (Taylor and Shields 1995) to the Midwest and Northeast U.S. *E. fabae* overwinter as adults in reproductive diapause but begin mating in late February (Taylor and Shields 1995). Initial migrants arriving in the north are female biased (Medler and Pienkowski 1966) and begin arriving sometime in May (Maredia et al. 1998). Arriving females are gravid (Medler and Pienkowski 1966) and continue to lay eggs for the duration of their lives (Decker et al. 1971), approximately 30 days in the wild (Delong 1938). Field studies indicate a female-biased sex ratio near 4:1 through most of the season until it approaches 1:1 towards the end of the growing season (Medler 1966 and Pienkowski, Decker et al. 1971, Flinn et al. 1990a, Emmen et al. 2004). In late summer, *E. fabae* enter reproductive diapause and return unmated to their overwintering habitat, assisted by northerly winds (Taylor 1989, Taylor and Shields 1995).

E. fabae feeds by inserting its stylet into plant vascular tissue, rupturing cells and ingesting phloem cell nutrients (Backus et al. 2005). Hopperburn is the term used to describe feeding injury symptoms, which occur from the combination of mechanical damage by the stylet puncturing the phloem tissue and exacerbated by salivary secretions (Ecale and Backus 1995, Backus et al. 2005). Hopperburn is expressed as triangular v-shaped yellowing at the leaflet tips. Other feeding injury symptoms include stunted growth, leaf tip-wilting, leaf chlorosis, and premature leaf-drop (Backus et al. 2005).

The first cutting of a season typically escapes damage due to the timing of spring migration. First crop is harvested before significant population buildup, but subsequent cuttings are at risk of intensive *E. fabae* pressure and damage. *E. fabae* feeding reduces alfalfa yield and crude protein content (Lamp et al. 1985). Left uncontrolled, *E. fabae* feeding can decrease the yield in the following cutting or year (Wilson et al. 1955, Vough et al. 1992) due to disruption in photoassimilate translocation to the roots and crown tissues where carbohydrates and N-containing compounds are stored (Flinn et al. 1990b, Lamp et al. 2001). Yield and quality impacts to alfalfa are greater for young plants, either in the seeding year or shortly after harvest during the regrowth phase (Kouskolekas and Decker 1968, Cuperus et al. 1983, Hower 1989).

The current pest management paradigm in alfalfa for *E. fabae* is to monitor the population using weekly sweep net samples and treat with foliar insecticide when economic threshold densities are reached (Degooyer et al. 1998, Cullen et al. 2012). Economic thresholds are management guidelines for insecticide treatment that allow for pest presence but initiate control action before economic crop injury occurs. Pest scouting, economic thresholds, and selective insecticide use to conserve natural enemies for biological control are basic tenets of insect integrated pest management (IPM) (Stern et al. 1959). A fully developed IPM program is comprised of multiple strategies for a given pest or pest complex in a cropping system incorporating host plant resistance, biological, cultural and physical controls when available, and chemical control when necessary (Pedigo 1999). Several IPM management strategies have been developed for *E. fabae* management in alfalfa.

One IPM strategy that has been developed is host plant resistance. Host plant resistance is compatible with chemical, biological and cultural controls. It refers to heritable traits that enable a plant to incur less damage than plants without the traits (Painter 1951, Teetes 2013). Host plant resistance traits can suppress an insect population through antibiosis or antixenosis, or increase plant tolerance to insect damage. Antibiotic traits act by increasing mortality or decreasing fecundity. Antixenotic traits make the plant less attractive to the insect thus modifying insect behavior so they are less likely to utilize the plant as food or ovipositional site. Tolerance traits are demonstrated when the plant has an increased ability to recover or withstand insect damage compared to a susceptible plant with an equivalent insect population (Teetes 2013).

In alfalfa, host plant resistance to *E. fabae* is expressed through the presence of glandular hairs on the stem and leaves (Elden and Elgin 1992), although presence of pubescence does not necessarily confer resistance (Taylor 1956). The pubescence trait is heterozygous and inherited quantitatively through a continuous range of phenotypes via multiple genes making it difficult to isolate and breed for the exact resistance mechanism (Taylor 1956). Initial screening for resistant germplasm was based on decreased incidence of leaf yellowing. This method has slowed progress in resistance breeding (Kindler et al. 1973, Elden and Elgin 1992) because resistant varieties can maintain green foliage without outperforming susceptible varieties (Lefko et al. 2000a).

Early work on potato leafhopper host plant resistance identified candidate germplasm, but the resistance mechanism varied by alfalfa clone tested (Jarvis and Kehr 1966). Some clones demonstrated tolerance to higher leafhopper numbers and reduced leaf yellowing, while others demonstrated antixenosis through a decreased presence of leafhoppers on the plants (Jarvis and Kehr 1966). Alfalfa lines from candidate germplasm with the densest pubescence consistently led to antixenosis of adult feeding and oviposition and nymphal anitbiosis (Elden and Elgin 1992).

The first alfalfa varieties marketed as potato leafhopper resistant were commercially released in 1997 (Miller 1998). Results from both field and lab studies of the market varieties continue to show variability in resistance mechanisms. Lefko et al. (2000b) demonstrated tolerance in the field for three different commercial cultivars. When resistant and susceptible varieties were caged in the field, they had similar yields even though the resistant cultivars were populated with up to 2.5 times the potato leafhoppers. Lefko et al. (2000b) also show that antibiosis was not the mechanism for these resistant cultivars because population growth was similar between both the resistant and susceptible varieties. Lamp et al. (2007) conducted laboratory and field assays and concluded that there is a physiological basis for a tolerance mechanism: when susceptible alfalfa is fed on by potato leafhoppers, the plant responds with reduced rates of photosynthesis and transpiration, while their tested resistant cultivars did not exhibit reduce rates.

However, there are several examples of antibiosis and antixenosis from field and laboratory studies in commercial potato leafhopper resistant varieties. In the field, Sulc et al. (2001) observed lower leafhopper nymph density on resistant cultivars than susceptible cultivars, indicating either antibiosis or antixenosis had a strong role in the resistance mechanism. In a laboratory assay, Ranger and Hower (2001) confirmed that antibiosis is expressed by observing complete nymphal mortality after 48 hours on a resistant alfalfa clone. By performing the same assay with the glandular trichomes removed, Ranger and Hower (2001) confirmed that the presence of trichomes is a key trait generating resistance. Host plant resistance to the first and second instars occurs mechanically when nymphs are entrapped by the glandular hairs and exudates, while resistance to adults and older instars appears to be solely chemical (Ranger and Hower 2001). Further study revealed that nonvolatile fatty acid amide extracts from the glandular trichomes also deter adult settling behavior suggesting antixenotic mechanisms (Ranger et al. 2004).

Economic advantages of resistant varieties vary depending on potato leafhopper pressure. Miller-Garvin et al. (1998) calculated that under high potato leafhopper pressure in the seeding year, resistant alfalfa cultivars earned farmers up to $31 more per acre in improved forage quality and yield, but yields were not significantly different under moderate leafhopper pressure. Similarly, McCaslin (1998) found a 10-20% yield advantage for resistant alfalfa varieties under moderate to high leafhopper pressure, but these same varieties had lower yields than susceptible alfalfa with insecticide treatment or in the absence of potato leafhoppers. Sulc et al. (2001) also observed a yield advantage in resistant alfalfa cultivars when leafhoppers exceeded threshold: when no insecticides were applied, resistant varieties yielded 1.0-1.2 Mg ha^{-1} $year^{-1}$ more than susceptible varieties. However, when populations greatly exceed established economic thresholds, the resistant varieties also incurred yield loss. Additional studies have reported yield drag in resistant alfalfa when leafhopper pressure was low (Hogg et al. 1998, Hansen et al. 2002). Much of this work was done on early releases (also called 1st generation) of resistant alfalfa, which displayed 35% resistance to *E. fabae* but continued improvement in breeding has raised the level of resistance up to 80% (Peterson 2003, Undersander pers. comm).

Host plant resistance also affects alfalfa forage quality. Hansen et al. (2002) compared forage quality of susceptible and resistant alfalfa cultivars over three years at three locations in New York. Resistant alfalfa cultivars had higher crude protein content and fewer visual symptoms of leafhopper damage. Sulc et al. (2004) found that resistant alfalfa had higher crude protein regardless of insecticide applications and lower neutral detergent fiber (NDF) than susceptible alfalfa when insecticides were applied. However, Dellinger et al. (2006) saw no significant difference between the crude protein nor acid detergent fiber (ADF) in the susceptible and resistant alfalfa tested when leafhopper populations were left uncontrolled.[2]

Though pure alfalfa stands are cultivated as high quality forage, forage grasses are promoted as an intercrop with alfalfa for the provision of increased digestible fiber and decreased non-fiber carbohydrates, which can help reduce incidence of ruminal acidosis (Lee, 2011). Alfalfa-grass intercrops provide several other benefits. Growing grass with alfalfa helps maintain a dense ground cover, thereby reducing weed pressure (Spandl et al. 1997). High density, fibrous grass roots also protect against soil erosion (Drolsom and Smith 1976). In contrast to growing

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[2] NDF and ADF are both inversely related to animal feed intake, such that lower amounts are desirable so that the animal eats and grows more (but ADF relates more to digestibility while NDF relates to potential intake). ADF is contained within NDF, which are structural carbohydrates. The NDF is composed of lignin, cutin, cellulose and hemicellulose and ADF excludes hemicellulose (Robinson 1999).

grass alone, alfalfa-grass mixtures produce higher grass biomass as nitrogen is transferred from alfalfa roots to grass roots. Concurrently, the presence of grass roots reduces soil nitrogen encouraging greater nitrogen fixation per unit weight of alfalfa (Briske 2007). However, some studies show that alfalfa-grass mixtures offer little, if any, yield benefit over alfalfa alone (Sleugh et al. 2000). Alfalfa-grass mixtures require careful management due to differential timing of alfalfa and grass maturation. Grass matures more quickly in the spring than alfalfa and its quality begins to diminish when alfalfa forage quality peaks (Spandl and Hesterman 1997).

Several studies have also observed that alfalfa-grass mixtures can be used for potato leafhopper management. Alfalfa stands containing 9% forage grasses, either smooth bromegrass (*Bromus inermis* Leyss) or orchardgrass (*Dactylis glomerata* L.), had 4-37% reduction in potato leafhopper densities compared to alfalfa monocultures (Roda et al. 1997). Degooyer et al. (1999) similarly observed significantly fewer leafhoppers in alfalfa stands intercropped with smooth bromegrass or orchardgrass compared to alfalfa monocultures but noted it was not enough to keep populations below economic thresholds. The above patterns may be due to higher leafhopper emigration out of plots containing grass (Roda et al. 1997), as well as potato leafhopper inability to reproduce on monocots (Lamp et al. 1994).

The present study examined the effects of alfalfa host plant resistance and orchardgrass intercrop on *E. fabae* densities in five field experiments conducted at two locations over a three year period (2010-2012). Corresponding alfalfa yield and forage quality data were analyzed at one location for three of the five field experiments years.

Effective IPM strategies aim to reduce the use of insecticides but chemical control is an integral part of most IPM plans to reduce economic loss (Summers, 1998). Therefore, this work also investigated yield response of reducing the current potato leafhopper economic thresholds in light of the increasing market value of the alfalfa crop. This was done by evaluating yield response to insecticide treatment at current economic threshold and half of the current economic threshold potato leafhopper populations.

Materials and Methods

Experimental sites. Multi-year field experiments were established in two locations: one at Arlington, WI Agricultural Research Station (AARS) (2010-2012) and two at the USDA Dairy Forage and Research Center (DFRC) in Prairie du Sac, WI (2012). The AARS field study was spring seeded and the two field studies established at DFRC included a spring and late summer seeding. At both AARS and DFRC, experiments were arranged in complete randomized block with a 2 x 2 factorial design (four total whole plot treatments). Factorial treatments were alfalfa variety (potato leafhopper-susceptible and -resistant) and orchard grass intercrop (alfalfa monoculture and alfalfa-grass intercrop).

Seeded May 17, 2010 at AARS, the whole plots were 26 m x 6.7 m (85 ft. x 22 ft.) and divided equally into three split plots, 8.5 m x 6.7 m (28 ft. x 22 ft.). Split plot treatments consisting of an untreated control, insecticide treatment at half the current potato leafhopper economic threshold (1/2 ET), and insecticide treatment at the current economic threshold (ET) (Table 1) were included to create a range in leafhopper density. The experiment was divided into four blocks with two replications per block for a total of eight replicates. Pioneer Hi-Bred International, Inc. (Arlington, WI) provided alfalfa seed including leafhopper-susceptible alfalfa 55V48 and resistant alfalfa 53H93 varieties. Profit Orchardgrass was purchased from Welter Seed & Honey Co. (Onslow, IA). Alfalfa was seeded at 20 kg ha^{-1} (18 lbs acre^{-1}) and grass was seeded at 4.5 kg ha^{-1} (4 lbs acre^{-1}). Due to low grass establishment during the first growing season, grass was reseeded September 10, 2010 at 6.7 kg ha^{-1} (6 lbs acre^{-1}).

At DFRC, the late summer seeding was planted August 16, 2011 and the spring seeding was planted April 12, 2012. Each whole plot was 18.3 m x 9.1 m (60 ft. x 30 ft.). No split plots were created at DFRC because this site was only intended to study potato leafhopper response to alfalfa variety and orchardgrass, and not intended to study yield response to insecticide treatment. Seed was provided by Forage Genetics, International (Nampa, ID) including leafhopper-susceptible alfalfa WL354HQ and resistant alfalfa WL353LH varieties. Profit Orchardgrass was purchased from Welter Seed & Honey Co. Alfalfa was seeded at 13.5 kg ha^{-1} (12 lbs acre^{-1}) and grass was seeded at 4.5 kg ha^{-1} (4 lbs acre^{-1}) as per University of Wisconsin-Extension recommended guidelines (Undersander et al. 2004).

Insect sampling and insecticide treatments. Potato leafhopper populations were monitored weekly in each experiment using a 38 cm (15-inch) diameter sweep net to collect 20 sweep net samples per split plot at AARS, and 20 sweeps per plot at DFRC. Sweep net sampling is widely used in Wisconsin management programs for potato leafhopper and has been shown to have strong correlation with absolute density (Fleischer et al. 1982). At AARS, the pyrethroid insecticide Warrior II (active ingredient lambda-cyhalothrin) was applied at 1.6 oz acre^{-1} when leafhopper densities reached 1⁄2 ET and ET in at least half of the respective split plots (Table 1).

Yield and forage quality. Yield data were collected from each plot at each harvest per growing season at AARS using an Almaco plot harvester through the center of the plot when plants reached approximately 10% bloom stage. Harvested plant subsamples were oven dried at 60°C and yields calculated on a dry matter basis. Alfalfa quality (crude protein and neutral detergent fiber) as well as grass percentage was analyzed by near-infra red reflectance (NIR) methods on dried and ground alfalfa samples. Yield and forage quality data were not collected at DFRC.

Statistical analysis. Potato leafhopper response to alfalfa variety and orchardgrass at both AARS and DFRC was determined by individual ANOVA for each sampling date with a random block effect (PROC MIXED, SAS Institute 2008). The percentage of orchard grass in alfalfa-grass intercrop plots was assigned to three levels for statistical analysis based on the NIR results at AARS and visual estimation at DFRC: low (0-9%), moderate (10-19%) and high (>20%). Significant differences between least square means were determined through Tukey-Kramer post-hoc test. Because no harvest data were collected at DFRC, visual estimations of grass percentage were recorded at the end of 2012 growing season. Initial analyses found no interactions between variety and orchardgrass percentage so only analyses of main effects (alfalfa variety, orchardgrass) are presented.

Repeated measures analyses were also initially conducted to examine potato leafhopper response as mixed effects models in R version 2.10.1 (R Development Core Team 2009) using Rpackage *nlme*. Fixed effects included in the model were alfalfa variety, orchardgrass presence, sample date, and interactions between sample date and alfalfa variety and orchardgrass presence respectively. Random effects were block and plot ID nested within block. Orchardgrass was analyzed as presence versus absence instead of as percentage levels because the percentage levels change per plot over time. However, whole plot treatments interacting with time precluded the interpretation of these results (see Appendix I-V).

Yield response to insecticide treatments was analyzed for the harvest at AARS only on July 26, 2010 because this was the only crop that both insecticide treatments (1/2 ET and ET) were applied (Table 1). Mixed model analysis was performed (PROC MIXED, SAS Institute 2008) with yield as the dependent variable, and insecticide treatment, alfalfa variety, and orchardgrass percentage as fixed effects, and a random block effect. Orchardgrass percentage was again assigned to three levels for statistical analysis based on the NIR results: low (0-9%), moderate (10-19%) and high (>20%). Interactions were tested and dropped when non-significant. Bonferroni corrections were used to determine significant differences of least squares means between yields within whole plot treatments.

Yield and forage quality response to potato leafhopper, alfalfa variety and grass was determined for each harvest at AARS by mixed model analysis with a random block effect (PROC MIXED, SAS Institute 2008). Significant differences between least square means were determined through Tukey-Kramer post-hoc test. Potato leafhopper effect was analyzed as average potato leafhopper counts by summing the potato leafhoppers recovered in 20 sweep net samples for each sample date in a crop and dividing by the number of sample dates per crop. As noted above, percentage orchardgrass in alfalfa-grass plots was analyzed by three levels based on NIR results: low (0-9%), moderate (10-19%) and high (>20%). The interaction between whole plot treatments and potato leafhoppers was examined and omitted for the harvests for which there was no significant interaction but retained when the interaction was significant.

The effect of alfalfa variety, orchardgrass and cumulative potato leafhoppers on the cumulative yield for 2010 and 2011 was also determined. Cumulative yield was determined by totaling yields for each cutting. Cumulative potato leafhoppers were determined by adding together the potato leafhopper values used for each harvest. For these analyses, orchardgrass was analyzed as presence versus absence instead of the percentage classes because orchardgrass composition varied each harvest. Analysis was completed with a mixed effects model using alfalfa variety, orchardgrass presence and cumulative potato leafhoppers as fixed effects and block as a random effect in R version 2.10.1 (R Development Core Team 2009) using Rpackage *nlme*. All interactions were checked and excluded when not significant.

Results

Potato Leafhopper Analysis.

AARS 2010. Potato leafhopper sampling was conducted 10 times in 2010 from June 10 to August 26. Potato leafhoppers reached the half-economic threshold treatment density (1/2 ET) during second crop on July 6; respective split plots were sprayed with insecticide July 7. On July 8, potato leafhoppers reached the economic threshold treatment density (ET) and respective split plots were sprayed with insecticide on July 9. Potato leafhopper populations in the resistant alfalfa plots were significantly lower on 4 of the 10 sampling dates: July 6 (df=1, 25; F=7.32; p=0.01), July 19 (df=1, 25; F=18.59; p<0.01), August 9 (df=1, 25; F=14.86; p<0.01) and August 26 (df=1, 25; F=6.87; p=0.01) (Fig. 1). Alfalfa-grass intercrop had a significant effect on June 10 (df=2, 25; F=4.29; p=0.03) (Fig. 2). On July 19, raw means used to produce figure 2 suggest that high orchardgrass suppressed potato leafhoppers but due to low orchardgrass establishment there were only n=2 data points for this orchardgrass class, and both of these data points are in susceptible plots so there are no significant differences in least square means.

AARS 2011. Potato leafhopper sampling was conducted 9 times in 2011 from June 7 to August 25. Potato leafhopper populations never reached economic threshold in 2011, but the half-economic threshold treatment density was reached twice; on July 14 and August 10. Respective split plots were sprayed with insecticide on July 15 and August 11. Potato leafhopper populations were significantly lower in resistant alfalfa plots at only one sample date: August 8 (df=1, 25; F=6.31; p=0.02) (Fig. 3). On July 12, orchardgrass had a significant effect on potato leafhopper abundance (df=2, 25; F=4.86; p=0.02) (Fig. 4).

AARS 2012. Potato leafhopper sampling was conducted 10 times in 2012 from May 22 to August 7. Potato leafhoppers reached the half-economic threshold treatment density during second crop on July 2 and respective split plots were sprayed with insecticides on July 3. Potato leafhoppers did not reach the economic threshold density for any cutting during the season. The potato leafhopper population was significantly lower in plots with resistant alfalfa on two sample dates: July 10 (df=1, 25; F=7.10; p=0.01) and July 16 (df=1, 25; F=11.33; p<0.01) (Fig. 5). Orchardgrass suppressed potato leafhopper abundance on three sample dates: May 22 (df=2, 25; F=3.57; p=0.04), June 12 (df=2, 25; F=5.98; p<0.01) and July 2 (df=2, 25; F=4.92; p=0.02) (Fig. 6).

DFRC 2012. Potato leafhopper sampling was conducted 10 times in 2012 from May 21 to August 7 in the spring and late summer seeded experimental plots, respectively. In the spring seeded experiment, potato leafhopper abundance was suppressed in resistant alfalfa plots on five dates: June 6 (df=1, 9; F=9.79; p=0.01), June 21 (df=1, 9; F=7.23; p=0.02), June 26 (df=1, 9; F=12.23; p<0.01), July 3 (df=1, 9; F=6.89; p=0.03) and August 7 (df=1, 9; F=9.36; p=0.01) (Fig. 7). There was no significant orchardgrass effect in the spring seeding, but there was a trend in which leafhoppers were more abundant in plots that had high orchardgrass (Fig. 8).

In the late summer seeded experiment, potato leafhopper abundance was lower in plots with resistant alfalfa on two dates: July 3 (df=1, 10; F=9.51; p=0.01) and August 7 (df=1, 10; F=8.83; p=0.01) (Fig. 9). There was no significant orchardgrass effect at any of the sample dates, however none of the alfalfa-orchardgrass intercrop treatment plots in the late summer seeded experiment had greater than 20% orchardgrass (Fig. 10).

Precipitation. The 2010 growing season had greater than average precipitation. Compared to historical monthly averages (1981-2010), May received 112%, June 162%, July 224% and August 121% (NOAA-NCDC 2013). The 2011 growing season had a drought: compared to the historical monthly averages (1981-2010), May received 59%, June received

87%, July received 60% and August 37% (NOAA-NCDC 2013). Lack of adequate moisture created drought stress in parts of the field experiment late in the season, and we omitted data from the effected four whole plots (12 split plots) in the northeast corner (three plots of susceptible alfalfa, one of which was interseeded with orchardgrass, and one plot of resistant alfalfa monoculture). In 2012, the experiment was harvested on June 20, July 17, and August 14. However, the 2012 growing season experienced an even more severe drought than 2011; compared to the historical monthly averages (1981-2010), May received 80%, June received 6%, followed by average rainfall for July and 74% of normal for August (NOAA-NCDC 2013).

Effect of insecticide timing on yield. Potato leafhopper populations only reached economic threshold in July 2010, therefore July 26, 2010 (seeding year, second cutting) was the only harvest for which we were able to compare both half- and full economic threshold insecticide timing split plot treatments to the untreated control across whole plots (variety x orchardgrass). Insecticide treatment timing had no significant effect on yield (df=2, 87; F=2.94; p=0.06) (Table 2). Untreated control split plots did have lower, though not statistically different, yields (M=1.40, SD=0.07); there was no statistical difference between plots receiving full economic threshold insecticide treatment (M=1.53, SD=0.07) and the ½ economic threshold treatment (M=1.50, SD=0.07).

Yield and Forage Quality Analyses. Yield and forage quality analyses are presented (Tables 3-14) for the Arlington Agricultural Research Station site for seven harvests over three growing seasons: July 26 and September 7, 2010, June 1, July 5, August 1 and September 1, 2011, and June 20, 2012. In 2010, the experiment was harvested three times, however no data were collected at first cutting (July 1) because the field had a significant weed population. This first harvest served to remove the annual weeds, and aid stand establishment. Yield and forage quality data were only collected on June 20 during the 2012 season because alfalfa expressed visible drought stress symptoms disparately across the field, such that parts of the stand never grew above six inches, and data may not reliably represent experimental treatments.

Effects of potato leafhopper on yield and forage quality. Although insecticide treatment had no significant effect on yield for the July 26, 2010 harvest, cumulative potato leafhopper population prior to harvest did have a significant negative impact on yield (df=1, 88; F=4.08; p=0.05) (Table 3). Cumulative potato leafhoppers had a significant negative effect on the total yield of 2010 (df=1, 89; F=4.20; p=0.04) (Table 4). Potato leafhoppers also had a negative impact on yield for the June 20, 2012 harvest (df=1, 88; F=7.22; p=0.01) (Table 3).

On August 1, 2011, the interaction of potato leafhoppers and variety on yield was significant; (df=1, 86; F=11.95; p<0.01); potato leafhoppers lowered yields of resistant alfalfa, but not of susceptible alfalfa. For the September 1, 2011 harvest, the effect of leafhoppers interacted significantly with orchardgrass presence (df=2, 74; F=6.09; p<0.01) (Table 3).

Potato leafhoppers decreased crude protein content, at one harvest: July 26, 2010 (df=1, 80; F=5.56; p=0.02) and had a significant interaction with orchardgrass on July 5, 2011 (df=2, 85; F=4.88; p=0.01) (Table 5). Potato leafhoppers did not have an effect on neutral detergent fiber (NDF) at any harvest, however they did have a significant interaction with orchardgrass on July 5, 2011 (df=2, 85; F=5.41; p<0.01) (Table 6).

Effects of alfalfa variety on yield and forage quality. Resistant alfalfa yields were 6-11% lower than susceptible alfalfa yields for four of the seven harvests: June 1, 2011 (df=1, 88; F=11.59; p<0.01), July 5, 2011 (df=1, 88; F=23.20; p<0.01), September 1, 2011 (df=1, 74; F=15.11; p<0.01) and June 20, 2012 (df=1, 88; F=12.55; p<0.01) (Table 7). For all of these harvests, the leafhopper population during the previous month never reached economic threshold. However, on July 26, 2010, resistant alfalfa yield was comparable to susceptible alfalfa yield when the potato leafhopper population reached economic threshold density (df=1, 88; F=0.60; p=0.44) (Table 7). In 2011, alfalfa variety had a significant effect on total yield (df=1, 77; F=26.56; p<0.01) (Table 8). Resistant alfalfa yielded about one half a ton acre^{-1} (1.2 Mg ha^{-1}) less than susceptible alfalfa over the 2011 season.

Resistant alfalfa had significantly higher crude protein content at five harvests: September 7, 2010 (df=1, 88; F=21.03; p<0.01), July 5, 2011 (df=1, 85; F=12.81; p<0.01), August 1, 2011 (df=1, 86; F=21.34; p<0.01), September 1, 2011 (df=1, 75; F=39.43; p<0.01) and June 20, 2012 (df=1, 81; F=21.61; p<0.01) (Table 9). However, susceptible alfalfa had significantly higher crude protein content than resistant alfalfa on July 26, 2010 (df=1, 80; F=4.13; p=0.05), the same harvest in which potato leafhopper economic thresholds were reached.

Susceptible alfalfa had statistically greater NDF content at four harvests: September 7, 2010 (df=1, 88; F= 22.44; p<0.01), August 1, 2011 (df=1, 86; F=4.49; p=0.04), September 1, 2011 (df=1, 75; F=8.71; p<0.01) and June 20, 2012 (df=1, 81; F=24.49; p<0.01) (Table 10). There was a significant variety x orchardgrass interaction effect on NDF content on June 1, 2011 (df=2, 86; F=7.22; p<0.01); orchardgrass increased NDF content more in resistant alfalfa than in susceptible alfalfa.

Orchardgrass effects on yield and forage quality. The impact of orchardgrass on yield was variable, having a significant effect at four harvests: June 1, 2011 (df=2, 88; F=3.21; p=0.05), August 1, 2011 (df=2, 86; F=4.66; p=0.01), September 1, 2011 (df=2, 74; F=5.32; p<0.01) and June 20, 2012 (df=2, 88; F=4.46; p=0.01) (Table 11). The presence of orchardgrass resulted in higher yield than alfalfa alone, regardless of variety, on June 1, 2011 but lower yield on August 1, 2011 and September 1, 2011. On June 20, 2012, yields were lower in plots with a moderate amount of orchardgrass than plots with either low or high orchardgrass presence. However, the presence of grass did not have a significant effect on cumulative yield in either 2010 (df=1, 89; F=2.04; p=0.16) or 2011 (df=1, 77; F=1.61; p=0.21) (Table 12).

Crude protein content decreased in both susceptible and resistant alfalfa with increasing orchardgrass presence at all harvests except for July 26, 2010 (first cutting of the seeding year): September 7, 2010 (df=2, 88; F=7.00; p<0.01), June 1, 2011 (df=2, 86; F=66.12; p<0.01), July 5, 2011 (df=2, 85; F=4.44; p=0.01), August 1, 2011 (df=2, 86; F=3.38; p=0.04), September 1, 2011 (df=2, 75; F=12.78; p<0.01), and June 20, 2012 (df=2, 81; F=38.53; p<0.01) (Table 13). There was a significant orchardgrass x variety effect on crude protein content on June 1, 2011 (df=2, 86; F=7.11; p<0.01).

Neutral detergent fiber content increased with orchardgrass presence at every harvest: July 26, 2010 (df=2, 80; F=4.16; p=0.02), September 7, 2010 (df=2, 88; F=13.63; p<0.01), June 1, 2011 (df=2, 86; F=69.18; p<0.01), July 5, 2011 (df=2, 85; F=8.04; p<0.01), August 1, 2011 (df=2, 86; F=5.45; p<0.01), September 1, 2011 (df=2, 75; F=8.98; p<0.01), and June 20, 2012 (df=2, 81; F=68.93; p<0.01) (Table 14).

Discussion

Host plant resistance suppressed potato leafhopper populations at different sampling points over the five site years, but most consistently in the seeding years (Figs. 1 and 7). In general, alfalfa variety had an erratic effect on potato leafhopper abundance during production years, but the decrease in abundance was most evident at peak leafhopper time points, as seen in 2012 at both the AARS site and the DFRC late summer seeding site (Figs. 5 and 9). However, the first production year at AARS only had one time point in which alfalfa variety had a statistically significant effect on potato leafhopper abundance: the first sample date after the August 1 harvest (Fig. 3). Although this does not appear biologically relevant due to the small potato leafhopper population, it may be economically relevant because small differences in leafhopper abundance immediately following a harvest (when regrowth is still short) could eliminate the need for an insecticide application if farmers are scouting and observing economic threshold.

Though there were times that potato leafhoppers were less abundant in plots seeded with resistant alfalfa, resistant alfalfa never out-yielded susceptible alfalfa in our study. This is congruent with the work of Hansen et al. (2002), in which fewer potato leafhoppers and lower visual damage was observed in resistant alfalfa stands, but a statistically greater yield was found in the resistant stands from only one of their four trials. At our AARS location, resistant alfalfa had similar yields to susceptible alfalfa at both harvests in 2010, but had significantly lower yields at three of the four harvests in 2011 and the one harvest measured in 2012. In 2011, this yield drag amounted to a total of about one half ton acre^{-1} less throughout the season which would be a significant economic loss for farmers. This is consistent with previous field studies in which potato leafhopper pressure ranged from low to moderate (Miller-Garvin et al. 1998, McCaslin 1998, Sulc et al. 2001). Overall, potato leafhopper pressure was low for the duration of our study at both AARS and DFRC locations. In contrast, Sulc et al. (2001) found that the yield advantage of growing resistant alfalfa increased under higher leafhopper pressure. In our study, economic thresholds were reached in the summer of 2010 at AARS in the seeding year, which is the only year for which resistant alfalfa yield was statistically equivalent to that of susceptible. Alfalfa breeding for leafhopper resistance and higher yields has been difficult because leafhopper populations are unpredictable from year to year and from location to location and in infestation timing relative to alfalfa growth (Miller 1998). This unpredictability in combination with alfalfa's complicated resistance genetics such as heterozygous, quantitative traits and mixed resistance mechanisms has made the quest for potato leafhopper resistant alfalfa a challenging process.

The effect of orchardgrass intercropped with alfalfa on potato leafhoppers was minimal. During the production years at AARS (2011 and 2012), there were a couple of time points in which potato leafhopper population was significantly lower in the alfalfa-orchard grass intercrop treatments (Figs. 4 and 6). There was almost no effect during seeding years except that plots with more grass showed a non-statistically significant trend of higher leafhopper numbers than plots with no grass at the DFRC location (Fig. 8). Even though this trend was not statistically significant, it does highlight the inconsistency in the effect of orchardgrass presence on leafhopper response. However, presence of orchardgrass in the plots on July 12, 2011 did significantly reduce potato leafhoppers (Fig. 4) and would have eliminated the need to apply insecticides if a farmer intended to treat at ½ economic threshold.

Previous researchers have noted inconsistencies in the relationship between grass presence and potato leafhopper suppression but other work on the effect of grass intercrops on leafhopper abundance has been more notable (e.g. Lamp et al. 1984a, Roda et al. 1997, Degooyer et al. 1999). It is possible that polycultures (alfalfa plus weeds) present a more consistent suppressive effect on potato leafhoppers than that of intentional forage grass interseeding (Lamp et al. 1984a, Straub et al. 2013). Straub et al. (2013) also suggest that a polyculture effect on potato leafhopper abundance may be mediated through more efficient predation by *Nabis* spp. (Hemiptera: Nabidae). The authors speculate that increased predation could be due to increased movement of potato leafhoppers in polycultures making them more vulnerable as prey, though further research would be needed to elucidate the mechanism. We did not monitor natural enemy abundance in our experiment so we cannot comment on the contribution (or lack thereof) of predation to the lack of grass intercrop effect on potato leafhopper abundance. Regardless, considering the positive effects of interseeding grasses and alfalfa such as reduction in weed pressure, protection from soil erosion, complementary nitrogen use, and health benefits for dairy cows, growing alfalfa-grass mixtures should still be considered for insect pest management even if the effect is inconsistent.

Potato leafhopper populations were different between the two seedings at DFRC; in the same growing season, the spring seeded experiment had higher leafhopper pressure than the late summer seeded experiment. Seeding time also interacted significantly with potato leahopper response to alfalfa variety (Fig. 11). Spring seeded susceptible alfalfa had the greatest potato leafhopper pressure. Importantly, spring seeded resistant alfalfa had lower leafhopper abundance, similar to both the susceptible and resistant alfalfa in the late summer seeded experiment. However, because the experiment at DFRC was not specifically designed to test the effect of seeding time on insect response (i.e., the seeding time was not randomized within the blocks), results from the statistical analyses should be inferred with caution.

There are other factors that could have led to this significant seeding time effect and seeding time by variety interaction but they do not seem to explain the observed results. For example, field location or management history would not explain the results because the trials were located adjacent to each other in a field with uniform cropping history and nutrient management history (Fig. 12). The only unique management between the two trials was that prior to the spring seeding, the area was sprayed with Roundup herbicide (active ingredient, glyphosate). Another possible factor for these results could be location within the field. Higher densities of potato leafhopper are found along field margins (Flinn et al. 1990b). However, both spring and late summer seeded trials were located within a late summer seeded field (Fig. 12) and the border between the field edge and the spring seeded trial had similar leafhopper pressure to the late summer seeded research trial so this does not seem a likely cause. Lastly, potato leafhopper abundance has been studied in relation to weed density. The spring seeding had considerably greater weed density (and higher leafhopper abundance) than the late summer seeding; however, the relationship between weed density and potato leafhoppers depends on weed species composition. The most prevalent weed by visual estimation was common lambsquarter (*Chenopodium album*) and research has shown this plant does not promote leafhopper growth (Lamp et al. 1984b).

Implications from these observations suggest that farmers might consider leafhopper management benefits of late summer planting. However, this comes with its own agronomic risk of more unpredictable fall precipitation to provide adequate moisture for good stand establishment (Rankin 2001). Further research would be beneficial to design a study specifically intended to compare potato leafhopper response to late summer versus spring seeding.

Regardless of the diversity of non-chemical pest management practices available, it is inevitable that insect pest populations will reach densities that impact yield and threaten economic profits in some years. Therefore, it is necessary to rely on pest scouting and current, research derived economic thresholds for timely insecticide application. The one opportunity that we had during these field trials to test a reduced economic threshold showed that there was no statistical difference in yield between the current economic threshold and a half economic threshold treatment, and only a marginal difference in yield between the full threshold plots and the control plots with no insecticide treatment (Table 2). Results from this study can be used in

UW Extension programming to demonstrate to farmers that reduced-threshold treatment timing is not economically advisable and to alleviate grower concerns of the need for reducing the economic threshold in light of increased alfalfa market value (Holin 2008). It is worth noting that both insecticide treatments occurred shortly after harvest, when alfalfa regrowth was minimal: in the 0-4 inch height category. The half economic threshold treatment for this crop height is 0.1 leafhoppers per sweep while established economic thresholds are already very low for that crop height, 0.2 potato leafhoppers per sweep (Table 1). These population densities were reached and insecticide treatment applied on July 7 and July 9, just two days apart. By contrast, when alfalfa is 8-12 inches the economic threshold is 1.0 potato leafhopper per sweep and the half threshold is 0.5 leafhoppers per sweep. This difference in pest pressure could have caused a significant difference on yield but leafhoppers never reached densities required to apply insecticide to split plot treatments at this greater alfalfa height. We can only begin to infer that there is no difference between threshold and half threshold treatments when alfalfa regrowth is very short and cannot comment at this time on the validity of reducing the current economic threshold in taller alfalfa.

Because potato leafhopper population densities can change quickly in the field, going from ½ threshold to threshold in a matter of 1 to 2 days, insecticides were applied for a given treatment when at least half of the plots in the treatment had reached the respective potato leafhopper density. In practice, this meant that some plots within a treatment were sprayed when leafhopper numbers were either above or below the target treatment density. This gray area of insecticide spray timing in field research on insect pests with explosive growth potential, such as potato leafhopper (Hogg 1985), makes it impossible to apply insecticide treatments when all plots are at the target density. This could explain why even though the effect of insecticide on yield was only marginally significant, we did see a statistically significant effect of cumulative potato leafhopper on yield for the harvest on July 26, 2010.

Considering that the July 2010 crop was the only one in which economic threshold populations were detected, it is apparent that low pest pressure was present for the majority of this study. Despite this low overall leafhopper pressure, alfalfa yield was still affected negatively affected by potato leafhoppers June 20, 2012 at AARS. The yield loss coefficient for potato leafhoppers at this harvest was -0.09, representing a 0.09 ton loss per acre alfalfa yield loss for each additional leafhopper found in 20 sweeps of a sweep net. On July 26, 2010 the yield loss coefficient was -0.04. Considering that economic thresholds were not reached during the crop prior to June 20, 2012 harvest, the stronger negative effect of potato leafhoppers on yield for the June 2012 harvest may have been a result of interactions between potato leafhopper damage and water stress from lack of rain in June 2012. There was only 0.26 inches of rain in June 2012, which is 6% of the historical average (NOAA-NCDC 2013). Potato leafhopper damage to alfalfa quality is additive with the effect of drought stress on alfalfa quality (Schroeder et al. 1988). Furthermore, Barta et al. (2002) observed a similar additive effect between potato leafhopper damage and drought stress on root dry matter accumulation. However, with regards to aboveground biomass, the two stressors were not additive; plants stressed by drought did not show additional effects of potato leafhopper damage (Barta et al. 2002).

The above yield loss coefficients are especially interesting considering the gain thresholds based on the current range of control costs and alfalfa market values. The gain threshold is the amount of yield loss when economic damage begins: when the cost of the insecticide application is equal to the market value of the yield lost. It is calculated by dividing the cost of control by the market value of the crop (Pedigo et al.1986). Average prices for high quality alfalfa forage throughout the upper Midwest range from \$200 ton^{-1} to \$300 ton^{-1} (Barnett 2013). The cost of insecticide application ranges from \$9.60 acre^{-1} to \$41.70 acre^{-1} (Cullen et al. 2012, USDA NASS 2011, USDA NASS 2013b). These costs produce a gain threshold ranging from 0.03 tons acre^{-1} (when value=\$300 and cost=\$9.60) to 0.21 tons acre^{-1} (when value=\$200 and cost=\$41.70). Thus, in July 2010, potato leafhoppers left untreated in the control plots surpassed economic threshold and made economic impact, with a yield loss coefficient of 0.04 tons acre^{-1} for each additional leafhopper in 20 sweeps of the sweep net. In June 2012, potato leafhopper damage surpassed the gain threshold even when economic thresholds were not reached, suggesting that the economic threshold should be re-evaluated during drought conditions and potentially lowered.

Leafhoppers had an interesting interaction with main plot effects (alfalfa variety, grass intercrop) at two different harvests. First, on August 1, 2011 a significant potato leafhopper x alfalfa variety interaction resulted in lower resistant alfalfa yield, yet a higher susceptible alfalfa yield. This occurred under drought conditions so it may be that resistant alfalfa is more negatively impacted by drought stress. Second, at the September 1, 2011 harvest, there was a significant potato leafhopper x orchardgrass effect on yield: plots with moderate grass composition (10-19%) had lower yields, whereas leafhoppers had no effect on yield at either low (0-9%) or high (>20%) grass composition.

Yield was highest in plots with more than 20% orchardgrass presence for the first cutting of 2011 (first production year). We expected a similar trend in the June 20, 2012 cutting considering previous work in which yield was highest in the mixed alfalfa-grass stands for the first harvest of each growing season (Spandl and Hesterman 1997), however this was not the case. Stands with the least amount of orchardgrass at the first cutting in 2012 had the greatest yield. It is possible, given the moisture stress from 2011 and continued drought conditions in 2012, that alfalfa could outcompete the grass due to its extensive taproot. It is interesting to note that yield was lowest at both of these harvest dates for plots that had a moderate (10-19%) orchardgrass. This, in combination with the fact that on September 1, 2011, yield was more negatively affected by potato leafhoppers in the moderate grass plots, suggests that if farmers plant mixed alfalfa-grass stands it may be advisable to manage for a relatively high, rather than moderate, grass composition. However, when looking at the effect of grass presence in plots over an entire summer, the effect of orchardgrass on yield becomes insignificant.

Hower and Flinn (1986) documented that potato leafhopper feeding decreases crude protein even before visual hopperburn injury is apparent. The fact that potato leafhopper only had a significant effect on crude protein for the July 26, 2010 harvest, when economic threshold densities were reached, reiterates that potato leafhopper populations were low for the majority of the study. Though changes in neutral detergent fiber and crude protein can occur with potato leafhopper feeding, Hutchins et al. (1989) determined that the greatest influence that potato leafhoppers have on forage quality is biomass reduction. They concluded that future pest management research on potato leafhopper should focus on alfalfa yield improvements rather than on forage quality.

There is an inverse relationship between whole plot factors that promote greater crude protein content and factors that promote greater neutral detergent fiber: resistant alfalfa had greater crude protein content at five of the seven harvests at AARS, while in four of the seven harvests, susceptible alfalfa had greater fiber content. The reason for this is unclear. It may be related to alfalfa physiological stages and resistant alfalfa yield drag. If resistant alfalfa demonstrates yield drag due to slower maturation rates than susceptible alfalfa, it would follow that if the two are harvested at the same time, resistant alfalfa would have less stem lignification and therefore less fiber and greater protein. However, Sulc et al. (2004) rated maturity of susceptible and resistant cultivars and saw that resistant alfalfa is more mature at harvest, so this explanation is not likely. Most previous studies on the matter found resistant alfalfa to have higher protein levels (Hansen et al. 2002, Sulc et al. 2004) while Dellinger et al (2006) saw no difference in crude protein between resistant and susceptible varieties. Regardless of alfalfa variety, crude protein content in our study was always above 20%, which exceeds the minimum recommended dietary amount for dairy cow growth, reproduction, fattening and lactation (Ball et al. 2001).

Similarly, percent orchardgrass composition had a consistent positive relationship with an increase in neutral detergent fiber content at every harvest, yet decreased crude protein at six of the seven harvests. The only harvest in which this pattern is not apparent was the first harvest of the seeding year, when economic threshold populations were reached. Though there was no significant effect of orchardgrass on potato leafhopper abundance for this crop (July 26 2010), there is a visual trend (Fig. 2) showing potato leafhoppers were less abundant in plots with greater than 20% grass. Recall that at this harvest, potato leafhoppers did have a negative effect on crude protein (Table 5) so it follows that if potato leafhopper feeding was reduced in plots with more orchardgrass, effects of leafhoppers on crude protein content could be mitigated. This may explain why this harvest is the only one that did not have a significant relationship between orchardgrass and crude protein.

Alfalfa farmers may be opposed to including grass in their alfalfa stands because increasing fiber content decreases animal intake (Ball et al. 2001). Although neutral detergent fiber content is increased by grass presence, the fiber in the grass is more digestible and therefore does not reduce animal intake in the same way that alfalfa fiber does (Peterson 2013). Additionally, even though orchardgrass decreased protein, there was still consistently ample protein among all orchardgrass percentages in our study, and the increase in fiber can increase available energy to dairy cows (Anderson 2009).

Conclusions. As seen in the results of this study, the economic and ecological advantages of glandular haired, resistant alfalfa are experienced fewer times than the economic loss based on small quantities of yield drag. Perhaps the profits lost via yield drag would be regained based on increased forage quality via higher crude protein content. Though in the past, resistant alfalfa seed was purchased at a price premium (Dellinger et al. 2006) this is no longer the case. By removing the price premium, resistant alfalfa becomes economically viable only when potato leafhopper populations approach economic thresholds. In geographic regions where populations are more predictably economically damaging, glandular haired resistant alfalfa is an integral part of a successful IPM plan.

The ecological values of orchardgrass intercroppings along with the added value in dairy herd health make this practice worthwhile, however it does not appear to have a consistent or great enough effect on potato leafhopper populations to warrant its inclusion in the IPM paradigm.

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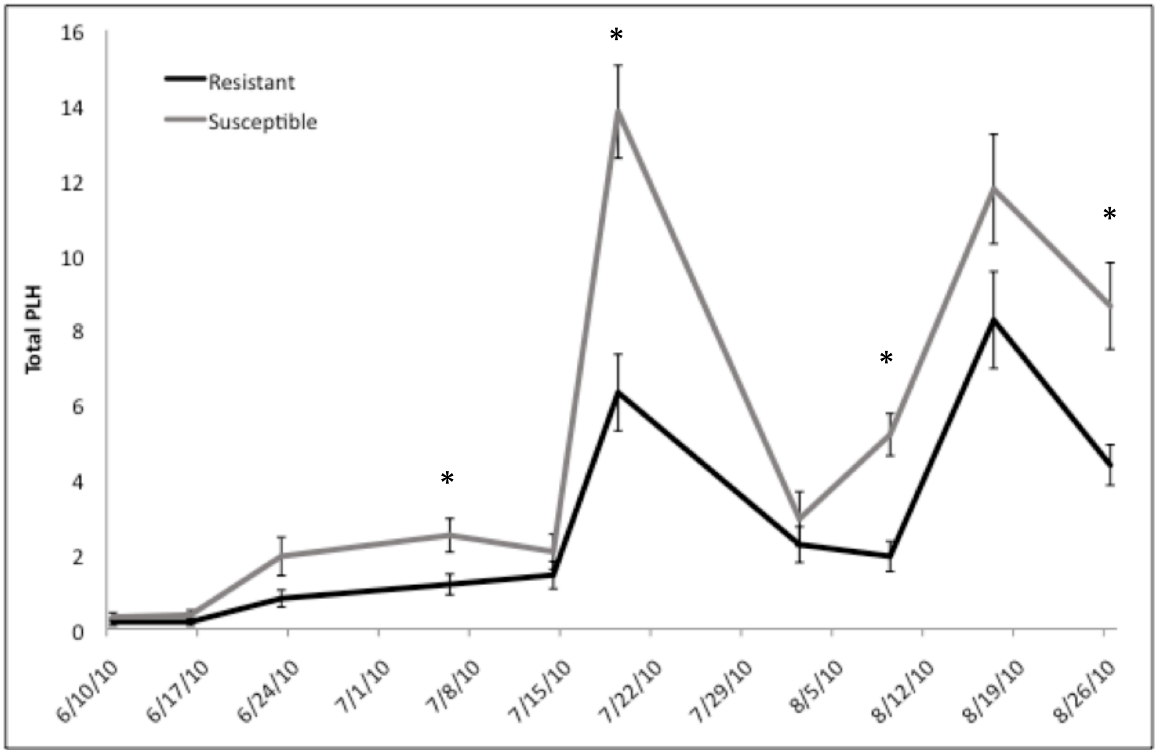

Figure 1. Potato leafhopper response to alfalfa variety at Arlington Agricultural Research Station in 2010 (seeding year). Total PLH is the number of potato leafhoppers found in 20 sweeps. Significant differences indicated with (*).

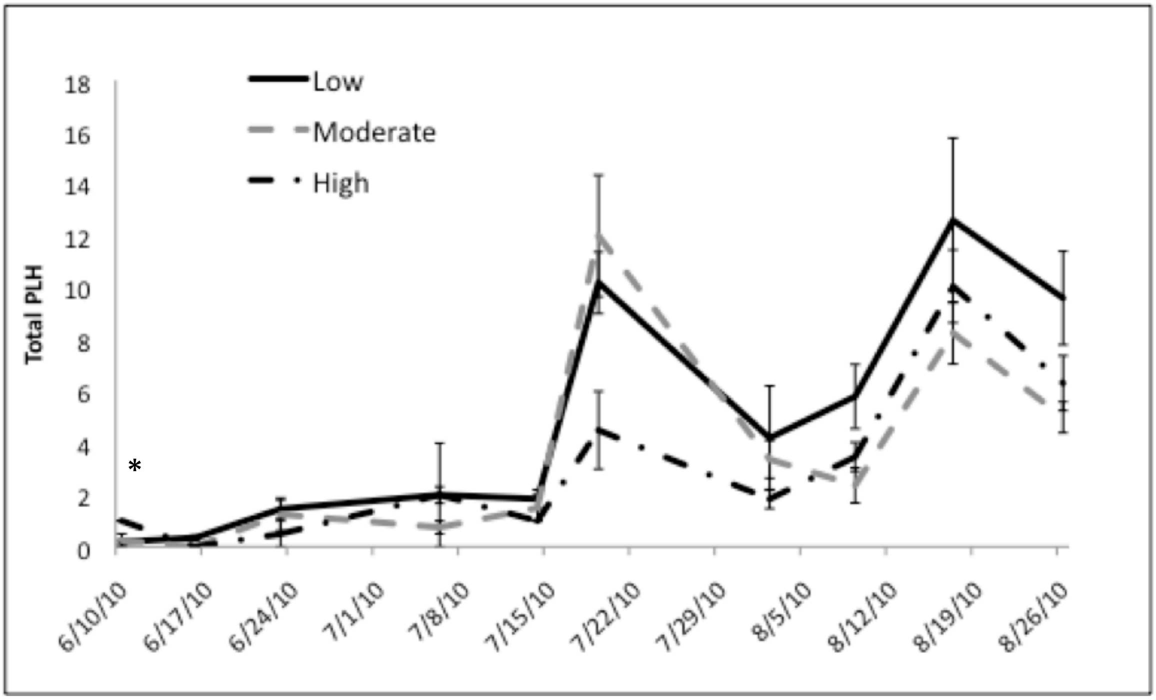

Figure 2. Potato leafhopper response to orchardgrass at Arlington Agricultural Research Station in 2010 (seeding year): low (0-9%), moderate (10-19%) and high (greater than 20%). Total PLH is the number of potato leafhoppers found in 20 sweeps. Significant differences indicated with (*).

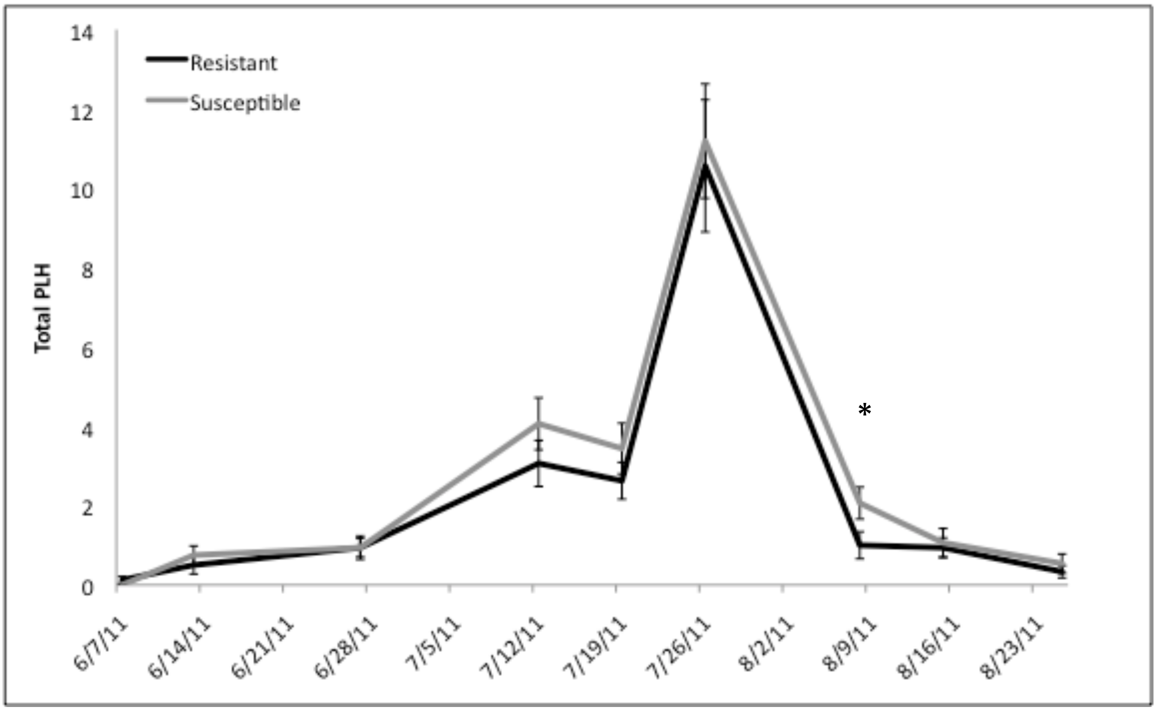

Figure 3. Potato leafhopper response to alfalfa variety at Arlington Agricultural Research Station in 2011 (first production year). Total PLH is the number of potato leafhoppers found in 20 sweeps. Significant differences indicated with (*).

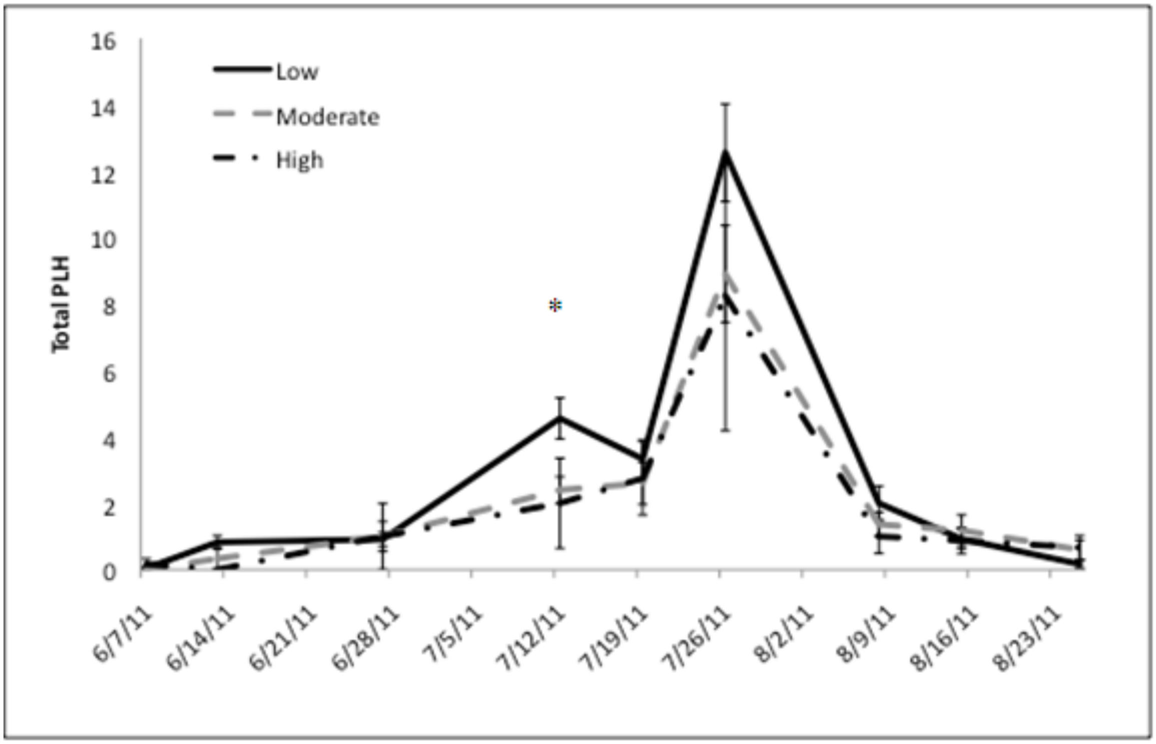

Figure 4. Potato leafhopper response to orchardgrass at Arlington Agricultural Research Station in 2011 (first production year): low (0-9%), moderate (10-19%) and high (greater than 20%). Total PLH is the number of potato leafhoppers found in 20 sweeps. Significant differences indicated with (*).

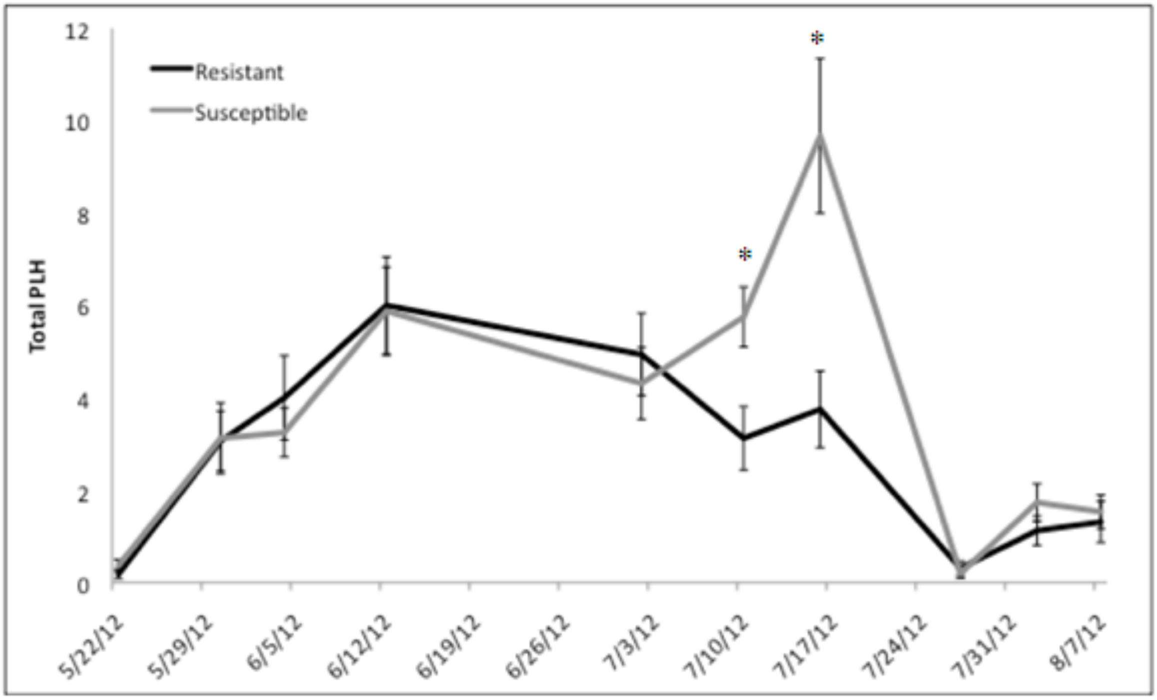

Figure 5. Potato leafhopper response to alfalfa variety at Arlington Agricultural Research Station in 2012 (second production year). Total PLH is the number of potato leafhoppers found in 20 sweeps. Significant differences indicated with (*).

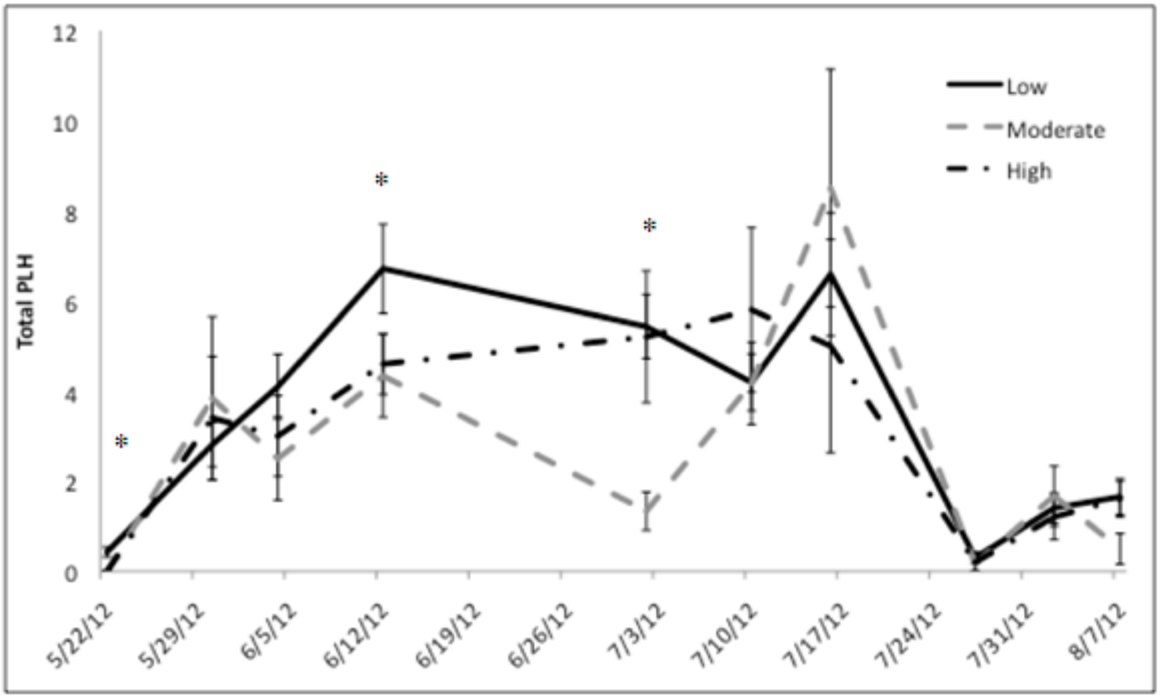

Figure 6. Potato leafhopper response to orchardgrass at Arlington Agricultural Research Station in 2012 (second production year): low (0-9%), moderate (10-19%) and high (greater than 20%). Total PLH is the number of potato leafhoppers found in 20 sweeps. Significant differences indicated with (*).

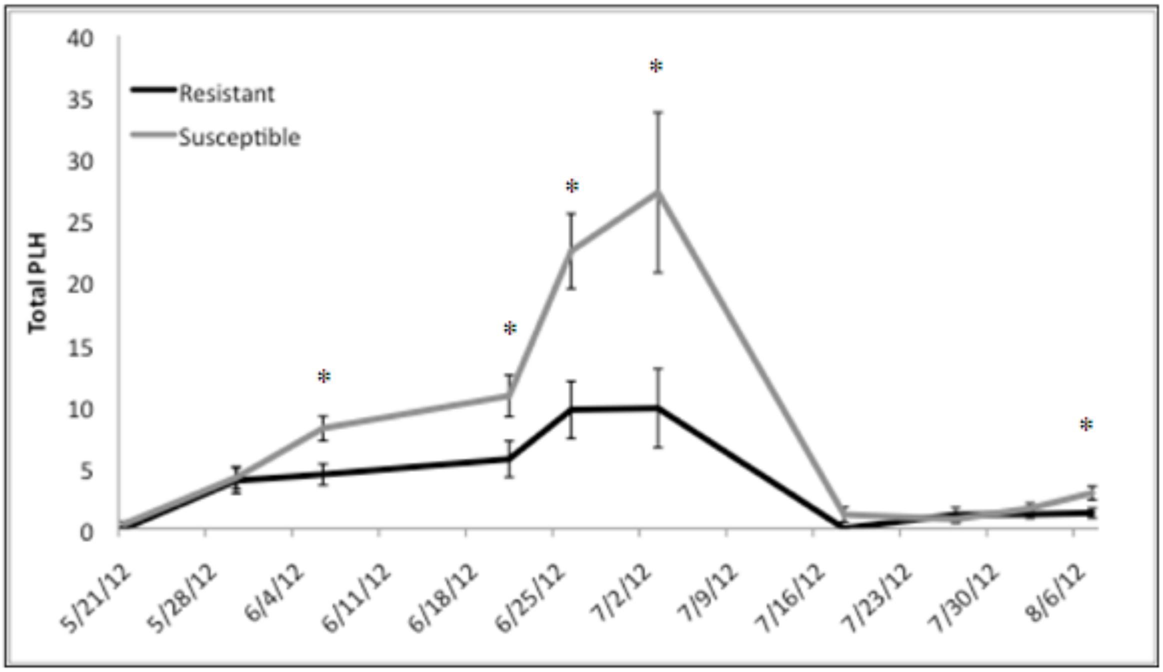

Figure 7. Potato leafhopper response to alfalfa variety at U.S. Dairy Forage Research Center spring seeding in 2012 (seeding year). Total PLH is the number of potato leafhoppers found in 20 sweeps. Significant differences indicated with (*).

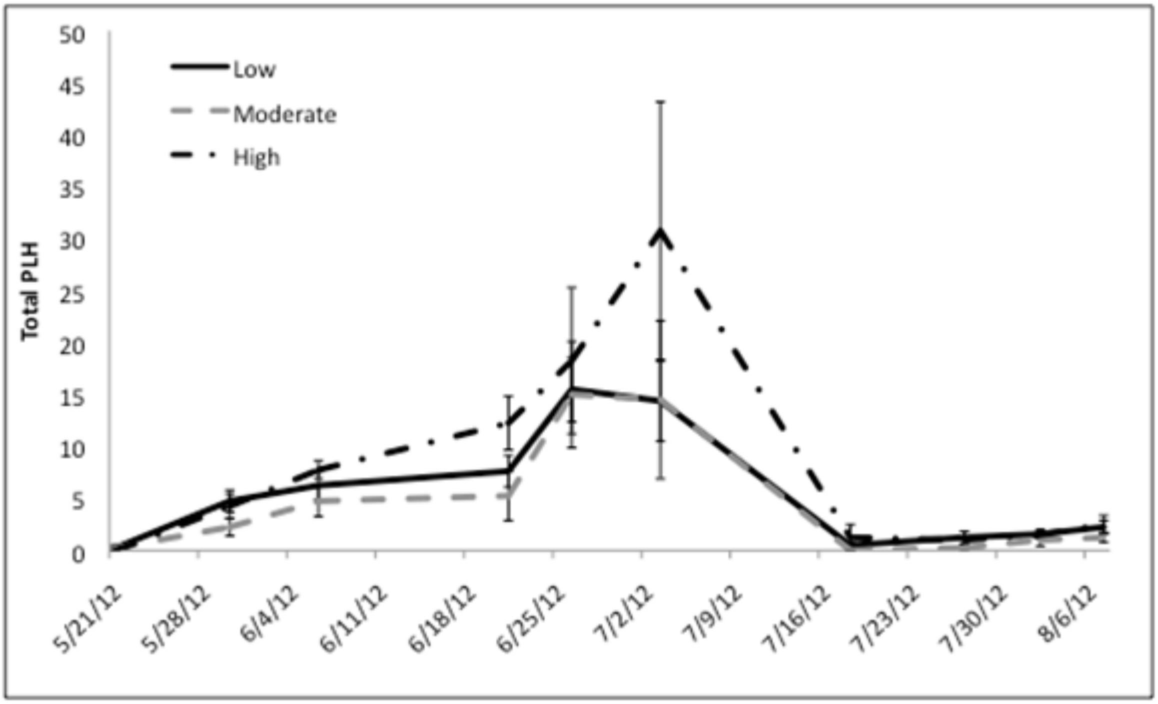

Figure 8. Potato leafhopper response to orchardgrass at U.S. Dairy Forage Research Center spring seeding in 2012 (seeding year): low (0-9%), moderate (10-19%) and high (greater than 20%). Total PLH is the number of potato leafhoppers found in 20 sweeps. No significant differences.

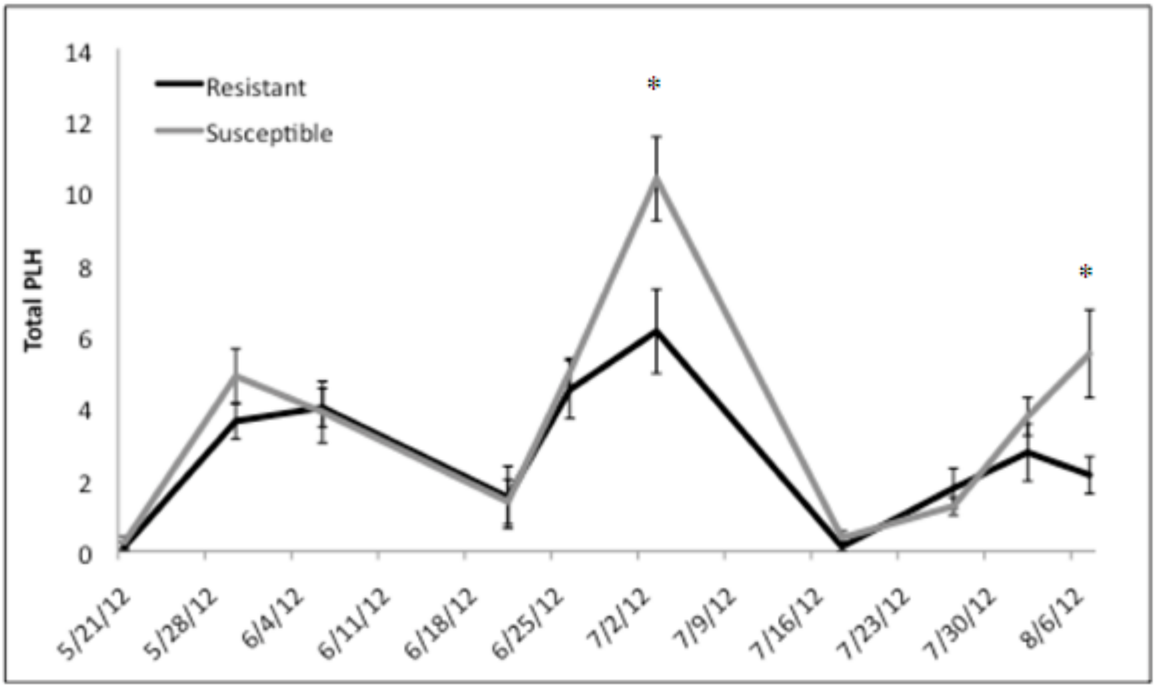

Figure 9. Potato leafhopper response to alfalfa variety at U.S. Dairy Forage Research Center late summer seeding in 2012 (production year). Total PLH is the number of potato leafhoppers found in 20 sweeps. Significant differences indicated with (*).

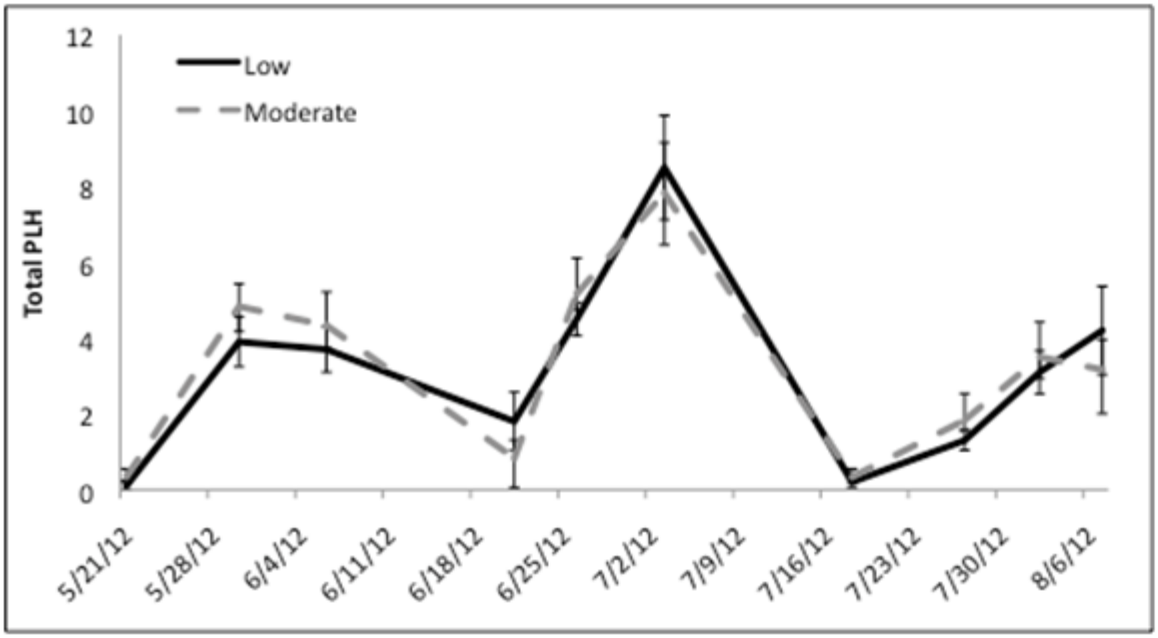

Figure 10. Potato leafhopper response to orchardgrass at U.S. Dairy Forage Research Center late summer seeding in 2012 (production year): low (0-9%) and moderate (10-19%). Total PLH is the number of potato leafhoppers found in 20 sweeps. No significant differences.

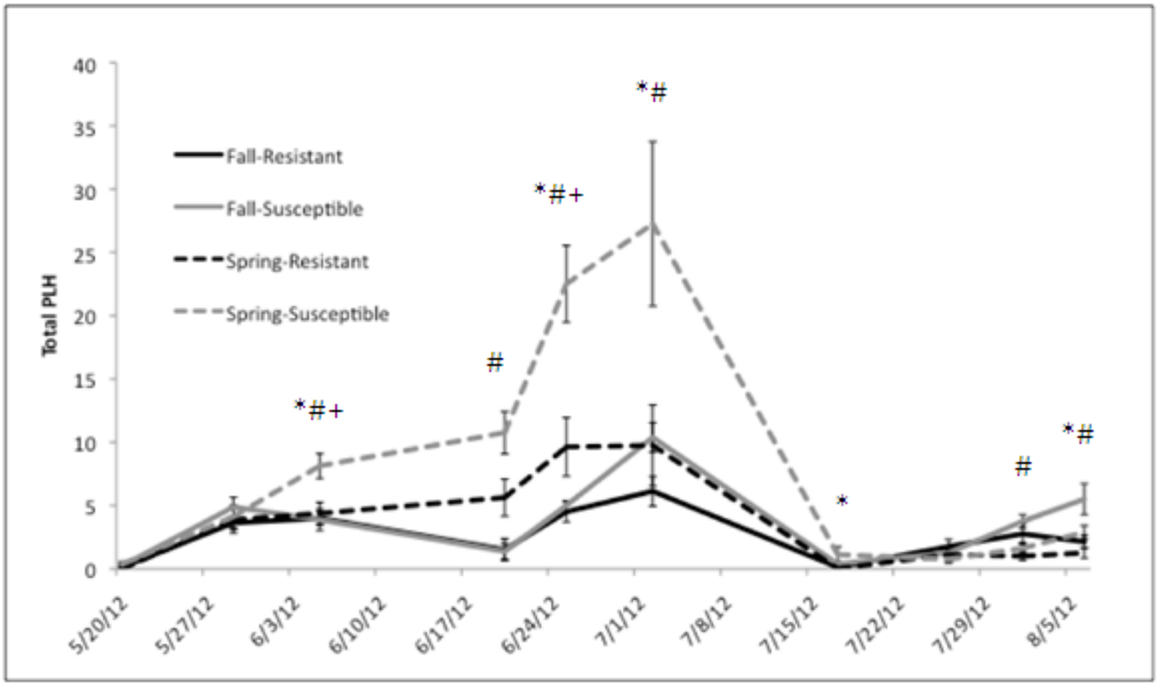

Figure 11. Potato leafhopper response to alfalfa variety and seeding time at U. S. Dairy Forage Research Center in 2012. Total PLH is the number of potato leafhoppers found in 20 sweeps. Significant differences indicated with (*) for variety, (#) for seeding time and (+) for interactions.

Figure 12. Aerial view of spring and late summer seeded trials at U. S. Dairy Forage Research Center. Surrounding field is late summer seeded alfalfa.

Table 1. Split plot insecticide treatments at Arlington Agricultural Research Station, adapted from Cullen et al. (2013).

Alfalfa Height, inches (cm)	½ ET	ET
	PLH/sweep	
0-4 (0-10)	0.1	0.2
4-8 (10-20)	0.3	0.5
8-12 (20-30)	0.5	1.0
12+ (30+)	1.0	2.0

Table 2. Effect of split plot insecticide treatments on yield on July 26, 2010 (second crop, seeding year) at Arlington Agricultural Research Station. Orchardgrass percentages are: low (0-9%), moderate (10-19%) and high (greater than 20%).

Treatment	Yield, tons acre^{-1} ± SE[a] (Mg ha^{-1})
Susceptible alfalfa - low orchardgrass	
No spray	1.30 ± 0.07a (2.91)
Economic Threshold[b]	1.32 ± 0.07a (2.96)
½ Economic Threshold[c]	1.35 ± 0.07a (3.03)
Susceptible alfalfa - moderate orchardgrass	
No spray	1.52 ± 0.16a (3.41)
Economic Threshold[b]	1.68 ± 0.16a (3.77)
½ Economic Threshold[c]	n/a[d]
Susceptible alfalfa - high orchardgrass	
No spray	n/a[d]
Economic Threshold[b]	n/a[d]
½ Economic Threshold[c]	n/a[d]
Resistant alfalfa - low orchardgrass	
No spray	1.31 ± 0.08a (2.94)
Economic Threshold[b]	1.48 ± 0.08a (3.32)
½ Economic Threshold[c]	1.43 ± 0.07a (3.21)
Resistant alfalfa - moderate orchardgrass	
No spray	1.31 ± 0.16a (2.94)
Economic Threshold[b]	1.49 ± 0.22a (3.34)
½ Economic Threshold[c]	1.56 ± 0.22a (3.50)
Resistant alfalfa - high orchardgrass	
No spray	1.38 ± 0.16a (3.09)
Economic Threshold[b]	1.78 ± 0.16a (3.99)
½ Economic Threshold[c]	n/a[d]

[a] Means followed by the same letter are not significantly different by Bonferroni adjustment (p=0.017).

[b] Economic threshold treatment sprayed on July 9; alfalfa height 0-4 inches; PLH/sweep=0.2.

[c] ½ Economic threshold treatment sprayed on July 7; alfalfa height 0-4 inches; PLH/sweep=0.1.

[d] No yield results because there were no treatments in this category based on NIR results.

Table 3. The effect of potato leafhoppers on yield at Arlington Agricultural Research Station for all crops in which yield was collected. Significant effects are in **bold**. Slope values represent yield, tons acre^{-1} (Mg ha^{-1}) loss or gain per potato leafhopper in the sweep net.

Date	Interactions		Slope	df	F	Pr>F
July 26, 2010	na		**-0.04 (-0.09)**	**1, 88**	**4.08**	**0.05**
September 7, 2010	na		-0.01 (-0.02)	1, 88	1.28	0.26
June 1, 2011	na		0.00 (0.00)	1, 88	0.05	0.82
July 5, 2011	na		0.04 (0.09)	1, 88	0.83	0.37
August 1, 2011				1, 86	0.27	0.60
	Variety			**1, 86**	**11.95**	**<0.01**
		Resistant	**-0.02 (-0.04)**			
		Susceptible	**0.02 (0.04)**			
September 1, 2011				1, 74	1.28	0.26
	Orchardgrass %			**2, 74**	**6.09**	**<0.01**
		0 to 9	**-0.05 (-0.11)**			
		10 to 19	**-0.14 (-0.32)**			
		Greater than 20	**0.07 (0.16)**			
June 20, 2012			**-0.09 (-0.20)**	**1, 88**	**7.22**	**<0.01**

Table 4. The effect of cumulative potato leafhoppers on total yield at Arlington Agricultural Research Station for all crops in which yield was collected. Significant effects are in **bold**. Slope values represent yield, tons acre^{-1} (Mg ha^{-1}) loss or gain per potato leafhopper in the sweep net.

Year	Interactions	Slope	df	F	Pr>F
2010	na	**-0.02 (-0.05)**	**1, 89**	**4.20**	**0.04**
2011	na	0.01 (0.02)	1, 77	1.06	0.31

Table 5. Potato leafhopper effect on crude protein content at Arlington Agricultural Research Station for all crops in which yield was collected. Significant effects are in **bold**. Slope values represent crude protein (%) loss or gain per potato leafhopper in the sweep net.

Date	Interactions	Slope	df	F	Pr>F
July 26, 2010	na	**-0.25**	**1, 80**	**5.56**	**0.02**
September 7, 2010	na	0.05	1, 88	1.19	0.28
June 1, 2011	na	-0.08	1, 86	0.97	0.33
July 5, 2011			1, 85	2.05	0.16
	Orchardgrass %		**2, 85**	**4.88**	**0.01**
	0 to 9	**0.60**			
	10 to 19	**1.44**			
	Greater than 20	**-0.92**			
August 1, 2011	na	-0.07	1, 86	3.75	0.06
September 1, 2011	na	0.00	1, 75	0.00	1.00
June 20, 2011	na	0.07	1, 81	0.30	0.59

Table 6. Potato leafhopper effect on neutral detergent fiber content at Arlington Agricultural Research Station for all crops in which yield was collected. Significant effects are in **bold**. Slope values represent neutral detergent fiber (%) loss or gain per potato leafhopper in the sweep net.

Date	Interactions	Slope	df	F	Pr>F
July 26, 2010	na	-0.42	1, 80	3.25	0.08
September 7, 2010	na	-0.20	1, 88	3.47	0.07
June 1, 2011	na	0.23	1, 86	1.80	0.18
July 5, 2011	na		1, 85	0.54	0.47
	Orchardgrass %		**2, 85**	**5.41**	**<0.01**
	0 to 9	**-1.3**			
	10 to 19	**-2.96**			
	Greater than 20	**2.88**			
August 1, 2011	na	-0.01	1, 86	0.01	0.93
September 1, 2011	na	-0.42	1, 75	1.84	0.18
June 20, 2012	na	-0.44	1, 81	2.72	0.10

Table 7. Effect of alfalfa variety on yield, tons acre^{-1} (Mg ha^{-1}) at Arlington Agricultural Research Station for all crops in which yield data was collected. Significant effects are in **bold**. Intercepts are estimates for linear regressions; slopes found in table 3.

Date	Variety	Intercept	LSMean	df	F	Pr>F
July 26, 2010				1, 88	0.60	0.44
	Resistant	1.64 (3.68)	1.48 (3.32)			
	Susceptible	1.60 (3.59)	1.45 (3.25)			
September 7, 2010				1, 88	0.00	0.95
	Resistant	1.16 (2.60)	1.13 (2.53)			
	Susceptible	1.16 (2.60)	1.13 (2.53)			
June 1, 2011				**1, 88**	**11.59**	**<0.01**
	Resistant	**2.94 (6.59)**	**2.87 (6.43)**			
	Susceptible	**3.11 (6.97)**	**3.04 (6.82)**			
July 5, 2011				**1, 88**	**23.20**	**<0.01**
	Resistant	**2.08 (4.66)**	**2.10 (4.71)**			
	Susceptible	**2.25 (5.04)**	**2.27 (5.09)**			
August 1, 2011				1, 86	1.23	0.27
	Resistant	1.51 (3.39)	1.54 (3.45)			
	Susceptible	1.45 (3.25)	1.64 (3.68)			
September 1, 2011				**1, 74**	**15.11**	**<0.01**
	Resistant	**1.02 (2.29)**	**1.10 (2.47)**			
	Susceptible	**1.14 (2.56)**	**1.21 (2.71)**			
June 20, 2012				**1, 88**	**12.55**	**<0.01**
	Resistant	**1.84 (4.13)**	**1.66 (3.72)**			
	Susceptible	**2.05 (4.60)**	**1.86 (4.17)**			

Table 8. Effect of alfalfa variety on total yield, tons acre^{-1} (Mg ha^{-1}) at Arlington Agricultural Research Station for all crops in which yield data was collected. Significant effects are in **bold**. Intercepts are estimates for linear regressions; slopes found in table 4.

Year	Variety	Intercept	df	F	Pr>F
2010			1, 89	0.97	0.33
	Resistant	2.68 (6.01)			
	Susceptible	2.63 (5.90)			
2011			**1, 77**	**26.56**	**<0.01**
	Resistant	**7.50 (16.81)**			
	Susceptible	**8.03 (18.00)**			

Table 9. Effect of alfalfa variety on crude protein content (%) at Arlington Agricultural Research Station for all crops in which yield data was recorded. Significant effects are in **bold**. Interactions are included when significant. Intercepts are estimates for linear regressions; slopes found in table 4.

Date	Variety	Interactions	Intercept	LSMean	df	F	Pr>F
July 26, 2010		na			**1, 80**	**4.13**	**0.05**
	Resistant		**26.34**	**26.34**			
	Susceptible		**26.78**	**26.78**			
September 7, 2010		na			**1, 88**	**21.03**	**<0.01**
	Resistant		**23.96**	**24.81**			
	Susceptible		**22.70**	**23.54**			
June 1, 2011					1, 86	0.57	0.45
		Orchardgrass %			**2, 86**	**7.11**	**<0.01**
	Resistant						
		0 to 9	**23.82**	**24.89**			
		10 to 19	**24.03**	**24.17**			
		Greater than 20	**22.20**	**21.06**			
	Susceptible						
		0 to 9	**22.20**	**24.21**			
		10 to 19	**22.20**	**23.28**			
		Greater than 20	**22.20**	**22.00**			
July 5, 2011		na			**1, 85**	**12.81**	**<0.01**
	Resistant		**22.15**	**22.47**			
	Susceptible		**21.60**	**21.92**			
August 1, 2011		na			**1, 86**	**21.34**	**<0.01**
	Resistant		**25.70**	**25.56**			
	Susceptible		**24.77**	**24.63**			
September 1, 2011		na			**1, 75**	**39.43**	**<0.01**
	Resistant		**28.38**	**29.08**			
	Susceptible		**27.03**	**27.73**			
June 20, 2012		na			**1, 81**	**21.61**	**<0.01**
	Resistant		**21.08**	**22.13**			
	Susceptible		**20.18**	**21.23**			

Table 10. Effect of alfalfa variety on neutral detergent fiber content (%) at Arlington Agricultural Research Station for all crops in which yield data were collected. Significant effects are in **bold**. Interactions are included when significant. Intercepts are estimates for linear regressions; slopes found in table 5.

Date	Variety	Interactions	Intercept	LSMean	df	F	Pr>F
July 26, 2010		na			1, 80	0.89	0.35
	Resistant		36.15	34.32			
	Susceptible		36.61	34.78			
September 7, 2010		na			**1, 88**	**22.44**	**<0.01**
	Resistant		**42.23**	**39.28**			
	Susceptible		**45.30**	**42.36**			
June 1, 2011					1, 86	1.34	0.25
		Orchardgrass %			**2, 86**	**7.22**	**<0.01**
	Resistant		41.70	37.27			
		0 to 9	**36.48**	**34.06**			
		10 to 19	**35.94**	**35.45**			
		Greater than 20	**39.91**	**42.29**			
	Susceptible		39.91	37.95			
		0 to 9	**39.91**	**35.71**			
		10 to 19	**39.91**	**37.64**			
		Greater than 20	**39.91**	**40.50**			
July 5, 2011		na			1, 85	2.68	0.11
	Resistant		40.96	40.05			
	Susceptible		41.56	40.65			
August 1, 2011		na			**1, 86**	**4.49**	**0.04**
	Resistant		**39.42**	**37.82**			
	Susceptible		**40.37**	**38.77**			
September 1, 2011		na			**1, 75**	**8.71**	**<0.01**
	Resistant		**31.76**	**30.00**			
	Susceptible		**33.03**	**31.25**			
June 20, 2012		na			**1**	**24.49**	**<0.01**
	Resistant		**45.59**	**41.31**			
	Susceptible		**47.79**	**43.51**			

Table 11. Effects of orchardgrass on yield, tons acre^{-1} (Mg ha^{-1}) at Arlington Agricultural Research Station for all crops in which yield data were collected. Significant effects are in **bold**. Intercepts are estimates for linear regressions; slopes found in table 3.

Date	Orchardgrass %	Intercept	LSMean	df	F	Pr>F
July 26, 2010				2, 88	1.95	0.15
	0 to 9	1.43 (3.21)	1.37 (3.07)			
	10 to 19	1.55 (3.48)	1.49 (3.34)			
	Greater than 20	1.60 (3.59)	1.54 (3.45)			
September 7, 2010				2, 88	0.44	0.65
	0 to 9	1.14 (2.56)	1.11 (2.49)			
	10 to 19	1.17 (2.62)	1.15 (2.58)			
	Greater than 20	1.16 (2.60)	1.13 (2.53)			
June 1, 2011				**2, 88**	**3.21**	**0.05**
	0 to 9	**3.05 (6.84)**	**2.96 (6.64)**			
	10 to 19	**2.98 (6.68)**	**2.89 (6.48)**			
	Greater than 20	**3.11 (6.97)**	**3.02 (6.77)**			
July 5, 2011				2, 88	0.54	0.58
	0 to 9	2.28 (5.11)	2.21 (4.95)			
	10 to 19	2.23 (5.00)	2.17 (4.87)			
	Greater than 20	2.25 (5.04)	2.18 (4.89)			
August 1, 2011				**2, 86**	**4.66**	**0.01**
	0 to 9	**1.62 (3.63)**	**1.66 (3.72)**			
	10 to 19	**1.56 (3.50)**	**1.61 (3.61)**			
	Greater than 20	**1.45 (3.25)**	**1.50 (3.36)**			
September 1, 2011				**2, 74**	**5.32**	**<0.01**
	0 to 9	**1.23 (2.76)**	**1.15 (2.58)**			
	10 to 19	**1.35 (3.03)**	**1.17 (2.62)**			
	Greater than 20	**1.14 (2.56)**	**1.14 (2.56)**			
June 20, 2012				**2, 88**	**4.46**	**0.01**
	0 to 9	**2.20 (4.93)**	**1.89 (4.24)**			
	10 to 19	**1.98 (4.43)**	**1.67 (3.74)**			
	Greater than 20	**2.05 (4.60)**	**1.74 (3.90)**			

Table 12. Effects of orchardgrass on total yield, tons acre^{-1} (Mg ha^{-1}) at Arlington Agricultural Research Station for all crops in which yield data were collected. No significant effects. Intercepts are estimates for linear regressions; slopes found in table 4.

Year	Orchardgrass	Intercept	df	F	Pr>F
2010			1, 89	2.04	0.16
	Present	2.61 (5.85)			
	Absent	2.68 (6.01)			
2011			1, 77	1.61	0.21
	Present	7.63 (17.10)			
	Absent	7.50 (16.81)			

Table 13. Effect of orchardgrass on crude protein content (%) at Arlington Agricultural Research Station for all crops in which yield data were collected. Significant effects are in **bold**. Interactions are included when significant. Intercepts are estimates for linear regressions; slopes found in table 4.

Date	Orchardgrass %	Interactions	Intercept	LSMean	df	F	Pr>F
July 26, 2010		na			2, 80	1.62	0.20
	0 to 9		27.59	26.94			
	10 to 19		27.26	26.61			
	Greater than 20		26.78	26.13			
September 7, 2010		na			**2, 88**	**7.00**	**<0.01**
	0 to 9		**23.70**	**24.54**			
	10 to 19		**23.62**	**24.45**			
	Greater than 20		**22.70**	**23.54**			
June 1, 2011					**2, 86**	**66.12**	**<0.01**
		Alfalfa variety			**2, 86**	**7.11**	**<0.01**
	0 to 9		**24.41**	**24.55**			
		Resistant	**23.82**	**24.89**			
		Susceptible	**22.20**	**24.21**			
	10 to 19		**23.48**	**23.72**			
		Resistant	**24.03**	**24.17**			
		Susceptible	**22.20**	**23.28**			
	Greater than 20		**22.20**	**21.53**			
		Resistant	**22.20**	**21.06**			
		Susceptible	**22.20**	**22.00**			
July 5, 2011		na			**2, 85**	**4.44**	**0.01**
	0 to 9		**22.23**	**22.79**			
	10 to 19		**21.40**	**22.37**			
	Greater than 20		**21.60**	**21.43**			
August 1, 2011		na			**2, 86**	**3.38**	**0.04**
	0 to 9		**25.34**	**25.48**			
	10 to 19		**24.75**	**24.89**			
	Greater than 20		**24.77**	**24.91**			
September 1, 2011		na			**2, 75**	**12.78**	**<0.01**
	0 to 9		**28.48**	**29.17**			
	10 to 19		**27.65**	**28.34**			
	Greater than 20		**27.03**	**27.71**			
June 20, 2012		na			**2, 81**	**38.53**	**<0.01**
	0 to 9		**22.31**	**22.91**			
	10 to 19		**20.78**	**21.37**			
	Greater than 20		**20.18**	**20.77**			

Table 14. Effect of orchardgrass on neutral detergent fiber content (%) at Arlington Agricultural Research Station for all crops in which yield data were collected. Significant effects are in **bold**. Interactions are included when significant. Intercepts are estimates for linear regressions; slopes found in table 5.

Date	Orchardgrass %	Interactions	Intercept	LSMean	df	F	Pr>F
July 26, 2010		na			**2, 80**	**4.16**	**0.02**
	0 to 9		**34.14**	**33.18**			
	10 to 19		**35.78**	**34.82**			
	Greater than 20		**36.61**	**35.64**			
Sept. 7, 2010		na			**2, 88**	**13.63**	**<0.01**
	0 to 9		**41.80**	**39.45**			
	10 to 19		**42.40**	**40.05**			
	Greater than 20		**45.30**	**42.96**			
June 1, 2011					**2, 86**	**69.18**	**<0.01**
		Alfalfa variety			**2, 86**	**7.22**	**<0.01**
	0 to 9		**35.12**	**34.89**			
		Resistant	**36.48**	**34.06**			
		Susceptible	**39.91**	**35.71**			
	10 to 19		**37.05**	**36.55**			
		Resistant	**35.94**	**35.45**			
		Susceptible	**37.05**	**36.55**			
	Greater than 20		**39.91**	**41.39**			
		Resistant	**39.91**	**42.29**			
		Susceptible	**39.91**	**40.50**			
July 5, 2011					**2, 85**	**8.04**	**<0.01**
	0 to 9		**39.28**	**38.36**			
	10 to 19		**41.36**	**40.03**			
	Greater than 20		**41.56**	**42.66**			
Aug. 1, 2011		na			**2, 86**	**5.45**	**<0.01**
	0 to 9		**37.60**	**37.10**			
	10 to 19		**38.44**	**37.93**			
	Greater than 20		**40.37**	**39.86**			
Sept. 1, 2011		na			**2, 75**	**8.98**	**<0.01**
	0 to 9		**30.72**	**29.73**			
	10 to 19		**31.07**	**30.08**			
	Greater than 20		**33.03**	**32.04**			
June 20, 2012		na			**2, 81**	**68.93**	**<0.01**
	0 to 9		**40.93**	**38.88**			
	10 to 19		**44.67**	**42.62**			
	Greater than 20		**47.79**	**45.74**			

Appendix I. Treatment effects on potato leafhopper response through repeated measures analysis for Arlington Agricultural Research Station, 2010. Significant effects are in **bold**.

Effect	df	F	Pr>F
Sample date	**9, 261**	**55.73**	**<0.01**
Alfalfa variety	**1, 26**	**40.14**	**<0.01**
Orchardgrass presence	1, 26	0.80	0.38
Alfalfa variety x sample date	**9, 261**	**5.57**	**<0.01**[a]
Orchardgrass presence x sample date	9, 261	0.57	0.82

[a] Significant alfalfa variety x sample date interaction signifies that the effect of alfalfa variety on potato leafhopper changes over time and the analysis proceeds by looking at dates individually as seen in figure 1.

Appendix II. Treatment effects on potato leafhopper response through repeated measures analysis for Arlington Agricultural Research Station, 2011. Significant effects in **bold**.

Effect	df	F	Pr>F[a]
Sample date	**8, 228**	**59.04**	**<0.01**
Alfalfa variety	1, 26	1.98	0.17
Orchardgrass presence	1, 26	2.71	0.11
Alfalfa variety x sample date	8, 228	0.26	0.98
Orchardgrass presence x sample date	8, 228	1.34	0.22

[a] Lack of significant interactions between sample date or treatments can be seen in figures 3 and 4.

Appendix III. Treatment effects on potato leafhopper response through repeated measures analysis for Arlington Agricultural Research Station, 2012. Significant effects in **bold**.

Effect	df	F	Pr>F
Sample date	**9, 250**	**19.57**	**<0.01**
Alfalfa variety	**1, 26**	**4.55**	**0.04**
Orchardgrass presence	**1, 26**	**6.55**	**0.02**
Alfalfa variety x sample date	**9, 250**	**3.41**	**<0.01**[a]
Orchardgrass presence x sample date	9, 250	0.91	0.52

[a] Significant alfalfa variety x sample date interaction signifies that the effect of alfalfa variety on potato leafhopper changes over time and the analysis proceeds by looking at dates individually as seen in figure 5.

Appendix IV. Treatment effects on potato leafhopper response through repeated measures analysis for spring seeding (seeding year) at U.S. Dairy Forage Research Center, 2012. Significant effects in **bold**.

Effect	df	F	Pr>F
Sample date	**9, 116**	**26.71**	**<0.01**
Alfalfa variety	**1, 10**	**11.83**	**<0.01**
Orchardgrass presence	1, 10	0.35	0.57
Alfalfa variety x sample date	**9, 116**	**5.66**	**<0.01**[a]
Orchardgrass presence x sample date	9, 116	1.14	0.34

[a] Significant alfalfa variety x sample date interaction signifies that the effect of alfalfa variety on potato leafhopper changes over time and the analysis proceeds by looking at dates individually as seen in figure 7.

Appendix V. Treatment effects on potato leafhopper response through repeated measures analysis for late summer seeding (production year) at U.S. Dairy Forage Research Center, 2012. Significant effects in **bold**.

Effect	df	F	Pr>F
Sample date	**9, 117**	**29.10**	**<0.01**
Alfalfa variety	**1, 10**	**12.20**	**<0.01**
Orchardgrass presence	**1, 10**	**6.86**	**0.03**
Alfalfa variety x sample date	**9, 117**	**3.06**	**<0.01**[a]
Orchardgrass presence x sample date	9, 117	0.91	0.52

[a] Significant alfalfa variety x sample date interaction signifies that the effect of alfalfa variety on potato leafhopper changes over time and the analysis proceeds by looking at dates individually as seen in figure 9.

Chapter 3: Revisiting the Economic Threshold Model for Potato Leafhopper in Alfalfa for Susceptible and Resistant Varieties

Abstract

The economic injury level for potato leafhopper, *Empoasca fabae* in alfalfa was evaluated over 30 years ago. In response to increasing market value of alfalfa, farmers and consultants are interested in reducing the economic threshold for potato leafhopper in alfalfa. To address this question, caged field trials were established on two consecutive crops in 2013. A range of potato leafhopper densities infested in cages in order to create a linear regression of alfalfa yield response. Leafhopper resistant and susceptible alfalfa varieties were used for the first trial, and the second trial consisted of susceptible alfalfa only. Resistant and susceptible alfalfa did not differ in their yield response to potato leafhoppers. Resistance mechanisms are discussed and antixenosis is the mechanism proposed to be functioning in the resistant cultivar tested in this study. The slopes, or yield loss per insect, for the linear regressions of both trials were used to calculate an economic injury level with a range of current alfalfa market values and control costs. The resulting economic thresholds demonstrate that there is no need to reduce the current economic threshold.

Keywords: IPM, economic injury level, host plant resistance, antixenosis, potato leafhopper

Alfalfa grown in the United States covers roughly 10 million ha; with one quarter of this area in Wisconsin, Minnesota, Iowa and South Dakota (Flanders and Radcliffe 2013). Alfalfa hay and haylage provide an important source of energy, protein and fiber for dairy cattle contributing to high quality dairy products (Jennings, 2006). As a perennial, nitrogen-fixing crop, alfalfa also provides valuable ecological services by enhancing soil structure and nitrogen content and providing habitat for nearly 1,000 arthropod species including insect natural enemies and pollinators (Flanders and Radcliffe 2013). The potato leafhopper, *Empoasca fabae* Harris, is a major pest of alfalfa and in most years is the only insect capable of causing economic damage. As a homopterous pest, the potato leafhopper injures alfalfa by inserting its stylet into phloem cells and disrupting translocation of photoassimilates, leading to leaf chlorosis and stunted plant growth (Backus et al. 2005). Reduced yields occur due to stunted growth and a delay in crop maturation time (Hutchins and Pedigo 1990). Alfalfa forage quality is also diminished through a decrease in crude protein content as a result of pototato leafhopper feeding (Cuperus et al. 1983, Hutchins and Pedigo 1990).

Integrated pest management (IPM) programs have a long history and are well developed for alfalfa management (Summers 1998). Pest scouting, economic thresholds, and selective insecticide use to conserve natural enemies for biological control are basic tenets of insect IPM (Stern et al. 1959). A fully developed IPM program is comprised of multiple strategies for a given pest or pest complex in a cropping system incorporating host plant resistance, biological, cultural and physical controls when available, and chemical control when necessary (Pedigo and Rice 2009). Though the objective of an IPM program is to reduce environmental and health risks of pesticide use, these programs function within economic and social constraints (Zalom 2010).

Therefore, IPM programs rely on a foundation of regular pest scouting and insecticide use when established economic thresholds are reached.

Economic threshold models support the concept that not all pest densities present in a cropping system will cause economic damage (Pedigo 2013). Economic thresholds provide guidelines to help farmers determine when insecticides are economically justified, that is when the pest population is large enough to create economic damage. Economic damage occurs when the market value of the crop yield lost to pest damage is greater than the cost of the control action. This quantity of yield is determined by calculating the cost of insecticide treatment per unit area divided by the market value of the crop, known as the gain threshold (Pedigo et al. 1986). The economic threshold is set below the insect density that will cause economic damage to the crop; it is set at the pest density at which insecticides should be applied in order to prevent an increasing pest population from reaching the economic injury level (EIL), allowing both for time to initiate control and for the control to take effect (Stern et al. 1959).

While the economic threshold is an approximation, it is based on the field research derived EIL for a particular pest-crop combination. The EIL is defined as the lowest number of insects that will cause economic damage, or the minimum number of insects that would reduce yield equal to the gain threshold (Pedigo and Rice 2009). The EIL is calculated as follows:

$$EIL = C/VIDK,$$

where C=cost of the control action (price per area unit), V=market value of the crop (price per unit weight), I=injury per insect unit, D=damage or yield loss associated with each injury unit and K=the proportion of yield loss that is reduced when treatment is applied. Although the above is the standard EIL model, modifications are necessary depending on the biology of specific pests and crop damage relationships. For example, for piercing-sucking pests such as the potato leafhopper that remove photosynthates via the vascular cells, the *I* and *D* variables are combined, as there is no direct way of measuring them separately. Instead, experiments are conducted to relate yield loss to insect density and the slope value from the resulting linear regression, representing yield loss per insect, is used in place of the combined I*D values (Pedigo et al. 1986). Identification of incremental damage to a host plant from increasing pest injury from these linear regressions is vital in order to create a useful model.

The economic injury level is dynamic because it is determined by market cost of insecticides and crops. When the market value of a crop increases, the EIL decreases because the application of the insecticide is economically justified. However, the economic threshold itself is not necessarily dynamic with market costs because it is set lower than the EIL and reductions in the threshold may result in insecticide treatments at insect densities lower than the damage boundary (Cullen 2009), that is the number of pests that cause detectable yield loss (Pedigo et al. 1986). The economic injury level and economic thresholds for potato leafhopper in alfalfa were established over 30 years ago (Cuperus et al. 1983) (Table 1). The economic thresholds for potato leafhopper in alfalfa vary by alfalfa height, taking into account that shorter alfalfa is more susceptible to damage (Kouskolekas and Decker 1968, Cuperus et al. 1983). Sampling for potato leafhopper is standardized through the use of a 38 cm (15-inch) diameter sweep net to collect 100 sweep samples in an alfalfa field comprised of five set of 20 samples taken from different areas of the field representing the total field area. Potato leafhopper insecticide treatment recommendations are based on the number of potato leafhoppers per sweep, the average of total leafhoppers in 100 sweep net samples (Cullen et al. 2012).

Over the last decade, widespread land use changes in the U.S. Corn Belt in combination with drought conditions have led to a decrease in alfalfa supply and thus the market value of alfalfa to increase (Barnett 2013, Gould 2013). In response to increasing alfalfa market value, growers and consultants are interested in reducing the current economic threshold (Holin 2008). This action is not advisable until up to date research is conducted examining the response of current alfalfa varieties to potato leafhopper feeding because there can be economic and environmental costs to treating below the pest damage boundary.

Host plant resistance is an excellent IPM tactic because it is compatible with other IPM tactics, such as cultural, chemical and biological control. In 1997 alfalfa seed companies released varieties bred for potato leafhopper resistance (Miller 1998). It is necessary to determine the response of resistant alfalfa varieties to potato leafhopper feeding in order to calculate economic thresholds for resistant alfalfa. Lefko et al. (2000) determined that resistant alfalfa could tolerate greater potato leafhopper populations before incurring yield loss. These revised economic thresholds were developed for the first generation releases of resistant cultivars, but new generations of cultivars are released each year. Over the last decade, traditional breeding has led to increased resistance ratings from 35% resistance in first generation up to 80% in current cultivars (Peterson 2003, Undersander pers. comm).

Host plant resistance refers to preadapted characteristics of the host plant that are reintroduced to the crop through traditional breeding. These characteristics act by reducing the pest ability to detect or accept the host plant, diminishing the crop nutritional value, or enhancing toxicity to the insect (Prokopy and Kogan 2009). Termed, respectively, antixenosis or anitbiosis, both of these characteristics decrease pest abundance on the resistant crop. Another resistant mechanism is tolerance, when the host plant has increased yield over susceptible varieties under similar pest pressures (Painter 1951, Teetes 2013). Potato leafhopper resistant, or glandular haired alfalfa, has demonstrated increased tolerance to potato leafhoppers (Lefko et al. 2000, Lamp et al. 2007) as well as antibiosis (Ranger and Hower 2001) and antixenosis (Ranger et al. 2004). When a host plant demonstrates tolerance as a resistance mechanism, a reevaluation of the EIL from what is used for susceptible alfalfa is required (Stern et al. 1959).

In light of increasing alfalfa market value and farmer interest in reducing the economic threshold, as well as advances in host plant resistant alfalfa breeding, research was conducted to update linear yield-loss models for alfalfa and potato leafhopper. Caged field trials were conducted in Wisconsin on susceptible alfalfa for two crops and resistant alfalfa for one crop. By examining alfalfa yield response across a wide range of potato leafhopper densities, regression model coefficients were used to re-evaluate the EIL and determine if the ET should be lowered in susceptible alfalfa. We also examined yield response of resistant alfalfa to potato leafhopper feeding in order to evaluate the need for a distinct threshold for the resistant cultivar used in this study.

Materials and Methods

Potato leafhopper maintenance. A potato leafhopper colony was maintained for the purpose of caged field infestations from field collected individuals recovered from alfalfa in 2011 and replenished with field collected individuals from 2012. Potato leafhoppers were reared on greenhouse grown ‘Henderson’ bush lima beans (*Phaseolus lunatus* L.). The colony was maintained in an environmental growth room set to 25° C (day and night) with a photoperiod of 16:8 (L:D) (Hunter and Backus 1989). When the insect colony grew too large for the environmental chamber space (mid-April 2012), additional cages were kept in a greenhouse, also with a photoperiod of 16:8 (L:D).

Potato leafhopper terrariums. Terrariums used for potato leafhopper transport from the colony to the field for cage infestation were constructed from wax-lined paper cups approximately 6 cm in diameter, in which potato leafhopper susceptible alfalfa (Magnitude, Farm Science Genetics, Nampa, ID) was grown in potting soil (Metro Mix, Agawam, MA), and a 1 cm hole was cut out of the bottom to allow for water drainage. Alfalfa was used in the terrariums in order to acclimate the potato leafhoppers to alfalfa for 24 hours in the greenhouse before transferring them to alfalfa in the caged field trials. On the day of aspirating, the cups were covered with clear plastic dome shaped lids with holes cut out of the top in order to insert the aspirator rubber stopper. When the appropriate number of leafhoppers was aspirated into a terrarium, the rubber stopper was taken out and the lid was covered with nylon socks.

Field plot establishment. This study was conducted during the 2013 growing season on a second year alfalfa stand. The alfalfa was seeded into plots 90’ x 30’ (27.5 m x 9.2 m) at 12 lbs acre^{-1} (13.5 kg ha^{-1}) on April 12, 2012 at the US Dairy Forage Research Center in Prairie du Sac, WI. Two alfalfa varieties were planted: potato leafhopper susceptible, non-glandular haired WL354HQ and potato leafhopper resistant, glandular haired WL353LH (Forage Genetics, International, Nampa, ID) in a complete randomized block design with three replicates. The plots were located on St. Charles silt loam soil (fine-silty, mixed, superactive, mesic Typic Hapludalfs).

Two trials were completed. Trial 1 was conducted on second crop and included both resistant and susceptible alfalfa varieties. Trial 2 was conducted on third crop and consisted of only the susceptible variety due to limited numbers of potato leafhoppers from the greenhouse colony.

Caged field trials. Caged field trials were initiated one day after the hay was removed from the field from the previous harvest. Pyganic (a.i. pyrethrins, 64 oz acre^{-1}) was applied to alfalfa stubble to kill any existing insect populations and allow a relatively quick (12 hour) field reentry interval to conduct field work. The following day, cages were placed over alfalfa regrowth. Square cages measuring 0.21 m^2 at the base (0.46 m on each side) x 0.75 m tall were assembled with schedule 40 PVC pipe. Custom cage netting was constructed out of "white no-see-um" netting with a zipper at the top seam along one side for inserting the potato leafhoppers (Skeeta Products, Bradenton, FL). Cages were built over plot sections with similar plant densities as determined by visual estimation. The day after cages were built and placed over alfalfa foliage, potato leafhoppers were aspirated from the greenhouse colony into individual terrariums for transport to the field for cage infestation.

Insect density treatments were chosen based on the work of Lefko et al. (2000) in which caged field trials were performed on susceptible and resistant alfalfa. Resistant alfalfa cages were infested with greater leafhopper densities than susceptible alfalfa cages due to known tolerance to potato leafhoppers compared to susceptible varieties (Lefko et al. 2000). Susceptible alfalfa was infested with 0, 15, 30, 45, 60, 75, 90, 105, 120 or 135 leafhoppers per cage. Resistant alfalfa was infested with 0, 20, 40, 60, 80, 100, 120, 140, 160 or 180 leafhoppers per cage. These ten densities were selected in order to increase power of the linear regression of yield loss and insect density, but only eight were used per repetition due to limitations in growing and maintaining a colony large enough to infest at all ten densities. The eight densities were purposefully chosen for each varietal replication so that each replication contained the lowest and highest densities and no density was omitted more than once. The eight densities were then assigned randomly to individual cages within each variety replication

The following day, when alfalfa regrowth was 7-12 cm (3-5 inches), terrariums were deposited and opened inside cages. After allowing one day for leafhoppers to disperse from terrariums onto the alfalfa, terrariums were checked for dead leafhoppers and then the cages were supplemented with potato leafhoppers in order to account for any dead individuals.

Cages were then left undisturbed until the day before harvest, at first bloom. At this time, a leaf blower retrofitted as a vacuum was used to recover potato leafhoppers from each cage in order to record leafhopper density at the end of the experiment. The vacuum was inserted into the cage through the zippered seam and moved through the foliage, methodically so that all stems were vacuumed for a total of about 30 seconds. Vacuum bags containing insects obtained from vacuum samples for each cage replicate were brought back to the lab, frozen at -80° C for 15 minutes and then stored at 0° C until samples were processed and potato leafhoppers nymphs and adults were counted.

At harvest, cages were removed and alfalfa from within the cages was hand-cut to 3 inches and biomass was recorded. Stems were counted to account for the effect of increasing stem density on increased yield. Biomass was oven dried at 60°C and yield was calculated based on this dry matter. Alfalfa quality (crude protein and neutral detergent fiber) was analyzed by near-infra red reflectance (NIR) methods on dried and ground alfalfa samples.

At the conclusion of trial 2, the alfalfa from the field areas that had been caged and infested for trial 1 was harvested again and processed as above in order to evaluate carryover effects from the potato leafhopper feeding during the previous harvest cycle. Carryover effects are defined as the effect of potato leafhoppers from the previous crop on yield and/or forage quality in the current crop. Dates of activities for each trial are listed (Table 2).

Vacuum recovery efficiency preliminary trial. In order to determine the efficiency of the vacuum recovery of potato leafhoppers from the cages and to ensure reliable insect recovery data, 14 cages were constructed and infested with a range of densities of adult leafhoppers in the same manner as the cages for the experiment. Cages were vacuum sampled one day after infestation, allowing a day for leafhopper dispersal from the terrarium to the caged field alfalfa. Vacuum bags were brought back to the lab, frozen at -80° C for 15 minutes and then stored at 0° C until potato leafhoppers were counted. Percent recovery calculated from this preliminary trial was used to determine the final adult population density of the cages from the main trials.

Statistical Analysis.

Vacuum recovery efficiency. A linear model forced through the origin was created to determine percent recovery by vacuum sample, with the number of leafhoppers collected in the preliminary trial by the number of leafhoppers infested. The resulting linear equation was used to convert the number of adults recovered in each vacuum sample from the main experiments into the absolute number of adult leafhoppers present at the conclusion of each trial.

Potato leafhopper response to alfalfa variety. The effect of alfalfa variety on potato leafhoppers was determined from trial 1 through mixed-effects linear models. Dependent variables were leafhopper adults or nymphs recovered at harvest. Fixed effects were the number of potato leafhoppers initially infested and alfalfa variety, with repetition as a random effect. Adult recovery was used as an indicator of mortality and nymph recovery was used as an indicator of fecundity.

Yield response to potato leafhoppers. The yield for each cage replicate recorded as dry grams per 0.21 m^2 was extrapolated to tons $acre^{-1}$. Yield was calculated as tons $acre^{-1}$ as per alfalfa production industry in the United States. Leafhopper values were analyzed as the total number of adults and nymphs recovered by vacuum sample, where adults were converted to absolute density from the linear model computed from the vacuum recovery experiment. The analysis was completed using a mixed-effects model in which total leafhoppers, alfalfa variety, and stem counts were regressed as fixed effects with a random effect of repetition against yield. This analysis resulted in a model of the form $y = a + bx_0 + cx_1$, where a, b and c are the estimated model constants, x_0 is the number of leafhoppers and x_1 is the number of stems. In order to create a linear model with the form $y = mx + b$, where x is the number of leafhoppers, the average number of stems, x_1 was multiplied by its coefficient, c and added to the y-intercept, a. The slope value (yield loss per insect) from the resulting regression was used in the EIL equation as the combined (I) and (D) variables, where I=injury per insect unit and D=damage or yield loss associated with each injury unit.

Forage quality response to potato leafhoppers. The effect of potato leafhoppers on crude protein and neutral detergent fiber (NDF) were computed as mixed-effects models by using alfalfa variety and the total leafhoppers (as explained above) as fixed effects with a random effect of repetition against the NIR output for each quality indicator.

All models above, linear and mixed, were created in R version 2.10.1 (R Development Core Team 2009) using *lm* and Rpackage *nlme*. Marginal R^2 (proportion of variance explained by the fixed factors alone) and conditional R^2 (proportion of variance explained by both the fixed and random factors) were calculated according to Nakagawa and Schielzeth (2013).

Values used in the calculation of the economic injury level. A range of alfalfa market values (V) for high quality alfalfa hay (Barnett 2013) and without regard to quality (USDA NASS 2013a) were used in the EIL equations to explore the model sensitivity to alfalfa price. The costs of insecticides recommended for use on potato leafhopper in alfalfa (Cullen et al. 2012) were found in USDA NASS (2013b), and obtained from informal telephone surveys of farm supply cooperatives. Application costs were estimated for 2013 from either the cost of operating and owning insecticide application equipment or the cost of hiring out the work (USDA NASS 2011). Cost of control (C) was then determined by summing insecticide and application costs and a range of values were used to assess model sensitivity. When an effective insecticide is used to suppress an insect population, the proportion of yield loss reduced from treatment (K) is assumed to be 100% because the insect population is reduced to a density below the damage boundary, or the population at which measurable yield loss occurs (Pedigo et al. 1986).

These values were then used in the standard EIL equation, EIL= C/VIDK. This produced a set of values representing the EIL as absolute density of potato leafhoppers in 0.21 m^2. These values were converted to potato leafhoppers per sweep using a linear regression equation for converting absolute density of adults and nymphs to the number of adults and nymphs found in 10 sweep net samples, $y=10.67+0.94x$, $R^2=0.72$ (Degooyer et al. 1998). The economic threshold was then calculated at 75% of the EIL (Lefko et al. 2000).

Because this economic threshold was based off of the number of leafhoppers present just before harvest, or when alfalfa was at its maximum height on the current threshold scale (greater than 12 inches), the economic thresholds for shorter alfalfa height classes were scaled according to the current economic threshold table. We adjusted potato leafhopper sweep^{-1} for each height class by the percent reduction in relation to that of 12+ inches (Table 3).

Results

The vacuum sample efficiency was 52%, $R^2 = 0.97$, $p < 0.01$ (Fig. 1). The number of adults recovered at the end of each trial in the vacuum sample were converted to absolute density with the linear model $y = 0.52x$, where x is the absolute density of leafhoppers in the cage at the end of each trial and y is the number of leafhoppers recovered in the vacuum.

Effect of alfalfa variety on leafhoppers in trial 1. The number of adults recovered was dependent on the number of adults infested (DF=1, 42; F=34.21; p<0.01), but neither alfalfa variety (DF=1, 42; F=0.00; p=0.99) nor the interaction of alfalfa variety and number of adults infested were significant (df=1, 42; F=0.42; p=0.52) (Fig. 2). The number of nymphs recovered was also dependent on the number of adults infested (DF=1, 42; F=5.98; p=0.02), but the interaction between alfalfa variety and number of adults infested was not significant for nymphal recovery (df=1, 42; F=0.09; p=0.77) nor was the main effect of alfalfa variety (DF=1, 42; F=0.00; p=0.99) (Fig. 3).

Trial 1: Resistant and susceptible alfalfa. Alfalfa variety did not have a significant effect on yield (df=1, 42; F=0.88; p=0.35), therefore alfalfa variety was not included in the linear regression model. Stem count and total potato leafhoppers (nymphs and adults) were significant (df=1, 43; F=43.42; p<0.01 and df=1, 43; F=4.64; p<0.01 respectively): tons acre^{-1} = 0.038 + 0.0088*(number of stems) - 0.0019*(total leafhoppers), marginal $R^2 = 0.51$, conditional $R^2 = 0.68$. When the average number of stems is multiplied by its coefficient (0.0088), $y = 0.93 - 0.0019x$, where y is tons acre^{-1} and x is the total number of potato leafhoppers (Fig. 4). Thus, 0.0019 tons acre^{-1} yield loss is associated with each additional potato leafhopper per cage.

Crude protein was not affected by alfalfa variety (df=1, 43; F=3.40; p=0.07), therefore alfalfa variety was not included in the linear regression. Potato leafhoppers did have a negative effect on crude protein (df=1, 44; F=81.8; p<0.01) (Fig. 5); $y = 24.82 - 0.034x$, $R^2 = 0.63$ (same for marginal and conditional), where y is percent crude protein and x is the total number of potato leafhoppers.

Neutral detergent fiber was not affected by alfalfa variety (df=1, 43; F=1.07; p=0.31) therefore variety was not included in the linear regression. Potato leafhoppers did have a negative effect on NDF (df=1, 44; F=13.47; p<0.01) (Fig. 6); $y = 32.16 - 0.030x$, marginal $R^2 = 0.17$, conditional $R^2 = 0.41$, where y is percent NDF and x is the total number of potato leafhoppers.

Trial 2: Susceptible alfalfa. Leafhoppers did not have an effect on yield (df=1, 18; F=3.12; p=0.09). However, there were two outliers; the lowest and highest caged alfalfa yields of the data set. The cage that yielded lowest (0.40 tons acre^{-1}) was harvested from a cage with comparatively very few leafhoppers. The cage with the highest yield (1.58 tons acre^{-1}) was harvested from a cage that had one of the highest leafhopper totals. When these two outliers are omitted from the data set (Fig. 7) potato leafhopper (df=1, 16; F=7.73; p=0.01) and stem count (df=1, 16; F=8.12; p=0.01) effects on yield were significant; tons acre^{-1} = 0.79 – 0.0022*(total leafhoppers) + 0.0052*(number of stems), marginal $R^2 = 0.44$, conditional $R^2 = 0.60$. When the average number of stems is multiplied by its coefficient (0.0052); $y = 1.19 - 0.0022x$ where y is tons acre^{-1} and x is the total number of potato leafhoppers. Thus, 0.0022 tons acre^{-1} yield loss is associated with each additional potato leafhopper per cage.

When analyzing the effect of leafhoppers on crude protein there were no outliers in the data set and leafhoppers had a significant negative effect on crude protein (DF=1, 19; F=61.08;

$p<0.01$); crude protein = 25.64 - 0.044*(total leafhoppers) (Fig. 8), marginal $R^2 = 0.72$, conditional $R^2 = 0.74$. Similarly, there were no outliers in the model observing leafhopper effect on NDF; leafhoppers had a significant negative effect on neutral detergent fiber (df=1, 19; F=11.23; $p<0.01$) (Fig. 9); NDF = 32.52 - 0.027*(total leafhoppers), marginal $R^2 = 0.28$, conditional $R^2 = 0.47$.

Leafhopper carryover effect. When alfalfa was harvested at the end of trial 2 (3^{rd} crop) from plots previously caged in trial 1, alfalfa variety did not have a significant effect on yield (df=1, 42; F=0.14; p=0.71) so the analysis proceeded without variety. Stem count from 3^{rd} crop harvest (df=1, 43; F=61.44; $p<0.01$) and leafhoppers from the previous 2^{nd} crop did have a significant negative effect on yield (df=1, 43; F=7.85; $p<0.01$) (Fig. 10); tons acre^{-1} = 0.13 + 0.0090*(number of stems) – 0.0015*(total leafhoppers), marginal $R^2 = 0.49$, conditional $R^2 = 0.77$. When the average number of stems is multiplied by its coefficient (0.0090); $y = 0.85 - 0.0015x$, where y is the yield (tons acre^{-1}) from 3rd crop and x is the total leafhoppers from trial 1 conducted during 2^{nd} crop.

Crude protein was affected by alfalfa variety (df=1, 43; F=6.20; p=0.02) but not by potato leafhoppers from the previous crop (df=1, 43; F=0.96; p=0.33). Resistant alfalfa had slightly higher crude protein (mean=27.47, SD=0.35) than susceptible alfalfa (mean=26.69, SD=0.31). Neither potato leafhoppers from previous crop (df=1, 43; F=0.27; p=0.61) nor alfalfa variety had a significant effect on NDF (df=1, 43; F=3.57; p=0.07).

EIL calculations. Yield loss per potato leafhopper from trial 1 is found in the linear regression from potato leafhopper effect on yield (Fig. 4); the slope is 0.0019. The slope for yield loss per potato leafhopper from trial 2 (Fig. 7) is 0.0022. Because these values were so similar, the average slope value (0.0021) was used to represent the incremental yield loss per insect in the

EIL equation, *I*D*, or *I*, injury per insect unit, and *D*, damage or yield loss associated with each injury unit.

There are at least 11 different insecticides registered for potato leafhopper control in alfalfa (Cullen et al. 2012) with a wide cost range from as little as \$1.90 acre^{-1} to as much as \$34.00 acre^{-1} (USDA NASS 2013b) (Table 4). The low, middle and highest values were used in the EIL calculations to represent the range of insecticide costs; Baythroid XL (active ingredient, cyfluthrin) at low label rate for potato leafhopper \$1.90, Imidan 70WP (active ingredient Phosmet) at high labeled rate \$15.16 and Besiege (active ingredients chlorantraniliprole + lambda-cyhalothrin) at the high labeled rate \$34.00. The average cost of application based on hiring an operator and machine with fuel in WI in 2010 was \$7.70 acre^{-1} (USDA NASS 2011). This cost was added to each of the insecticide costs in order to obtain the costs of control as seen in table 5.

The market value of alfalfa throughout the United States without regard to forage quality ranged from \$115 to \$245 ton^{-1} with an average of \$200 ton^{-1} (USDA NASS 2013a). The market value of high quality forage in the upper Midwest ranged from \$127.50 to \$370 ton^{-1} with the average value at \$230 ton^{-1} (Barnet 2013). From these values, the low, middle and highest (\$115, \$230 and \$370) were chosen to represent the range of alfalfa market values in the EIL equation as obtained in table 5.

From the combinations of control costs (*C)* and alfalfa market values (*V)* above, a range of gain thresholds were calculated. The lowest gain threshold was 0.03 tons acre^{-1} when V=\$370 and C=\$9.60 and the highest was 0.36 tons acre^{-1} when V=\$115 and C=\$41.70 (Table 5). The respective EILs were 2 leafhoppers sweep^{-1} and 20 leafhoppers sweep^{-1}. From that, the economic threshold was calculated for alfalfa at height greater than 12 inches. Table 6 provides an example of the calculations for percent adjustment based on the current economic thresholds per alfalfa height class.

Discussion

The results presented in this study show that potato leafhoppers have a significant negative effect on alfalfa yield (Figs. 4 and 7). This is not surprising as there is already a wealth of information corroborating this data (Cuperus et al. 1983, Lamp et al. 1985, Hutchins and Pedigo 1990). Potato leafhoppers feed on alfalfa stems and leaves by inserting their stylet into vascular bundles, and disrupting movement of plant nutrients through the vascular bundles through a combination of mechanical damage and a saliva-enhanced wound response (Ecale and Backus 1995, Backus et al. 2005). Feeding damage results in decreased plant height and alfalfa yield (Lamp et al. 1985).

Crude protein content and neutral detergent fiber are important alfalfa forage quality indicators. Crude protein content is negatively affected by potato leafhopper feeding (Hower and Flinn 1986, Sulc et al. 2004). Hutchins and Pedigo (1990) determined that this trend is mediated through the effect that potato leafhoppers have on alfalfa maturation. Alfalfa that is infested early in the regrowth cycle matures roughly 30% slower than uninfested alfalfa, resulting in decreased daily accumulation of dry matter and nutrients. This may explain the decrease in crude protein observed in this study from over 26% in cages with zero leafhoppers to less than 20% in cages with the highest leafhopper density (Figs. 5 and 8).

The effect of potato leafhoppers on neutral detergent fiber (NDF) is less straightforward. In this study, potato leafhoppers had a significant negative effect on fiber (Figs. 6 and 9). Sulc et al. (2004) also concluded that potato leafhoppers negatively impact NDF from their observation of an increase in NDF when insecticides were used. Hutchins et al. (1989) found no significant relationship between leafhopper density and NDF content in the alfalfa plant overall. However, in the leaves they found higher NDF concentrations from plots that sustained heavy leafhopper feeding. The authors attribute this to an increased cell-wall concentration in the necrotic areas and the resulting cytoplasm leaking out of the leaf at the PLH feeding sites.

Host plant resistance (alfalfa variety) had no effect on crude protein or NDF in trial 1. Previous research found that resistant alfalfa has greater crude protein content and lower neutral detergent fiber than susceptible alfalfa (Hansen et al. 2002, Sulc et al. 2004, E.M.C.,unpublished data from dissertation chapter 2). Dellinger et al. (2006) saw no significant difference in crude protein between susceptible and resistant alfalfa varieties when leafhopper populations were left uncontrolled. However, the present study did not detect a carryover effect of potato leafhoppers from previous crop on subsequent crop forage quality, at which point resistant alfalfa did have greater crude protein content than susceptible alfalfa. It may be that increased forage quality typically present in resistant alfalfa diminishes under high leafhopper pressure. For example, Sulc et al. (2004) attributed differences in forage quality between resistant and susceptible alfalfa varieties to variation in leafhopper density.

We did see that potato leafhopper injury from the previous crop had a carryover effect of decreased yield in the subsequent crop harvest (Fig. 10). Other researchers have documented this phenomenon and even observed the carryover effect into the next growing season (Wilson et al. 1955, Vough et al. 1992). This occurs due to alfalfa use of carbohydrate energy reserves in the root tissues and its movement throughout the alfalfa plant. Carbohydrates are transported from alfalfa root reserves to the tissues of young stems after harvest or defoliation and back down to root and crown tissues about 20 days postharvest (Neilson et al. 1999, Lamp et al. 2001). Potato leafhopper feeding damage disrupts carbohydrate translocation and diminishes root reserves and thus mobilization of carbohydrates postharvest (Lamp et al. 2001). Lamp et al. (2001) also suggest that potato leafhopper carryover effect on alfalfa regrowth is mediated through a decrease in nitrogen root reserves based on nitrogen's important role in regrowth (see Volenec et al. 1996).

It is interesting to note that we did not observe differences between resistant and susceptible alfalfa varieties in any of our statistical analyses; leafhopper mortality and fecundity, yield or carryover effect. Host plant resistance can be demonstrated by antibiosis, antixenosis and/or tolerance. Depending on the mechanism of resistance, differential economic thresholds may be necessary for resistant and susceptible alfalfa varieties. Antibiosis host plant mechanisms act by either increasing insect mortality or decreasing fecundity (Teetes 2013). In this study, the numbers of adult and nymph potato leafhoppers recovered by vacuum sample are used as indicators of mortality and fecundity, respectively. If mortality rate increased or fecundity rate decreased on resistant alfalfa, then there would be reason to believe that the resistant variety demonstrated antibiosis. This was not the case as the interaction between alfalfa variety and number of leafhoppers infested did not have a significant effect on the number of leafhoppers recovered (Figs. 2 and 3) as indicated by the fact that there is no difference in slope between the resistant and susceptible alfalfa varieties. There is no evidence for antibiosis in the resistant cultivar as tested in the present study. Lefko et al. (2000) also found that nymph production did not differ between resistant and susceptible alfalfa in caged field trials and concluded that antibiosis was not a likely resistance mechanism in the resistant cultivar they tested. However, in no-choice laboratory experiments, Ranger and Hower (2001) observed higher nymphal mortality for leafhoppers caged on a resistant cultivar stem than a susceptible one. Thus, antibiosis may be functioning at an individual plant level rather than a alfalfa field population level, or simply in resistant cultivars other than the one used in our study.

By nature of the experimental design of caged trials, we could not test for leafhopper preference, or antixenotic resistance mechanisms. Previous field experiments in open plot, natural leafhopper infestations with the same resistant cultivar used in the present study (WL353LH) found lower potato leafhopper abundance in plots planted with resistant alfalfa (E.M.C., unpublished data from dissertation chapter 2). In these experiments, potato leafhoppers had a "choice" between alfalfa plots. The lack of evidence in the present study for antibiosis in conjunction with the decrease in potato leafhopper presence in the resistant alfalfa plots (chapter 2) supports antixenosis as a mechanism of resistance in this alfalfa cultivar. That is, there is a characteristic of this cultivar that is less attractive to the potato leafhopper than that of the susceptible variety planted alongside it in the field (Painter 1951).

Antixenosis has been noted previously in resistant alfalfa cultivars. For example, Ranger and Hower (2002) observed that adult potato leafhoppers in laboratory choice experiments settle and feed more on susceptible alfalfa than resistant alfalfa. Furthermore, Ranger et al. (2004) extracted compounds from trichomes of resistant and susceptible alfalfa stems and compared adult potato leafhopper settling behavior on sachets prepared with the extracts and artificial diet. There were consistently more adults found on sachets with the extracts from susceptible alfalfa than resistant alfalfa trichomes.

The last host plant mechanism to be considered is tolerance. This is expressed when the resistant alfalfa variety has similar yields to the susceptible alfalfa under increased potato leafhopper density, or when the yield of resistant alfalfa does not decrease at the same rate as susceptible alfalfa under similar pest pressure. There were no differences in yield or rates of yield loss per insect in the present study between resistant and susceptible varieties. Our results differ with those of a caged field study by Lefko et al. (2000). They found that several leafhopper resistant alfalfa cultivars produced equivalent yields to the susceptible variety when they infested the cage with twice the leafhoppers. However, Lefko et al. (2000) did not account for adult mortality in their experiment as we did by recovering potato leafhoppers with the vacuum at the end of each trial. Based on the methods described, it is possible that after infesting their cages with adult leafhoppers, they had higher mortality in the cages with resistant alfalfa. Though they did measure population growth based on nymphal count from each cage and found no differences in population growth between susceptible and resistant alfalfa cages.

Based on results of the present study, neither antibiosis nor tolerance can be confirmed for the resistant alfalfa cultivar (WL353LH), but in conjunction with previous work (E.M.C., unpublished data from dissertation chapter 2) it seems likely that antixenosis may be functioning in this cultivar. While antibiosis and antixenosis act on the pest population, tolerance is a change in how the plant responds to the pest. This change in plant response represents a change in one of the variables necessary for the determination of the economic injury level; D, or yield loss associated with each injury unit (Stern et al. 1959). The lack of evidence for tolerance in the cultivar in the present study led us to calculate an economic injury level from field data obtained for combined average yield response to potato leafhopper density for susceptible and resistant alfalfa.

Though the current potato leafhopper economic thresholds (Table 1) were originally derived from a curvilinear regression of yield loss per insect (Cuperus et al. 1983), there was no evidence of a curvilinear relationship of yield response to potato leafhopper density in the present study (Figs. 4 and 7). Additionally, both Lefko et al. (2000) and Hutchins and Pedigo

(1998) assumed a linear relationship for the purposes of calculating the EIL for potato leafhopper in alfalfa.

Calculating the gain threshold is an important first step for determining the EIL because market value of alfalfa is dynamic, and control costs vary by farmer practices (Pedigo et al. 1986). The gain threshold is the break-even point when the cost of the insecticide treatment is equal to the economic damage imposed on the cropping system. It is expressed in terms of yield and calculated by dividing the cost of control by the market value of the crop.

As seen in table 5, the gain threshold varies depending on alfalfa price and control cost at any given time, ranging from 0.03 tons acre^{-1} to 0.36 tons acre^{-1}. For practical purposes, a farmer or crop consultant interested in determining the economic threshold can easily calculate the gain threshold based on their control costs and anticipated value of alfalfa crop. By selecting the gain threshold from table 5 that is most similar to their own, they can determine appropriate economic thresholds for their circumstance.

For the purpose of this discussion, the market value of alfalfa in the middle of the range on table 5 (\$230 ton^{-1}) most closely represents the current alfalfa prices regardless of forage quality throughout the United States (Gould 2013). At this alfalfa hay price and the cheapest control cost (\$9.60 acre^{-1}), economic thresholds calculated in the present study differ only slightly from current economic thresholds (Table 1). For alfalfa heights 0-4 inches, the threshold remains the same, but at 4-8 inches, the threshold is one tenth of a leafhopper higher than the current practice (0.6 leafhoppers sweep^{-1} compared to 0.5 leafhoppers sweep^{-1}). For alfalfa 8-12 inches, the threshold is two tenths of a leafhopper greater than the current practice (1.2 leafhoppers sweep^{-1} compared to 1.0 leafhoppers sweep^{-1}), and for alfalfa greater than 12 inches the threshold is a half of a leafhopper per more than the current practice (2.5 leafhoppers sweep^{-1}

compared to 2.0 leafhoppers sweep^{-1}) (table 5). The prices used to calculate this series of economic thresholds (C=\$9.60 and V=\$230) give rise to one of the lowest gain thresholds calculated (0.04 tons acre^{-1}). The lowest gain threshold of 0.03 tons acre^{-1} is calculated when alfalfa prices increase to \$370 ton^{-1} and cost of control remains \$9.60 acre^{-1}. However, even this lowest gain threshold does not validate decreasing the current economic threshold because it is so similar to the current economic threshold (Tables 1 and 5). At the lowest gain threshold, the ET for alfalfa 0-4 inches and 4-8 inches are the same as the current ET (0.2 leafhoppers sweep^{-1} and 0.5 leafhopper sweep^{-1} respectively). For alfalfa 8-12 inches, the ET decreases by just one-tenth of a leafhopper (0.9 leafhoppers sweep^{-1} compared to 1.0 leafhoppers sweep^{-1}) and for alfalfa greater than 12 inches the ET decreases by just two-tenths of a leafhopper sweep^{-1} (1.8 leafhoppers sweep^{-1} compared to 2.0 leafhoppers sweep^{-1}). Farmers currently in the practice of reducing the ET when alfalfa market values increase are most likely incurring unnecessary economic costs as well as increasing environmental risk of insecticides.

The methods employed in this study to re-evaluate potato leafhopper economic thresholds were adapted from Lefko et al. (2000) in which EILs were calculated for resistant and susceptible alfalfa varieties. However, differences in their study design included the number of different initial leafhopper densities in the cages and the calculation of the economic threshold. Lefko et al. (2000) used four densities of leafhoppers in each cutting experiment (instead of the ten in this study) and instead of regressing leafhopper density on yield, they estimated percentage yield loss for each cage compared to a cage with zero potato leafhoppers from which linear models were created for average percent loss for each level of infestation. The authors used a modified economic injury level equation (Pedigo et al. 1986) in which the gain threshold, typically defined as C/V, is calculated as a percentage of yield loss ($100*C/V*Y_P$, where Y_P is the yield potential) rather than the actual yield. Their EIL model also incorporates the y-intercept of the aforementioned yield loss equations, to account for differences in damage boundaries between cultivars, or the number of leafhoppers that create yield loss. In our work, we created a large enough gradient of potato leafhopper densities to give statistical power to the linear yield loss regression in order to utilize the original EIL equation (Pedigo et al. 1986).

Hutchins and Pedigo (1998) calculated economic injury levels for potato leafhopper on alfalfa with an emphasis on management for nutritional value based on type of animal for which the feed is intended. Incorporating variables of insect injury on forage quality characteristics (Hutchins and Pedigo 1990) and different animal nutrition needs, they determined that the economic injury level is lowest for alfalfa hay intended for sheep or horses, medium for a beef or dairy cows, and highest for beef steer. In the present study, we determined the relationship between potato leafhopper injury and crude protein content. Our results could be used to inform an EIL based on animal need for protein. In trials 1 and 2, crude protein declined 0.033 and 0.044 percent, respectively, for each additional leafhopper in the caged study area (Figs. 4 and 8). However, such work is beyond the scope of this project. Additionally, EILs based on livestock nutrient needs have not been adopted in practice.

The most frequently used insecticides for potato leafhopper in alfalfa are pyrethroids. These insecticides are broad-spectrum and affect natural enemy or pollinator populations just as they do pest populations. Though pyrethroids have improved mammalian and avian toxicity profiles compared to forbearers such as organochlorines, carbamates and organophosphate insecticides they are acutely toxic to fish and aquatic invertebrates (Lopez et al. 2005, Devine and Furlong 2007).

Previous researchers have suggested that standard EIL formula (Stern et al. 1959, Pedigo and Rice 2009) should be revised to include environmental costs (Prokopy and Kogan 2009). Even the seminal publication (Stern et al. 1959) introducing the EIL concept promoted the idea that deleterious environmental effects must be accounted for as a cost function of the EIL:

> *When chemical control is used, the damage from the pest species must be sufficiently great not only to cover the cost of the insecticide but also the possible deleterious effects such as the harmful influence of the chemical on the ecosystem* (p.88, Stern et al. 1959).

Integrated control combines biological and chemical control; when applying chemical control it is important to take biological control agents into consideration. This pertains to biological control of key pests as well as other pests within the agroecosystem. With regard to alfalfa, this idea is especially pertinent considering that alfalfa is habitat for so many arthropods, including pollinators and natural enemies of pests in neighboring crops (Flanders and Radcliffe 2013) thus it provides a large contribution of pest suppression to the surrounding agricultural landscape.

There is a significant ecological cost associated with the loss of non-target invertebrates such as pollinators and natural enemies. Though it is beyond the scope of this paper to incorporate these costs into the EIL, a comprehensive study estimated $520 million of crop losses and increased insecticide costs due to natural enemy populations lost from pesticide use. The study estimated an additional $252 million in annual crop losses due to pollination services lost by pesticides in the United States (Pimental 2005). Certainly when costs such as these are taken into account, the economic injury level increases. However, economic thresholds based on this expanded EIL model would place a disproportionate economic burden on farmers.

The economic injury level concept is inherently limited for reasons such as the externalization of ecological costs. However, the research data used to derive realistic economic thresholds is also limited. Insecticide treatment decision support models are based on data derived from field trials under specific abiotic conditions (such as soil or climate) that have great influence on biological variables such as crop response to insect injury. For example, the data presented in this study represent alfalfa response to potato leafhopper during a dry summer. As such, yields are lower than typical. Hopperburn appears more frequently during summer droughts (Hoffman et al. 1991) likely due to an additive effect of leafhopper feeding and drought stress on alfalfa's physiological response (Schroeder et al. 1988). Additionally, potato leafhoppers do not cause uniform damage over their lifetime. First and 2nd instars and adult males cause less damage than 3-5th instars and female adults are cause more damage yet (Onstad et al. 1984). However, addressing complexities in both weather and potato leafhopper life tables would add a level of complexity to the model that would reduce its ability to be implemented.

Conclusion. Re-evaluation of current economic thresholds for potato leafhopper in alfalfa in the current study provides evidence that there is no need to reduce the current economic threshold in response to the increase in alfalfa market value (Holin 2008). Depending on insecticide control costs and market value of alfalfa, there is evidence that the economic threshold could be increased. Future work should test the validity of thresholds from our caged trials in the present study under natural potato leahopper infestation field conditions across multiple sites and years.

The mechanism of resistance shown in the resistant cultivar used in this study (WL353LH) can best be described as antixenosis, which did not warrant differential EIL calculation for resistant and susceptible alfalfa based on results of our study. Previous work has found that some resistant cultivars demonstrate tolerance to potato leafhopper feeding (Lefko et al. 2000). Based on the variability in resistance mechanisms demonstrated in different resistant cultivars, it may be that there is no singular EIL adjustment for leafhopper resistant alfalfa. It would be prudent to determine the mechanism of resistance for each potato leafhopper cultivar and then prescribe an EIL and ET based on those findings.

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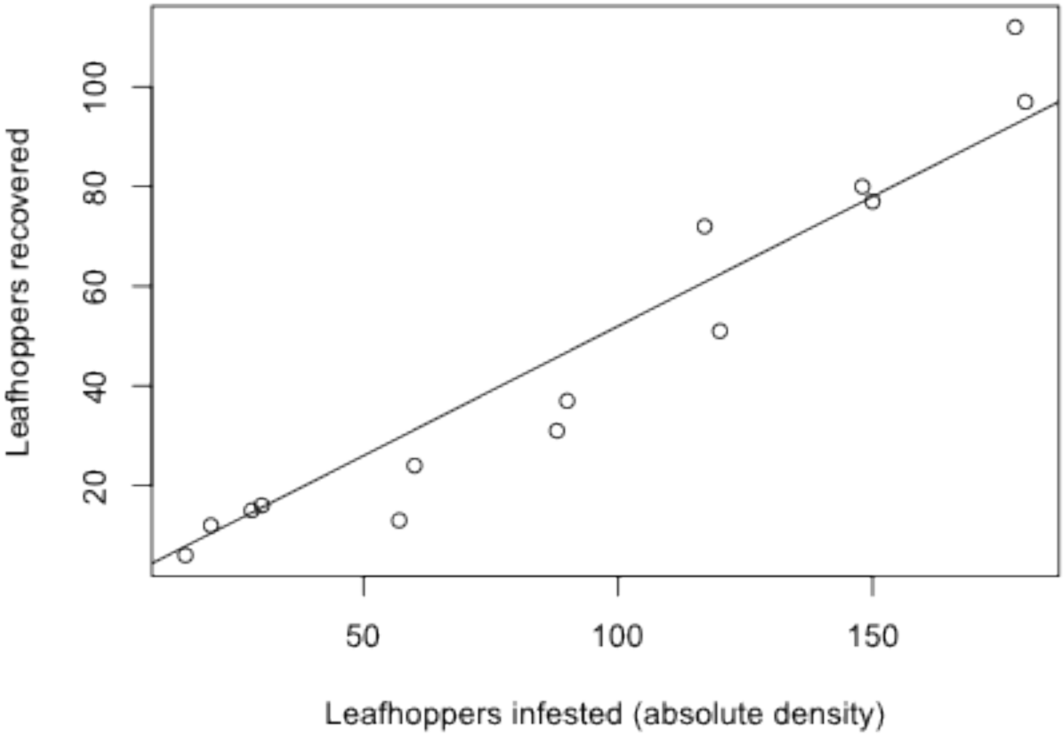

Figure 1. Efficiency of potato leafhopper vacuum recovery versus potato leafhopper infestation; $y = 0.52\ x$, $R^2 = 0.97$.

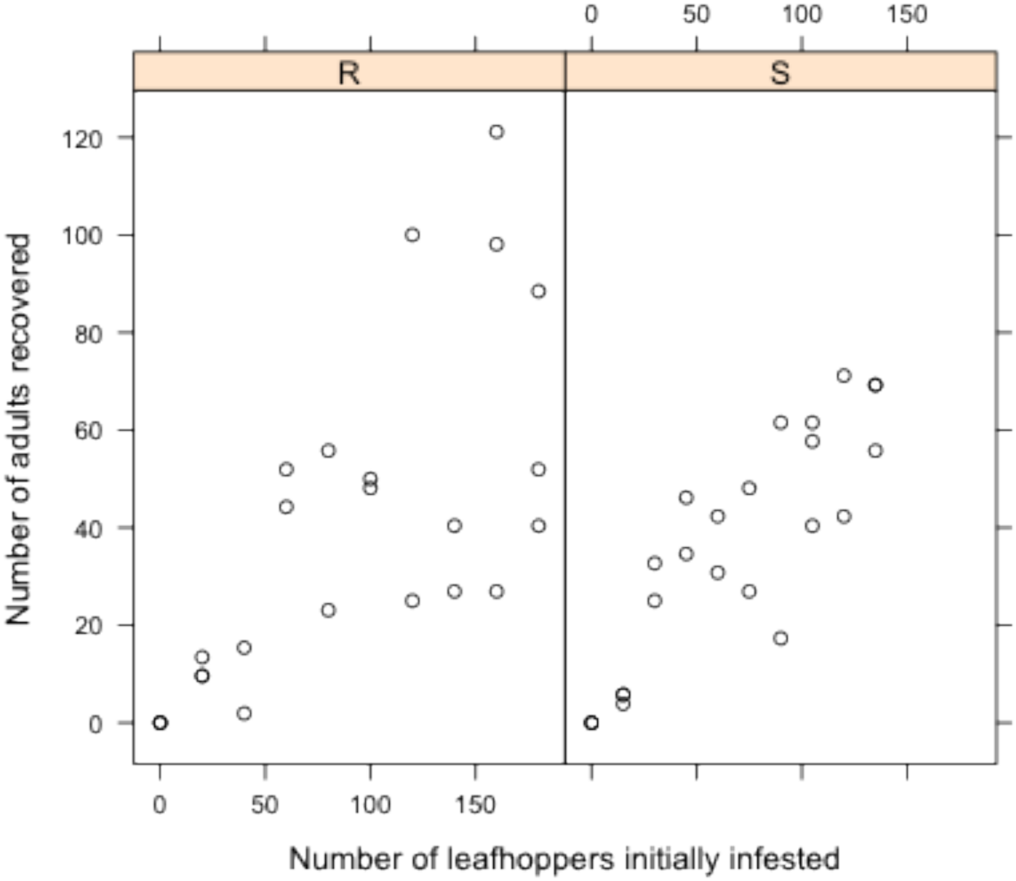

Figure 2. The number of potato leafhopper adults recovered from each alfalfa variety in trial 1 (R=resistant, S=susceptible) as a factor of initial adult infestation density. Slopes are not significantly different (df=1, 42; F=0.42; p=0.52).

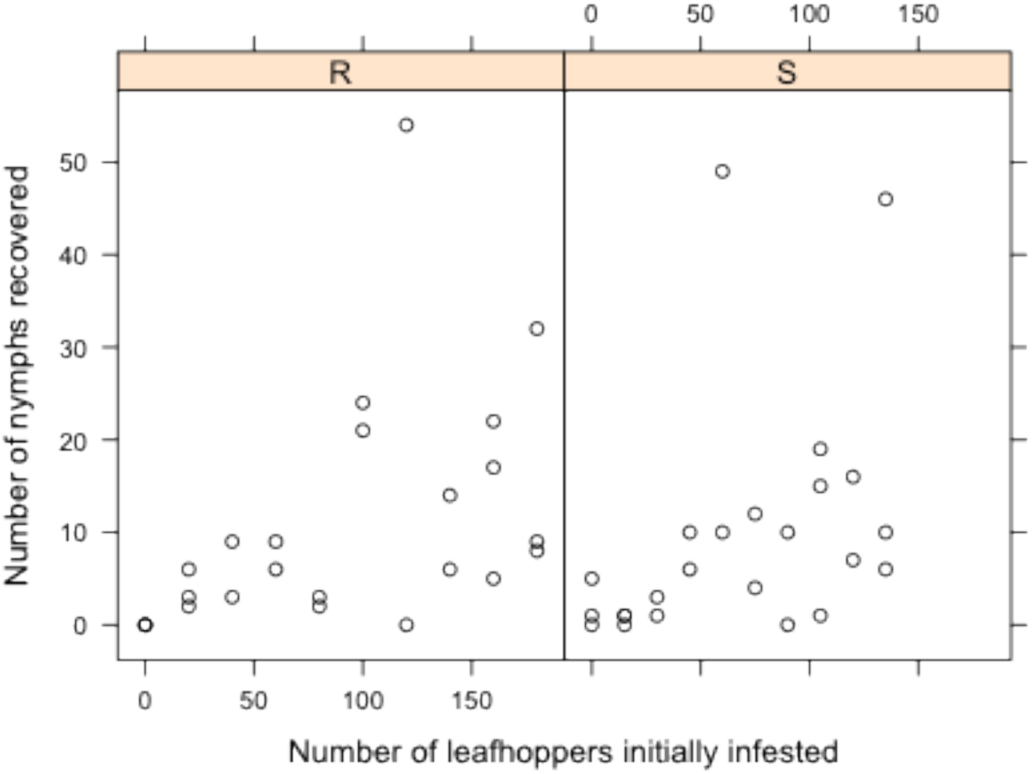

Figure 3. The number of nymphs recovered from each alfalfa variety in trial 1 (R=resistant, S=susceptible) as a factor of initial adult infestation density. Slopes are not significantly different (df=1, 42; F=0.09; p=0.77).

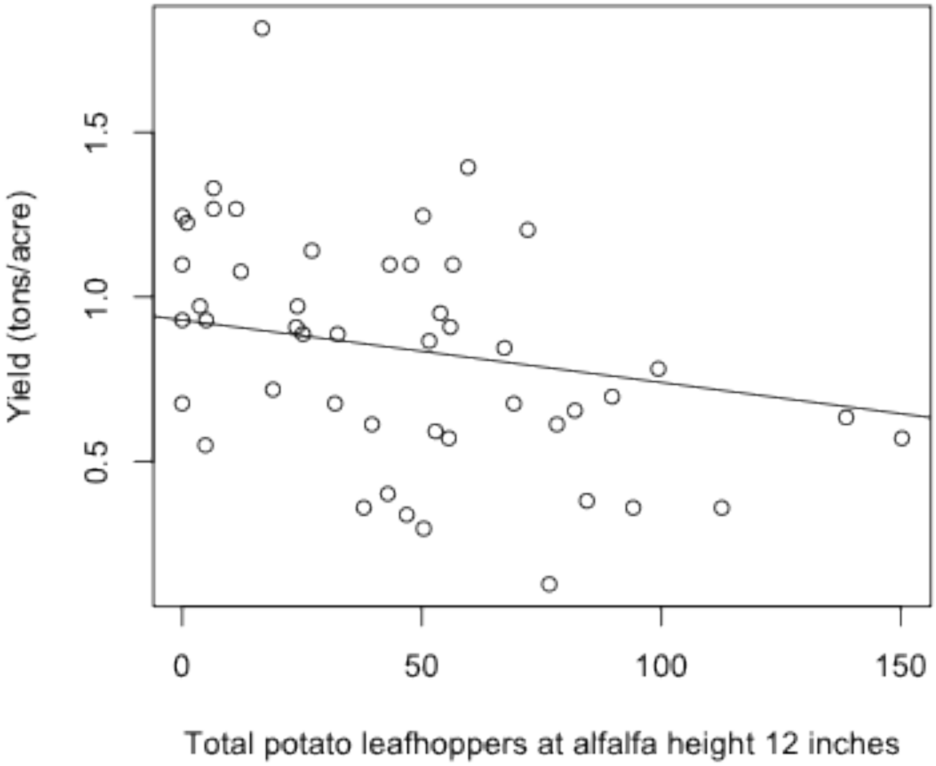

Figure 4. Effect of total potato leafhoppers on alfalfa yield (tons acre^{-1})[a] in trial 1 for susceptible and resistant alfalfa; y = 0.93 - 0.0019x, marginal $R^2 = 0.51$, conditional $R^2 = 0.68$.

[a] English units are presented here as per alfalfa production industry in the United States.

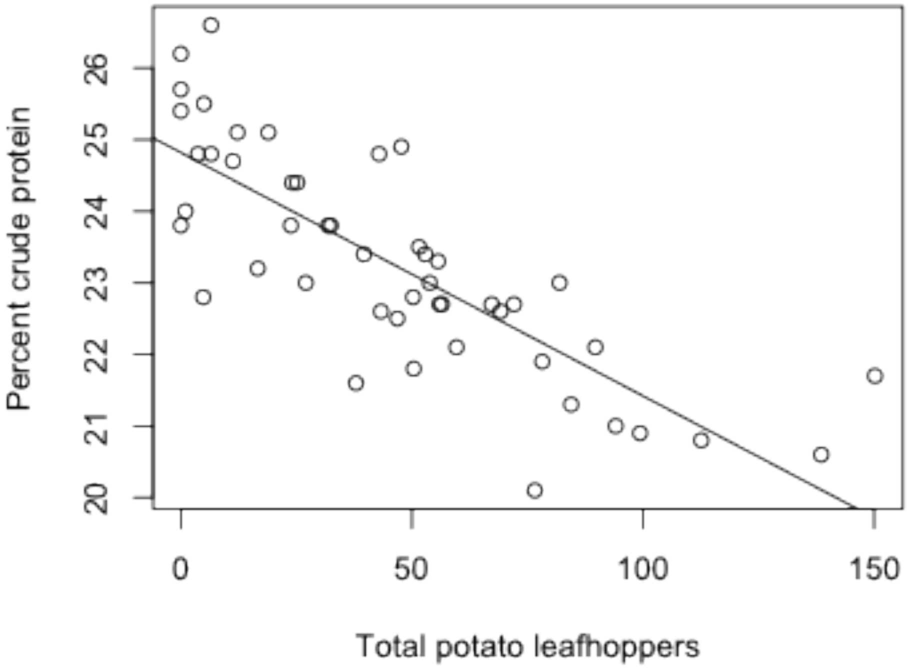

Figure 5. Effect of total leafhoppers on crude protein from trial 1; y = 24.82 - 0.033x, R^2 = 0.63 (same for marginal and conditional).

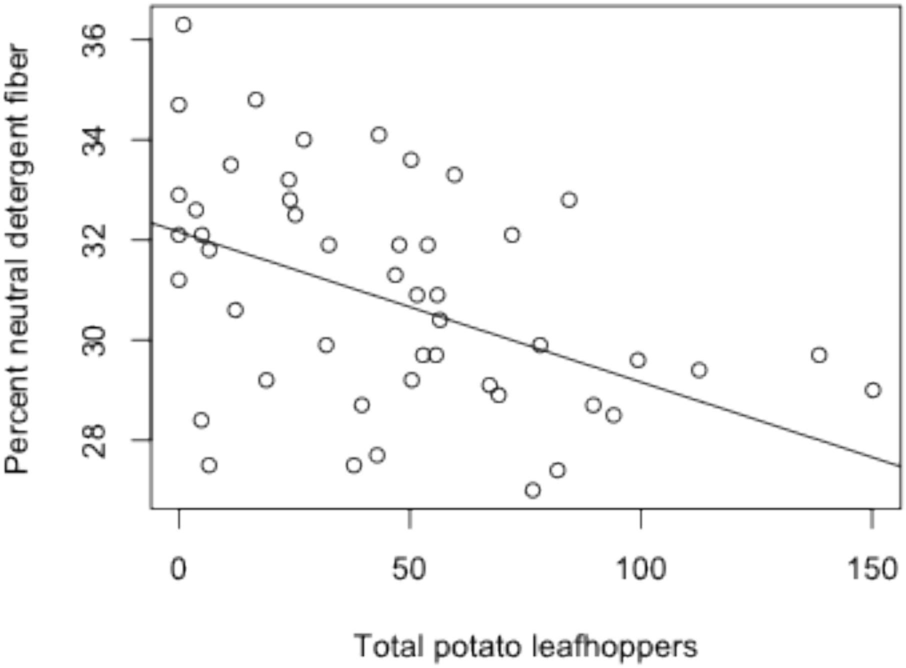

Figure 6. Effect of potato leafhoppers on neutral detergent fiber from trial 1; y = 32.16 -0.030x, marginal R^2 = 0.17, conditional R^2 = 0.41.

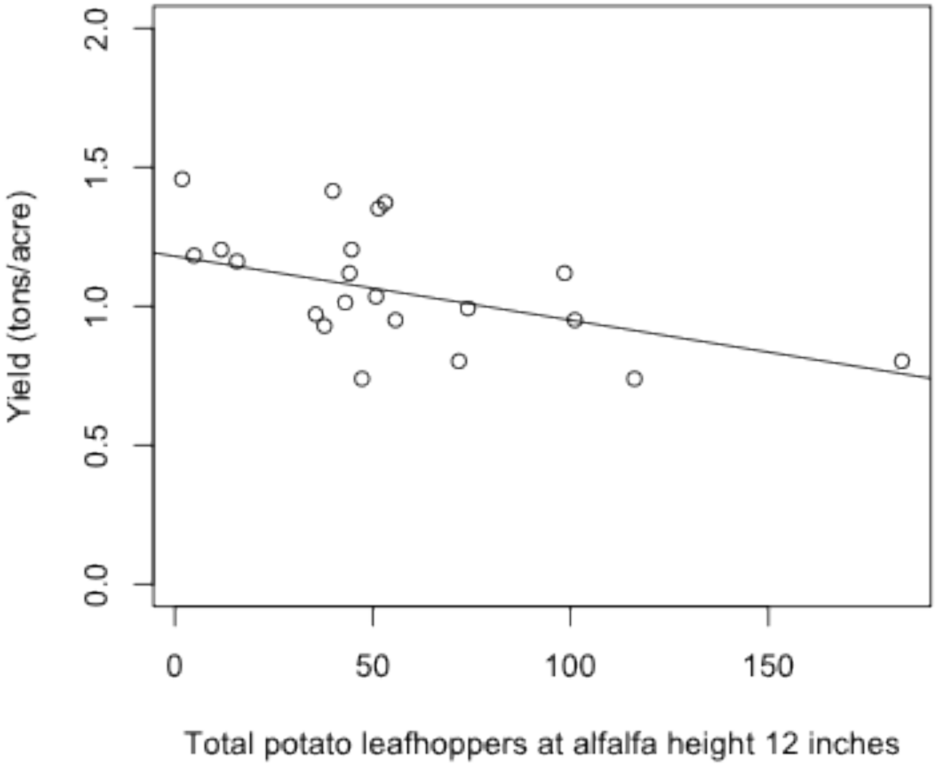

Figure 7. Effect of total leafhoppers on susceptible alfalfa yield (tons acre^{-1})[a] in trial 2 (omitting outliers)[b]; y = 1.19 – 0.0022x), marginal R^2 = 0.44, conditional R^2 = 0.60.

[a] English units are used as per alfalfa production industry in the United States.

[b] Sample size n=24 but one sample was omitted due to a broken vacuum bag reducing sample size to n=23 prior to omission of outliers for a total of n=21.

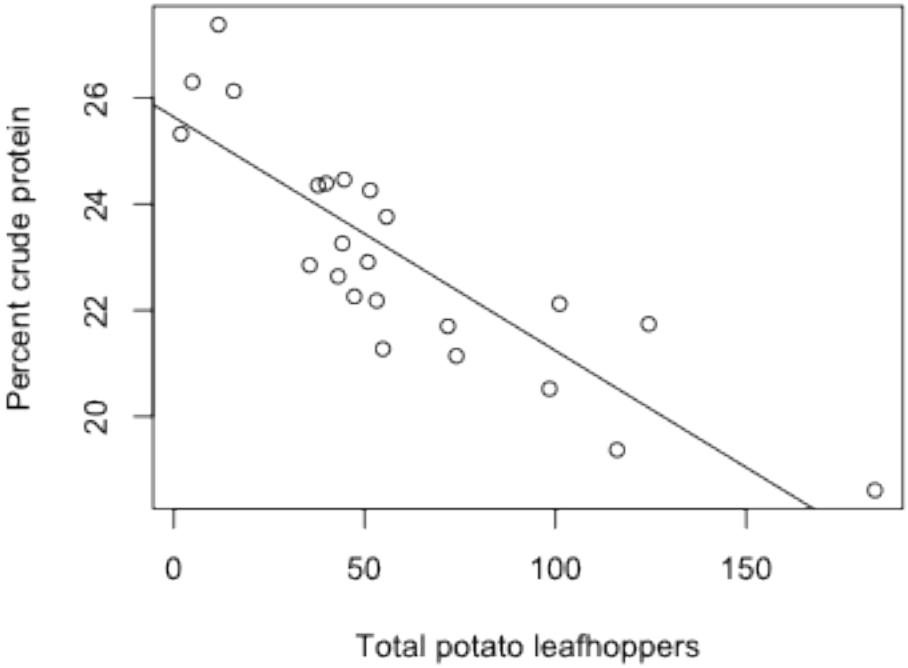

Figure 8. Potato leafhopper effect on crude protein for susceptible alfalfa in trial 2; y = 25.64 – 0.044x, marginal $R^2 = 0.72$, conditional $R^2 = 0.74$.

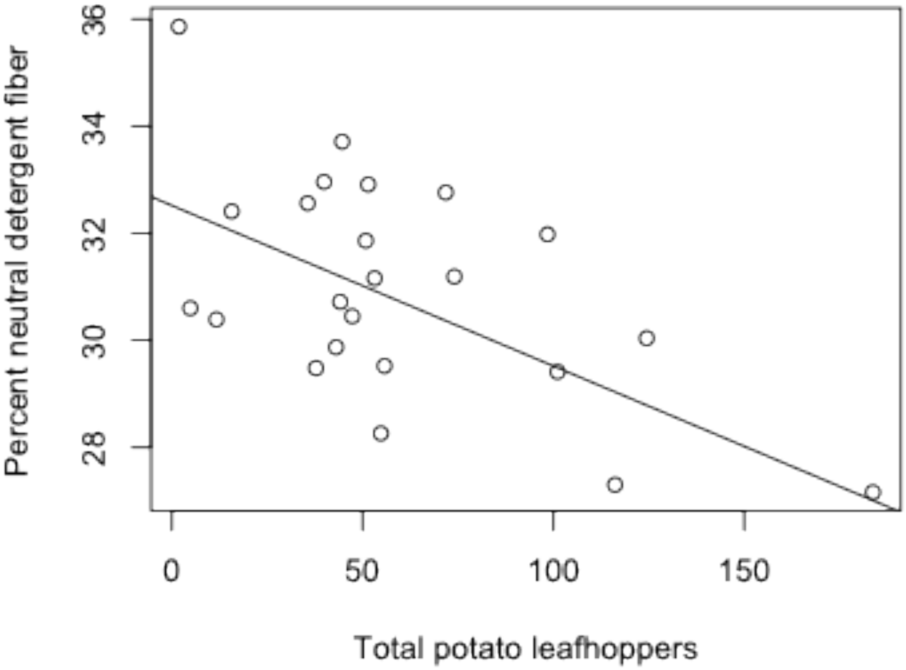

Figure 9. Effect of leafhoppers on neutral detergent fiber for susceptible alfalfa in trial 2; y = 32.52 – 0.027x, marginal R^2 = 0.28, conditional R^2 = 0.47.

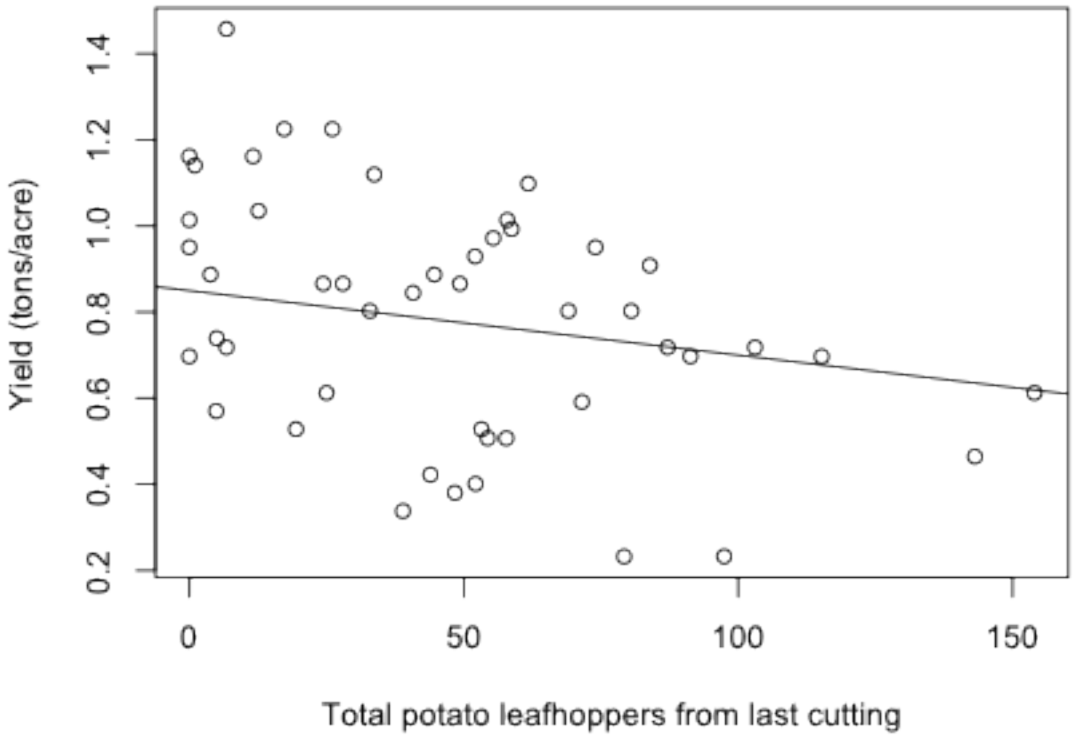

Figure 10. Carryover effect of potato leafhoppers on yield (tons acre^{-1})[a]; y = 0.85 - 0.0015x, marginal R^2 = 0.49, conditional R^2 = 0.77.

[a] English units are used as per alfalfa production industry in the United States.

Table 1. Current potato leafhopper (PLH) economic thresholds based on sweep net sample method (adapted from Cullen et al. 2012).

Alfalfa Height, inches (cm)	PLH sweep^{-1}
0-4 (0-10)	0.2
4-8 (10-20)	0.5
8-12 (20-30)	1.0
12+ (30+)	2.0

Table 2. Dates of experimental method activities for caged field experiments, trial 1 and trial 2. U.S. Dairy Forage Research Center, Prairie du Sac, WI, 2013.

Activity	Trial 1[a]	Trial 2[b]
Previous crop harvest	June 5	July 2
Insecticide application[c]	June 10	July 8
Cages installed	June 11	July 9
Leafhoppers aspirated	June 12	July 10
Cages infested	June 13	July 11
Leafhopper mortality assessed	June 14	July 12
Cages supplemented with new adults	June 17	July 15
Leafhopper vacuum recovery	July 1	July 29
Harvest	July 2	July 30[d]

[a] Completed during second crop of 2013 on susceptible and resistant alfalfa.

[b] Completed during third crop of 2013 on susceptible alfalfa.

[c] Pyganic (a.i. pyrethrin) applied at 64 oz acre^{-1}.

[d] Alfalfa from trial 1 harvested again to analyze carryover effects.

Table 3. Percent adjustments used to relate economic thresholds for all alfalfa heights based on current economic thresholds.

Alfalfa Height, inches (cm)	Current ET (PLH $sweep^{-1}$)	Percent Adjustment
0-4 (0-10)	0.2	10
4-8 (10-20)	0.5	25
8-12 (20-30)	1.0	50
12+ (+30)	2.0	100

Table 4. Insecticide costs for insecticides labeled for potato leafhopper in alfalfa for both low and high application rates.

Insecticides labeled for potato leafhopper in alfalfa[a]	Cost at low rate ($ acre^{-1})[b]	Cost at high rate ($ acre^{-1})[b]
Ambush 2E	2.33	9.33
Baythroid XL	1.90	3.79
Besiege	11.13	34.00
Cobalt Advanced	2.63	5.68
Dimethoate	3.10	6.20
Imidan 70WP	11.40	15.16
Lorsban Advanced	2.43	4.85
Mustang Max	2.35	4.19
Sevin XLR Plus	32.53	32.53
Stallion	4.10	9.64
Warrior II	2.95	4.91

[a] (Cullen et al. 2012)
[b] (USDA NASS 2013b)

Table 5. Economic injury levels (EIL), economic thresholds (ET) and gain thresholds (GT) for potato leafhopper control in alfalfa calculated for a range of alfalfa market values (V) and costs of control (C) with the average yield loss per insect from trial 1 and trial 2. Economic thresholds for each alfalfa class based off of the percent adjustments as per table 3.

Alfalfa market value (V) (\$ ton^{-1})	Insecticide Cost (C) (\$ acre^{-1})	Gain threshold (C/V) (tons acre^{-1})	EIL (PLH sweep^{-1})	ET 12+ in (30+ cm) (PLH sweep^{-1})	ET 8-12 in (20-30 cm) (PLH sweep^{-1})	ET 4-8 in (10-20 cm) (PLH sweep^{-1})	ET 0-4 in (0-10 cm) (PLH sweep^{-1})
115.00	9.60	0.08	6	4.1	2.1	1.0	0.4
	22.86	0.20	12	8.7	4.4	2.2	0.9
	41.70	0.36	20	15.3	7.6	3.8	1.5
230.00	9.60	0.04	3	2.5	1.2	0.6	0.2
	22.86	0.10	6	4.8	2.4	1.2	0.5
	41.70	0.18	11	8.0	4.0	2.0	0.8
370.00	9.60	0.03	2	1.8	0.9	0.5	0.2
	22.86	0.06	4	3.3	1.6	0.8	0.3
	41.70	0.11	7	5.3	2.7	1.3	0.5

Table 6. Example of percent adjustment for economic thresholds of potato leafhopper (PLH) at different alfalfa heights, based on the current economic threshold (ET), for the ET calculated when gain threshold (GT) is 0.04 tons acre^{-1} (0.09 Mg ha^{-1}).

Alfalfa Height, inches (cm)	Current ET (PLH sweep^{-1})	Percent Adjustment	Revised ET (PLH sweep^{-1})
0-4 (0-10)	0.2	10	0.2
4-8 (10-20)	0.5	25	0.6
8-12 (20-30)	1.0	50	1.2
12+ (30+)	2.0	100	2.5

Chapter 4: Potato Leafhopper Response to Alfalfa Treated with Liquid Dairy Manure in Laboratory Assay and Field Experiments

Abstract

Wisconsin alfalfa growers have observed, and communicated to county agents, a connection between the application of liquid dairy manure to their fields and a decreased incidence of economically damaging populations of potato leafhoppers. The objective of this study was to follow up on grower anecdotal evidence in order to provide scientific data with regards to this farmer observation. Potato leafhopper response to liquid dairy manure application, synthetic N-P-K-S application and a control treatment with no soil amendment was studied in the summers of 2011 and 2012 at Arlington Agricultural Research station. In 2011, we saw that when potato leafhopper populations peaked, manure did suppress potato leafhopper populations. In 2012 we repeated the experiment in two fields at Arlington Agricultural research station. Results from one field showed an increase in potato leafhopper abundance in manure plots, before potato leafhopper populations peaked later in the summer, and no difference between treatments when populations reached peak abundance. Results from the other field show no significant difference between treatments at any point in the summer. Concurrently, laboratory assays were conducted to determine female potato leafhopper ovipositional preference on alfalfa grown in potting soil with or liquid dairy manure treatment. Nymph emergence was counted as a proxy for ovipositional preference and no significant differences were detected. The results we observed are inconclusive with regards to the goal of this study. Explanations for inconsistent results between 2011 and 2012 and the laboratory assays are explored. Future work could explore effects on potato leafhoppers due to differences in the microbial communities of the soil and manures.

Keywords: IPM, cultural control, soil fertility management, potato leafhopper

Integrated pest management (IPM) programs combine ecologically based pest management tactics with the goal of improved economic and environmental sustainability and social acceptance (Mitchell and Hutchison 2008). IPM practices can be biological, physical, cultural or chemical (Hamerschlag 2007). One measure of a successful IPM program is reduced reliance on pesticides. Organic farmers have reported decreased insect herbivory in cropping systems when soil is treated with organic nutrient sources, as compared to synthetic nutrient sources (Altieri and Nicholls 2003). Though not accounted for in IPM literature, soil fertility is a potential form of cultural control. Cultural control is defined as the purposeful manipulation of the environment with the goal of reducing pest abundance (Prokopy and Kogan 2009). One of the functional mechanisms of cultural control is the creation of adverse biotic conditions that reduce the survival of pests (Ferro 2013). Previous research attributes patterns of decreased pest incidence in crops fertilized by organic nutrient sources to an increase in plant defenses or changes in palatability and foliar nitrogen content (Phelan et al. 1996, Altieri and Nicholls 2003, Staley et al. 2010). These changes in plant chemistry are an example of adverse biotic conditions that reduce pest abundance, making organic soil fertility management a candidate for a cultural control. Organic nutrient sources refer to substances added to the soil coming from plant or animal material, as compared to commercial synthetic or inorganic fertilizers.

Palatability of crops to insect herbivores is determined by structural and chemical defenses (Coley et al. 1985). Plant defense compounds are byproducts of primary metabolism; they are plant secondary metabolites that cause illness, injury or mortality to another organism when the organism encounters the compound through ingestion or physical contact (Berenbaum 1995). Inherent to this physiological production is a trade off for plants between growth and defense (Herms and Mattson 1992). Agricultural crops have morphological and biochemical plasticity that take advantage of resource pulses from inputs, such as fertilization, resulting in increased growth or defense (Coley et el. 1985).

Crops respond to fertilizer inputs by increasing growth and yield. Plants chemical composition also changes in response to nutrient sources. Changes in chemical composition can vary depending on whether the nutrient source is organic or synthetic. For example, when Staley et al. (2010) fertilized *Brassica oleracea* in the field with either ammonium nitrate (NH_4NO_3) or organic amendments composed of chicken manure plus green manure, foliar nitrogen content was greater for the ammonium nitrate treated plants and glucosinolate defense compounds were greater in plants treated with manures. Rao (2002) observed that groundnut, *Arachis hypogaea* had higher nitrogen content when fertilized synthetically than when treated with organic amendments such as farm yard manure, neem cake and vermicompost; in contrast, defense compounds such as phenols and tannins were higher in plants fertilized organically (Rao 2002). In both studies, the increase in defense compounds corresponded to reduced incidence of insect herbivore pests: an increase in glucosinolates was linked to suppression of green peach aphid, *Myzus persicae* and diamondback moth, *Plutella xylostella* (Staley et al. 2010), and increased phenols and tannins correlated to suppression of leafhopper, *Empoasca kerri*, and cowpea aphid, *Aphis craccivora* (Rao 2002). Similar patterns of increased plant defense compounds or decreased pest incidence when plants are fertilized organically compared to synthetically are well documented (Phelan et al. 1996, Yardim et al. 2006, Balakrishnan 2007, Cardoza 2011).

Patterns of increased insect abundance on crops fertilized with synthetic nitrogen arise from increased plant nitrogen content (Facknath and Lalljee 2005, Staley et al. 2010). Insects need nitrogen for growth and reproduction. Because plants consist mostly of carbohydrates, nitrogen is a limiting nutrient for insect herbivores (Mattson 1980). Synthetic fertilizers provide a more readily available nitrogen source for plant uptake and therefore nitrogen content increases more quickly in crops treated with synthetic fertilizer than crops treated with organic fertilizers. For example, conversion of nitrogen from urea ($CO(NH_2)_2$ to ammonium (NH_4^+) is rapid and more complete than from organic nitrogen sources such as alfalfa pellets, chicken manure and blood meal (Agehara and Warncke 2005). Because nitrogen from organic sources must first undergo biological decomposition, the nitrogen takes longer to mineralize into NH_4^+ (Chae and Tabatabai 1986, Agehara and Warncke 2005). Plants amended with organic nitrogen sources therefore do not demonstrate sharp increases in nitrogen content; rather, nitrogen is available in smaller concentrations over longer periods of time.

Decreased pest incidence is not always associated with organic nutrient sources. The opposite pattern is often found when an insect pest specializes on a particular crop. For example, the aphid *Brevicoryne brassicae* that specializes on brassica crops was more abundant on cabbage *Brassica oleracea* var. *capitata* fertilized organically with green manure or chicken manure pellets than with ammonium nitrate (Staley et al. 2010). Letourneau et al. (1996) found greater pest damage on tomatoes when crops had been fertilized with a range of organic amendments than when crops were fertilized synthetically. Garratt et al. (2011) performed a meta-analysis of insect pest responses to organic fertilizers compared to synthetic fertilizer and found no significant difference. However, upon more detailed analysis they found that pests are more frequently suppressed by animal manures than by plant compost when compared to synthetic fertilizers (Garratt et al. 2011).

Some Wisconsin farmers have noted a correlation between application of liquid dairy manure to established alfalfa stands after the first and/or second harvest and a decrease in economically damaging populations of potato leafhopper, *Empoasca fabae* when compared to similar fields on their farms that did not receive manure (M. Rankin, pers. comm.). Leguminous crops may not be as likely to exhibit patterns of foliar nitrogen increase when fertilized with synthetic fertilizer or increase of plant defense compounds when amended with organic soil treatments because these plants fix atmospheric nitrogen through symbiotic relationships with nitrogen fixing bacteria, *Rhizobium*. In contrast to the above pest suppression studies, synthetic nitrogen fertilizer is not typically applied to alfalfa. However, when ammonium-nitrogen (NH_4^+) or nitrate-nitrogen (NO_3^-) are readily available in the soil, as found in synthetic fertilizers or when organic nitrogen is mineralized from manure, its presence inhibits biological nitrogen fixation because it costs less energetically for the plant to uptake nitrate directly from the soil (Zahran 1999). This difference in energy expenditure by the plant for nitrogen uptake could lead to differences in alfalfa secondary metabolite synthesis when manure or synthetic nitrogen fertilizers are applied to the field.

Alternatively, a reduction in potato leafhopper abundance after the application of liquid dairy manure may be due to the volatilization of ammonia and resulting toxicity. Approximately 50-60% of the nitrogen content in liquid dairy manure may be found as ammonium (Beauchamp et al. 1982). Beauchamp et al. (1982) recorded 24-33% volatilization of the ammonium within one week of application. However, most of these emissions occur within the first few hours after manure slurry application (Pfluke et al. 2011). Considering that adult potato leafhoppers emigrate from alfalfa fields after harvest (Poston and Pedigo 1975) and the time needed to re-immigrate after harvest (Emmen et al. 2004) as well as that fact that insects have a greater tolerance to ammonia than other animals (Weihrauch et al. 2012), this explanation does not seem viable.

Alfalfa synthesizes several secondary metabolites that act as insect deterrents; derivatives of phenolic compounds in the form of tannins and flavonoids, and the triterpenoid saponin (Stochmal 2001, Agrell 2003 and Golawska 2009). Tannins and flavonoids are harmful to insect consumers because they bind to protein, inhibiting digestion. Flavonoids also inhibit digestive enzymes in insect herbivores (Treutter 2006). Saponins act on the plant surface, soap-like including both a lipophilic and hydrophilic end. They act by binding to sterols, inhibiting insect development (Bernays, 1994). Golawska (2009) studied pea aphid *Acyrthosiphon pisum* response to two difference alfalfa cultivars of different saponin concentrations. The cultivar with higher saponin concentration correlated with lower reproduction and survival of the aphids (Golawska, 2009).

No previous work has been done examining the putative relationship between manure applications on alfalfa fields and decreased potato leafhopper abundance, but a few studies have evaluated potato leafhopper response to synthetic fertilizers applied to alfalfa. Shaw et al. (1986) observed a decrease in potato leafhopper population in alfalfa fields receiving phosphorus (70 kg P ha^{-1}) and potassium (400 kg K ha^{-1}) compared to unfertilized fields, with exchangeable levels of 21 kg P ha^{-1} and 235 kg K ha^{-1}. However, in another study, increasing rates of potassium fertilization did not reduce potato leafhopper injury to alfalfa (Kitchen et al. 1990). Thus, potato leafhopper response is unclear with regard to individual plant nutrients as well as to manure.

In light of a growing body of literature supporting the correlation between manure applications and decreased insect pest abundance, experiments were conducted to provide scientific data to validate farmer observations in alfalfa regarding decreased potato leafhopper abundance after applications of liquid dairy manure. Field trials were conducted at the Arlington Agricultural Research Station over two growing seasons to observe potato leafhopper response to liquid dairy manure and synthetic fertilizer treatments. In addition, greenhouse bioassays were performed to test female ovipositional preference between alfalfa grown with or without liquid dariy manure soil amendment.

Materials and Methods

Field Trials.

Field site establishment. Two field sites were selected at the University of Wisconsin Arlington Agricultural Research Station (AARS), Arlington, WI. Field A was managed for two growing seasons, beginning in 2011 on a second year alfalfa stand, seeded May 17, 2010 with Pioneer 55V48 at 20.2 kg ha^{-1} (18 lbs acre^{-1}). Field A was located on Ringwood silt loam soil (fine-loamy, mixed, superactive mesic Typic Argiudoll). Field B was managed for one growing season in 2012 on a third year alfalfa stand, seeded April 13, 2010 with a mixture of alfalfa seed varieties at 16.8 kg ha^{-1} (15 lbs acre^{-1}). Field B was located on Ripon silt loam soil (fine-silty, mixed, superactive mesic Typic Arguidoll). Each trial was arranged in a randomized complete block design with three nutrient sources. Field A included six replications and Field B included 4 replications. All plots were 9.2 x 9.2 m (30 x 30 ft.). In total, three field trials were completed. Field A, 2011 will be referred to as trial 1; field A, 2012 will be referred to as trial 2, and field B, 2012 will be referred to as trial 3.

Treatments for all three field trials included 1) liquid dairy manure, applied by tanker after the first and second harvests (and after third harvest for trials 2 and 3) at a rate of approximately 46.8 kL ha^{-1} (5,000 gallons acre^{-1}) (table 1) and, 2) synthetic N applied as urea and ammonium sulfate, P applied as triple super phosphate, K applied as potassium chloride and S applied as ammonium sulfate, all hand applied at rates derived to match nutrient composition of a manure sample collected in 2010 (table 1) and, 3) control with no nutrient application.

Manure samples from each treatment application were sent to University of Wisconsin-Madison Soil and Forage Analysis Laboratories (Marshfield, WI) for nutrient analysis. Based on plant deficiency symptoms (white spots on lower leaflets) and management recommendations for annual fertilization (Undersander et al. 2011) at the end of the growing season in 2011 (September 13), Field A received 448 kg KCl ha^{-1} (400 lbs KCl $acre^{-1}$) and 135 kg K_2SO_4 ha^{-1} (120 lbs K_2SO_4 $acre^{-1}$) for field upkeep.

Trial 1 was initiated after first harvest 2011 when the first treatments were applied June 9. Treatments were applied again on July 8, after harvest July 6. Yield and forage quality were collected from harvests on July 6, August 1 and September 1.

Trial 2 treatments were applied after the first, second and third harvests on May 17, June 22 and July 20, 2012. Due to difficulties with the manure applicator, manure was applied on May 17 at roughly 65.5 kL ha^{-1} (7,000 gallons $acre^{-1}$). Alfalfa was harvested on June 20 (however only blocks 1-5 are represented because block 6 was missed during harvest), July 18 (weight was not recorded and samples were not taken due to severe plant stunting from drought stress) and August 14.

Trial 3 began when treatments were applied on May 17, 2012. Harvest data was collected June 20 and August 14. The July 18 harvest data was not collected because alfalfa plants were severely stressed due to drought. Treatments were applied after the first, second and third harvests (May 17, June 22 and July 20). Due to the same difficulties with manure tank machinery as above, manure was applied on May 17 at roughly 65.5 kL ha^{-1} (7,000 gallons $acre^{-1}$).

Data collection. Potato leafhopper populations were monitored weekly in each experiment using a 38 cm (15-inch) diameter sweep net to collect 20 sweep net samples per plot. Yield data was taken using an Almaco plot harvester through the center of the plot when plants reached approximately 10% bloom stage. Harvested plant subsamples were oven dried at 60°C and yields calculated on a dry matter basis. Alfalfa forage quality (crude protein and neutral detergent fiber) was analyzed by near-infra red reflectance (NIR) methods on dried and ground alfalfa samples.

Female oviposition choice bioassays

Colony maintenance. A colony of potato leafhoppers was maintained in an environmental growth room set to 25° C (day and night) and a photoperiod of 16:8 (L:D) (Hunter and Backus 1989). The colony was maintained on greenhouse grown 'Henderson' bush lima beans (*Phaseolus lunatus*). All leafhoppers were moved to alfalfa plants 24 hours prior to assay initiation in order to acclimate the leafhoppers to alfalfa.

Plant preparation. Alfalfa plant clones (Pioneer 55V48) were provided by Pioneer Hi-Bred International, Inc. (Arlington, WI) to ensure genetic uniformity. Plant clones were propagated by clipping stems off of an individual plant and placing in perlite growing medium with a root growth promoting hormone. When root growth was substantial, individual plants were transplanted into pots (15 cm diameter) with potting soil (Metro Mix, Agawam, MA) and watered daily, with 20-10-20 (N-P-K) Peter's peat-lite special fertilizer (Earth City, MO).

Assay: Five paired tests were conducted simultaneously until a total of n=24 replicates were completed. Prior to each assay, alfalfa clones were paired for each replicate based on similarity in height and number of stems at bud stage in the greenhouse and then cut to approximately 7 cm (3 inches). One plant for each replicate received 80 mL of manure, topically applied to the pots, to simulate the field application rate of 46.8 kL ha^{-1} (5,000 gal $acre^{-1}$). When alfalfa plants grew to approximately 20 cm (8 inches) they were moved to an environmental growth room with the same conditions as the potato leafhopper growth room (25° C and 16:8 (L:D)).

In the growth room, paired plants were placed inside a cage. Cages were 45 cm x 45 cm x 45 cm consisting of a front clear vinyl side with zipper and arm sleeve for access, and three sides and the top made of polyester mesh netting (Bugdorm, Taiwan). Each cage contained one experimental unit of a paired choice assay.

For each replicate, 10-15 newly developed adult females and 2-5 males were placed in a cage with two plants, one treated with manure and one control. Both plants in each cage replicate were watered daily with 25 ml of water and Peter's peat-lite special as in the greenhouse. Excess water that drained through the potting mix was collected in a drainage pot and reused to ensure that plant utilized the same amount of nutrients.

One week after insect introduction, plants were checked daily for nymphal emergence. One week after detection of the first nymph, each assay was terminated by methodically examining the plants and counting each nymph. In order to avoid recounting nymphs, plant material was cut and removed as the examination proceeded. Number of nymphs was recorded and used as a proxy for ovipositional preference.

Statistical analysis.

Field study. Potato leafhopper response to nutrient source was analyzed using individual linear mixed effects models for each sweep net sample date, in which nutrient source is a fixed effect and block is random. Repeated measures analyses were initially conducted to determine leafhopper response to treatment as mixed effects models in R version 2.10.1 (R Development Core Team 2009) using Rpackage *nlme*. Fixed effects included in the model were treatment, sample date, and sample date by treatment interaction. Random effects were block and plot nested within block. However, the interaction between treatment and sample date in trial 1 precluded the interpretation of these results (see Appendix I-III).

Yield and forage quality response to treatment and potato leafhoppers from the crop prior to harvest were analyzed as mixed effects models where treatment and the cumulative potato leafhoppers, determined by summing the total potato leafhoppers recovered from the sample dates from the crop prior to harvest, were fixed effects and block was a random effect. Tukey's post-hoc test was used to determine significant differences of treatment means (Hothorn et al. 2008).

Oviposition bioassy. A paired T-test was conducted to compare the nymphal emergence from alfalfa grown in pots with manure to alfalfa grown in pots without manure. Nymph abundance was used as a proxy for female ovipositional preference. Follow up tests were completed using linear mixed effects models, in order to determine if any alfalfa plant variables confounded the treatment effect; fixed effects were treatment, number of alfalfa stems per plant and tallest stem height per pot, cage ID was a random effect. All mixed models were created in R version 2.10.1 (R Development Core Team 2009) using R package *nlme*.

Results

Field Trials.

Trial 1. Potato leafhoppers were sampled eight times from June 13-August 25, 2011. On most sample dates, there was no significant effect of nutrient source on potato leafhopper abundance. Control plots had the greatest leafhopper abundance throughout July, but were only statistically greater than other treatments on July 26 (df= 2, 10; F=5.45; p=0.03) (Fig. 1). Potato leafhopper abundance was significantly greater in untreated control plots (Mean= 9.3, SE=1.3) than plots treated with manure (Mean=3.3, SE=1.85) or synthetic fertilizer (Mean=5.3, SE=1.85). There was no significant difference between plots treated with synthetic fertilizer and those treated with manure.

Neither nutrient source nor potato leafhoppers had an effect on alfalfa yield at any harvest for trial 1 (Table 2). There was a significant effect of nutrient source on crude protein for the August 1, 2011 harvest (df=2, 7; F=5.07; p=0.04) (Table 3). Alfalfa in plots treated with synthetic fertilizer had significantly greater crude protein (M=30.95, SE=2.45) than control plots (M=23.14, SE=1.61), but plots receiving manure were intermediate (M=27.03, SE=2.30) and not statistically different from either of the other treatments. The interaction of soil treatment and potato leafhoppers was also significant (df=2, 7; 5.44; p=0.04) such that potato leafhoppers reduced crude protein content most in alfalfa from plots with synthetic fertilizer and least in alfalfa from control plots. There was no significant effect of potato leafhoppers or soil amendment on neutral detergent fiber content at any harvest in 2011 (Table 4).

Trial 2. Potato leafhoppers were sampled eight times from June 4 to August 7. On June 4, nutrient source had a significant effect on potato leafhopper abundance (df=2, 10; F=9.90; p=0.004) (Fig 2). Manure application results in statistically greater potato leafhopper abundance (M=5.66, SE=0.76) compared to application of synthetic fertilizer (M=2.66, SE=0.76) or no nutrient applications (M=2.83, SE=0.74). Nutrient source had a significant effect on potato leafhopper abundance again on July 2 (df=2, 10; F=5.29; p=0.03). Again, manure applications resulted in statistically greater leafhopper abundance (M=6.83, SE=1.07) than applications of synthetic fertilizer (M=4.16, SE=1.12) or no nutrient applications (M=3.33, SE=1.07).

There were no significant effects of potato leafhoppers or nutrient source on yield in 2012 (Table 5). There was a significant effect of nutrient source on crude protein for the August 14 harvest (df=2, 9; F=22.70; p<0.01) (Table 6). Manure applications produced statistically greater crude protein content (M=29.68, SE=0.23) than synthetic fertilizer applications (M=28.23, SE=0.22) or no nutrient applications (M=28.62, SE=0.27). There were no significant effects of potato leafhoppers or nutrient source on neutral detergent fiber content at either harvest (Table 7).

Trial 3. Potato leafhoppers were sampled nine times from May 30 to August 7. There was no significant effect of nutrient source on potato leafhopper abundance at any sample date (Fig. 3).

Nutrient source had a significant effect on yield on June 20, 2012 (df=2, 5; F=7.74; p=0.03) (Table 8). Both untreated control plots (M=2.54, SE=0.39) and plots that received manure applications (M=2.43, SE=0.10) had statistically higher yields than plots treated with synthetic fertilizer (M=2.17, SE=0.10). Untreated control and manure plots were not significantly different from each other.

Nutrient source had a significant effect on crude protein content on August 14 harvest (df=2, 5; F=5.50; p=0.05) (Table 9). There was no significant difference in crude protein between plots with manure (M=28.84, SE=0.87) and plots with synthetic fertilizer (M=28.42, SE=0.82), but both treatments had statistically greater crude protein than control plots (M=26.25, SE=1.02). There was no significant effect of nutrient source or potato leafhoppers on neutral detergent fiber at either harvest in trial 2 (Table 10).

Female oviposition choice assays. There was no significant difference in nymphal emergence from alfalfa plants grown in potting soil treated with manure (M=34.4, SD=30.4) and pots without manure (M=42.8, SD=29.9); t(23)=0.67, p=0.51. Subsequent analyses by mixed model confirmed that tallest stem height per pot and numbers of stems per plant did not confound the manure treatment effect which was still non-significant (df=1, 21; F=0.39; p=0.54).

Discussion

Potato leafhopper response to nutrient source in the field trials was inconsistent. Throughout July 2011 in trial 1, untreated control plots had greater potato leafhopper abundance and on one sample day this trend was statistically significant (Fig. 1). This pattern is consistent with farmer anecdotal reports. In 2012, neither trial 2 nor trial 3 showed a similar trend to trial 1. In fact, in trial 2, the trend reversed at two sample dates such that plots treated with manure had the greatest leafhopper abundance (Fig. 2). At trial 2, there were no significant differences at any sample date (Fig. 3). The female choice oviposition assays showed no significant results.

Though data from this study do not support the conclusion that manure applications to alfalfa help to suppress the potato leafhopper, there are factors that necessitate further investigation. For example, both temperature and precipitation varied between the years of the field trials (Table 11). Though soil moisture was below average for both 2011 and 2012, this was much more severe in 2012. In June 2012, fields at Arlington Agricultural Research Station received only 0.7 cm (0.26 inches) of rain, or 6% of the historical monthly average (NOAA NCDC). The lack of rainfall early in June 2012 had noticeable effects on the soil and crops that were visually apparent in stunted growth throughout the 2012 growing season. The summer of 2012 also had exceptionally high temperatures. This is apparent in the number of days throughout the growing seasons in which temperatures exceeded 32° C (90° F). In 2011, there were 14 days in total from May through August in which this was the case (NOAA 2013). However, in 2012, there were 18 days in July alone and 33 days from May through August in which this was the case (NOAA 2013).

Both soil moisture and temperature have significant impacts on the fate of applied nutrients in the soil. Lack of soil moisture affects plant nutrient uptake by changing the soil solution nutrient concentration. For example, diffusion is the main mechanism of potassium and phosphorus movement from soils to roots and this process is dependent on soil water content (Marschner 1995). Plant nitrogen uptake may also be reduced in dry conditions (Wild et al. 2011).

Soil moisture and temperature significantly alter the rate of nitrogen availability from organic sources. Plant uptake of nitrogen is either in the form of ammonium (NH_4^+) or nitrate (NO_3^-). The transformation of nitrogen into either of these compounds is a biological process, mediated by bacteria and fungi. Microbial activity is decreased in dry conditions due to decreased diffusion of soil nutrients throughout the soil matrix (Griffin 1981). Therefore, biological nitrogen transformations such as ammonification and nitrification are suppressed when soil moisture is lacking. However, these transformations are hindered more when nitrogen is supplied from organic sources than when the nitrogen is supplied in a synthetic form likely because of a higher carbon to nitrogen ratio (Agehara and Warncke 2005).

Increased temperatures increase microbial activity (Zak et al. 1999). Changes in microbial activity disproportionately affect nitrogen availability from organic sources compared to synthetic sources. For example, urea hydrolysis is consistently more rapid than nitrogen mineralization from organic sources such as chicken manure and blood meal regardless of temperature, but mineralization of nitrogen from organic sources is significantly enhanced as temperatures increase (Agehara and Warncke 2005). Interactions between the effect of temperature and effect of drought on nitrogen transformations were distinct for the different soil treatments. Future work should study these effects via soil samples in which microbial communities and soil nitrogen are quantified as well as plant samples to determine nitrogen recovery.

The lack of biological communities in the soil could have altered the manner in which manure impacted the alfalfa in the greenhouse bioassays. Because laboratory plants were grown in potting soil mix and not in field soil, without inoculations nitrogen fixing *Rhizobium*, there was no presence of soil microbial communities. Some biological interactions were not accounted for that could have impacted the results. For example, using *Arabidopsis thaliana*, Pineda et al. (2012) show that mycorrhizal fungi and rhizobacteria can mediate induced plant susceptibility to generalist phloem feeders such as aphids. Complexities that arise from multitrophic interactions such as these were not accounted for in either the field or laboratory experiments. In fact, the manure used in the bioassays was frozen before applying it, which could have altered the microbial composition (Yanai et al. 2004). It may be that interactions with micribiota are at least partly responsible for the induced resistance to insect herbivores by organically fertilized plants. Cardoza (2011) found that survival of corn earworms, *Helicoverpa zea* on *A. thaliana* treated with sterilized vermicompost (frozen or autoclaved) was intermediate between survival on untreated control plants and plants treated with unsterilized vermicompost.

In the field trials, synthetic fertilizer applied at nitrogen, phosphorus, potassium and sulfur rates similar to those found in manure. This treatment was included to determine if potato leafhoppers would respond differently to nutrients supplied synthetically or organically. In trial 1, when nutrient source had a significant effect on potato leafhopper abundance in late July, 2011 (Fig. 1.), leafhopper abundance was greatest in control plots and both nutrient sources suppressed potato leafhopper abundance. Although there was no statistical difference in leafhopper abundance between nutrient sources, mean potato leafhopper abundance was lowest in plots with manure. This pattern is similar to that found by Cardoza (2011) as the sterilized vermicompost suppressed *H. zea* at an intermediate level between untreated plants and plants treated with biologically active vermicompost. By sterilizing the vermicompost, Cardoza (2011) eliminates the biological component and is able to test for the effect of the nutrients alone; though there are organic components remaining in the sterilized vermicompost, it is analogous to the synthetic fertilizer in the present study.

Eigenbrode and Pimental (1988) found greater densities of flea beetles, *Phyllotreta* spp., imported cabbage worm, *Pieris rapae*, and diamond back moth, *Plutella xylostella* larvae on unfertilized brassica plants than plants grown in soil treated with either synthetic fertilizers or cow manure. They attributed plant nutrient stress in the untreated plants to the observed increase in pest susceptibility. Nutrient stress is unlikely to be the cause of increased potato leafhopper abundance in untreated plots in trial 1 (Fig. 1) because there was no statistical difference in yield in 2011 between any of the treatments (Table 2).

The only time throughout this study that treatment had a significant effect on alfalfa yield was in trial 3, June 20, 2012 (Table 8). Interestingly, plots treated with synthetic fertilizer had the lowest yield. June 2012 was extremely dry, receiving only 0.7 cm of rain (NOAA 2013). During a drought, plants fertilized with manure may have improved yields over plants fertilized synthetically because organic fertilizers increase soil organic matter and soil water-holding capacity (Schjonning et al. 1994). Though increased organic matter and water holding capacity are not typically measurable after just one application of manure, the higher yield due to liquid dairy manure application it could be simply due to extra water on the plots from the liquid manure itself that contributed to the difference. The application of roughly 65.5 kL ha^{-1} of liquid manure on May 17 would have provided an extra 0.63 cm of water. Oddly though, plots that were untreated also had higher yield than plots with fertilizer. Without information regarding soil nutrients we cannot conclude how this may have occurred.

Soil treatment had a significant effect on crude protein content at three harvests (trial 1: August 1, 2011, August 14, 2012; trial 2: August 14, 2012). Crude protein content is a measure of nitrogen content in which crude protein is calculated as *6.25*N* (Undersander et al. 2011). At all three harvests, untreated control plots had the lowest crude protein content. Depending on the trial and harvest, plots with the highest crude protein content were either those receiving synthetic fertilizer or manure (Tables 3, 6 and 9). For example, alfalfa from plots that received synthetic fertilizer had the greater crude protein content on August 1, 2011 (trial 1, Table 3), but plots that had been treated with manure had the greatest crude protein content on August 14, 2012 (trial 2, Table 6). Vasquez-Vasquez (2010) found no differences in crude protein between alfalfa treated with rates ranging from 0 to 160 tons ha^{-1} manure or 30 and 100 kg synthetic nitrogen and phosphorus ha^{-1}. But Cherney et al. (1995) found crude protein concentrations increased with increasing nitrogen from 0, 112, 224 to 336 kg ha^{-1} (applied as ammonium nitrate) on alfalfa. Daliparthy et al. (1994) did not see a statistically significant increase in nitrogen content between plots receiving no nitrogen and plots receiving 336 kg N ha^{-1} as ammonium nitrate. Low rates of manure (112 kg N ha^{-1}) also did not raise nitrogen content in the plants (Daliparthy et al. 1994).

Though increases in nitrogen content are often followed by increases in insect herbivore pests (Phelan et al. 1996, Facknath and Lalljee 2005, Ren 2013), in this study potato leafhopper abundance did not correlate with increased nitrogen, or crude protein. Potato leafhoppers did have a significant interaction with soil treatment on crude protein in trial 2, August 1, 2012. Plots with synthetic fertilizer had the greatest crude protein content but it was most negatively affected by potato leafhoppers, while crude protein was the lowest in untreated control plots and not significantly impacted by potato leafhoppers (Table 6). Potato leafhoppers in July were more abundant in control plots (Fig. 1). This suggests a disproportionate decrease of crude protein per leafhopper between alfalfa from control plots and alfalfa from synthetically fertilized plots. Even though there was no significant impact of potato leafhoppers on yield (Table 5), the negative effects on the crude protein seen in the alfalfa treated with synthetic fertilizer could be a precursor to negative effects on yield. Hower and Flinn (1986) concluded that potato leafhopper feeding decreases crude protein before visual hopperburn injury or yield loss is apparent. Their conclusion along with the present results could imply that the potato leafhoppers were nitrogen limited and so therefore took advantage of the greater amount of available protein in the alfalfa fertilized synthetically.

Conclusion. Overall, results from this study are inconclusive with regard to the effect of liquid dairy manure on potato leafhopper abundance. These results are important because they can be used to show farmers that manure applications have potential to alter potato leafhopper abundance but this is not consistent and there are other factors involved. Outcomes of the application of manure and synthetic fertilizer to alfalfa stands are more complicated than applications to non-leguminous crops because of interactions with nitrogen fixing soil bacteria. When alfalfa takes nitrogen up from the soil directly rather than through symbiotic relationships with *Rhizobium* there may be additional energy available to the plant (Zahran 1999). Additional work is needed in order to distinguish effects of synthetic and organic nutrient applications and the effect of manure and soil microbial community on potato leafhopper response in alfalfa. This study was not designed to look at the microbial activity of the manure but considering the effects of low precipitation and high heat of 2012 and freezing of the manure sample prior to use in the bioassay on the microbiota in the manure, more work should be done that specifically aims to account for these interactions.

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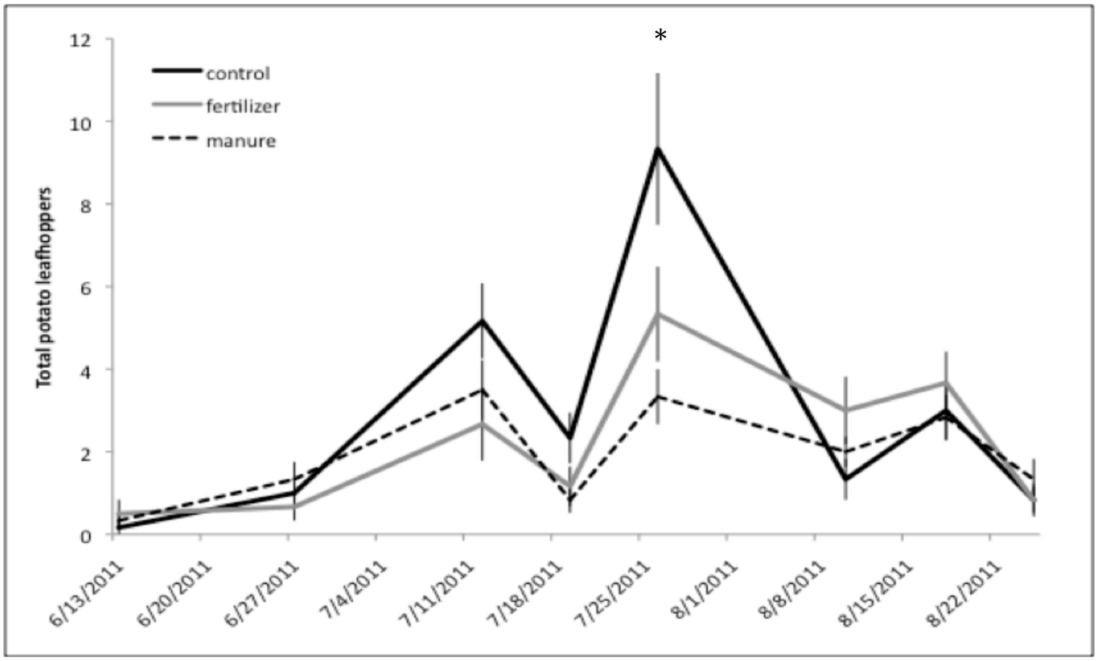

Figure 1. Effect of soil treatments on potato leafhoppers for trial 1 (field A, 2011). Total potato leafhoppers are the mean of total found in 20 sweep net samples per plot. Error bars indicate standard error. Significant differences marked with (*).

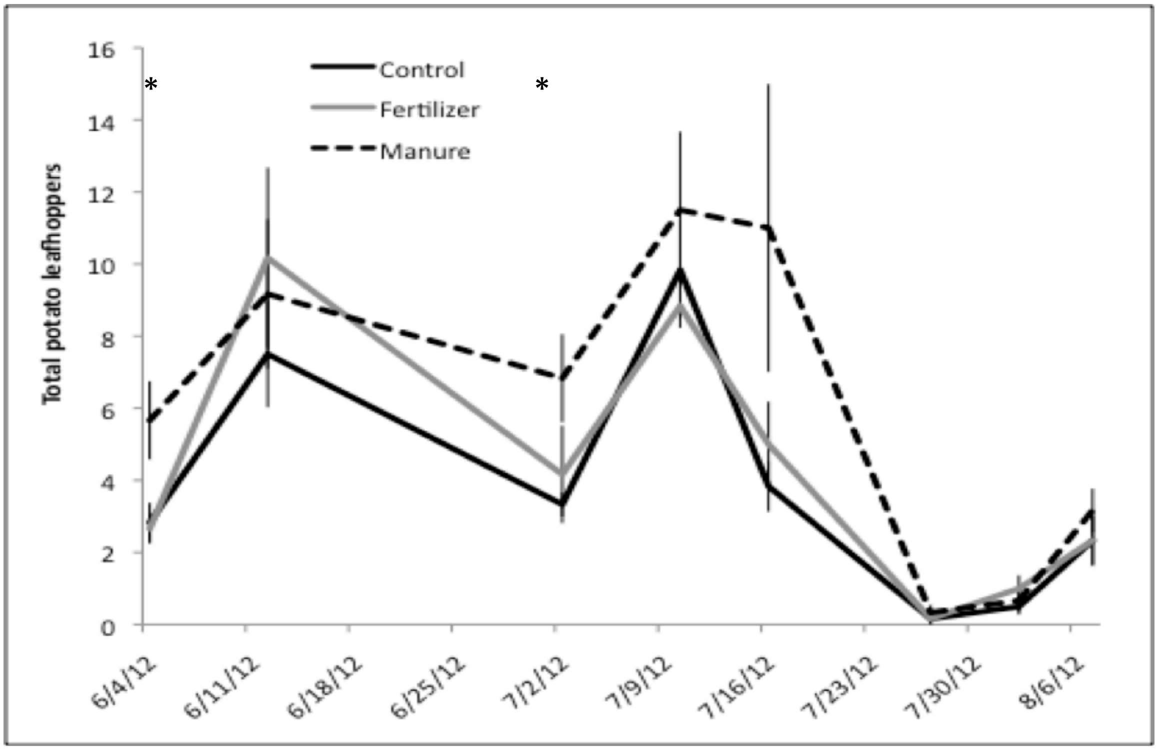

Figure 2. Effect of soil treatments on potato leafhoppers for trial 2 (field A, 2012). Total potato leafhoppers are the mean of total found in 20 sweep net samples per plot. Error bars indicate standard error. Significant effects marked with (*).

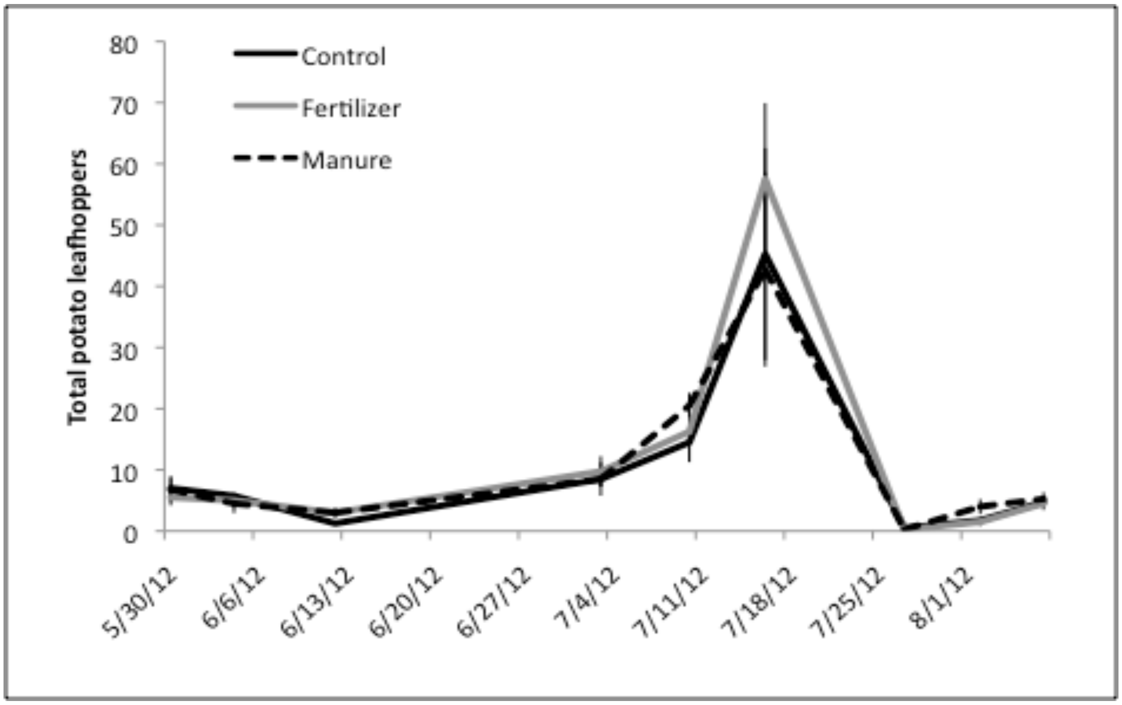

Figure 3. Effect of soil treatments on potato leafhoppers for trial 3 (field B, 2012). Total potato leafhoppers are the mean of total found in 20 sweep net samples per plot. Error bars indicate standard error. No significant effects.

Table 1. Soil treatments applied to field A in 2011 and 2012 and field B for 2012 growing seasons. Synthetic fertilizer treatment was used for 2011-2012[+]. Nutrient components of manure treatments (liquid dairy) are based on sample analysis.

Treatments	N kg ha^{-1} (lbs acre^{-1})	P_2O_5 kg ha^{-1} (lbs acre^{-1})	K_2O kg ha^{-1} (lbs acre^{-1})	S kg ha^{-1} (lbs acre^{-1})
Synthetic Fertilizer	33.0[a] (29.4)	17.6[b] (15.7)	69.1[c] (61.7)	4.1[d] (3.7)
2011 Manure[*]	33.6 (29.9)	26.7 (23.8)	90.5 (80.8)	4.1 (3.7)
2012 Manure[*]	35.4 (31.6)	29.7 (26.5)	83.8 (74.7)	3.6 (3.2)

[+] Synthetic fertilizer treatments derived to match mineral components of 2010 manure sample analysis for nutrients available within first year of application.

[*] Manure sample analysis results for nutrients available within first year application.

[a] Nitrogen applied as a combination of urea and ammonium sulfate.

[b] Phosphorus applied as triple super phosphate.

[c] Potassium applied as potassium chloride.

[d] Sulfur applied as ammonium sulfate.

Table 2. Effects of potato leafhoppers (PLH) and soil treatments on yield, Mg ha^{-1} (tons acre^{-1}) in trial 1 (field A, 2011). Estimates for PLH are slope values and for treatments are y-intercepts. No significant effects or interactions.

Harvest Date	Effect	Estimate, Mg ha^{-1} (tons acre$^{-1)}$	df	F	Pr>F
July 6, 2011	PLH	0.00 (0.00)	1, 9	0.01	0.94
	Treatment		2, 9	0.32	0.73
	Control	3.83 (1.71)			
	Fertilizer	4.06 (1.81)			
	Manure	3.90 (1.74)			
August 1, 2011	PLH	0.00 (0.00)	1, 9	0.17	0.69
	Treatment		2, 9	1.44	0.29
	Control	4.06 (1.81)			
	Fertilizer	4.19 (1.87)			
	Manure	3.99 (1.78)			
September 1, 2011	PLH	-0.02 (-0.01)	1, 9	0.21	0.66
	Treatment		2, 9	0.27	0.77
	Control	3.07 (1.37)			
	Fertilizer	2.98 (1.33)			
	Manure	3.14 (1.40)			

Table 3. Effects of potato leafhoppers (PLH) and soil treatments on crude protein content (%) in trial 1 (field A, 2011) Estimates for PLH are slope values and for treatments are y-intercepts. Significant effects in **bold**. Interactions included when significant.

Harvest Date	Effect	Estimate	df	F	Pr>F
July 6, 2011	PLH	-0.11	1, 9	0.17	0.69
	Treatment		2, 9	0.03	0.97
	Control	25.22			
	Fertilizer	25.36			
	Manure	25.32			
August 1, 2011	PLH		1, 7	1.97	0.20
	PLH*Treatment		**2, 7**	**5.47**	**0.04**
	PLH*Control	**0.13**			
	PLH*Fertilizer	**-0.57**			
	PLH*Manure	**-0.22**			
	Treatment		**2, 7**	**5.07**	**0.04**
	Control	**23.15**			
	Fertilizer	**30.95**			
	Manure	**27.03**			
September 1, 2011	PLH	0.02	1, 9	0.08	0.78
	Treatment		2, 9	0.66	0.54
	Control	28.74			
	Fertilizer	28.33			
	Manure	28.36			

Table 4. Effects of potato leafhoppers (PLH) and soil treatments on neutral detergent fiber content (%) in trial 1 (field A, 2011). Estimates for PLH are slope values and for treatments are y-intercepts. No significant effects or interactions.

Harvest Date	Effect	Estimate	df	F	Pr>F
July 6, 2011	PLH	0.20	1, 9	0.29	0.61
	Treatment		2, 9	0.36	0.71
	Control	34.76			
	Fertilizer	35.48			
	Manure	35.22			
August 1, 2011	PLH	0.04	1, 9	0.04	0.85
	Treatment		2, 9	0.54	0.60
	Control	34.27			
	Fertilizer	35.42			
	Manure	36.21			
September 1, 2011	PLH	-0.02	1, 9	0.11	0.74
	Treatment		2, 9	2.55	0.13
	Control	27.73			
	Fertilizer	28.45			
	Manure	28.52			

Table 5. Effect of potato leafhoppers and soil treatment on yield, Mg ha^{-1} (tons $acre^{-1}$) in trial 2 (field A, 2012). Estimates for PLH are slope values and for treatments are y-intercepts. No significant effects or interactions.

Harvest Date	Effect	Estimate, Mg ha^{-1} (tons $acre^{-1)}$	df	F	Pr>F
June 20, 2012	PLH	-0.02 (-0.01)	1, 7	0.16	0.70
	Treatment		2, 7	1.96	0.21
	Control	5.43 (2.42)			
	Fertilizer	5.78 (2.58)			
	Manure	5.99 (2.67)			
August 14, 2012	PLH	-0.07 (-0.03)	1, 9	1.23	0.30
	Treatment		2, 9	0.95	0.42
	Control	2.51 (1.12)			
	Fertilizer	2.69 (1.20)			
	Manure	2.87 (1.28)			

Table 6. Effects of potato leafhoppers (PLH) and soil treatments on crude protein content (%) in trial 2 (field A, 2012). Estimates for PLH are slope values and for treatments are y-intercepts. Significant effects in **bold**. No significant interactions.

Harvest Date	Effect	Estimate	df	F	Pr>F
June 20, 2012	PLH	-0.01	1, 6	0.02	0.90
	Treatment		2, 6	0.64	0.56
	Control	22.85			
	Fertilizer	22.50			
	Manure	22.37			
August 14, 2012	PLH	-0.08	1, 9	1.71	0.22
	Treatment		**2, 9**	**22.70**	**<0.01**
	Control	**28.62**			
	Fertilizer	**28.23**			
	Manure	**29.68**			

Table 7. Effects of potato leafhoppers (PLH) and soil treatments on neutral detergent fiber content (%) in trial 2 (field A, 2012). Estimates for PLH are slope values and for treatments are y-intercepts. No significant effects or interactions.

Harvest Date	Effect	Estimate	df	F	Pr>F
June 20, 2012	PLH	-0.06	1, 6	0.35	0.58
	Treatment		2, 6	1.84	0.23
	Control	40.28			
	Fertilizer	41.40			
	Manure	42.65			
August 14, 2012	PLH		1, 9	1.03	0.34
	Treatment		2, 9	2.68	0.12
	Control	28.46			
	Fertilizer	30.44			
	Manure	29.77			

Table 8. Effect of potato leafhoppers and soil treatment on yield, Mg ha^{-1} (tons acre^{-1}) in trial 3 (field B, 2012). Estimates for PLH are slope values and for treatments are y-intercepts. Significant effects in **bold**. No significant interactions.

Harvest Date	Effect	Estimate, Mg ha^{-1} (tons acre$^{-1)}$	df	F	Pr>F
June 20, 2012	PLH	-0.04 (-0.02)	1, 5	0.79	0.42
	Treatment		**2, 5**	**7.74**	**0.03**
	Control	**5.69 (2.54)**			
	Fertilizer	**4.87 (2.17)**			
	Manure	**5.45 (2.43)**			
August 14, 2012	PLH	-0.02 (-0.01)	1, 5	0.09	0.78
	Treatment		2, 5	0.48	0.64
	Control	2.31 (1.03)			
	Fertilizer	2.13 (0.95)			
	Manure	1.97 (0.88)			

Table 9. Effects of potato leafhoppers (PLH) and soil treatments on crude protein content (%) in trial 3 (field B, 2012). Estimates for PLH are slope values and for treatments are y-intercepts. Significant effects in **bold**. No significant interactions.

Harvest Date	Effect	Estimate	df	F	Pr>F
June 20, 2012	PLH	0.06	1, 5	0.31	0.60
	Treatment		2, 5	0.34	0.73
	Control	21.57			
	Fertilizer	21.23			
	Manure	22.00			
August 14, 2012	PLH	0.06	1, 5	0.28	0.62
	Treatment		**2, 5**	**5.60**	**0.05**
	Control	**26.25**			
	Fertilizer	**28.42**			
	Manure	**28.84**			

Table 10. Effects of potato leafhoppers (PLH) and soil treatments on neutral detergent fiber content (%) in trial 3 (field B, 2012). Estimates for PLH are slope values and for treatments are y-intercepts. No significant effects or interactions.

Harvest Date	Effect	Estimate	df	F	Pr>F
June 20, 2012	PLH	-0.18	1, 5	0.56	0.49
	Treatment		2, 5	0.83	0.49
	Control	41.02			
	Fertilizer	43.67			
	Manure	41.69			
August 14, 2012	PLH	-0.02	1, 5	0.01	0.93
	Treatment		2, 5	1.07	0.41
	Control	30.76			
	Fertilizer	29.07			
	Manure	29.75			

Table 11. Monthly total precipitation and total number of days exceeding 32° C (90° F) for growing seasons in 2011 and 2012.

Month	2011		2012		Historical means	
	Precipitation (cm)	Days > 32° C	Precipitation (cm)	Days > 32° C	Precipitation (cm)	Days > 32° C
May	5.5	0	7.5	2	8.7	0.2
June	10.4	3	0.7	8	10.3	2.3
July	6.3	7	10.8	18	9.8	3.7
August	3.7	4	7.3	5	10.8	2.4

Appendix I. Nutrient source treatment effects on potato leafhopper response through repeated measures analysis for trial 1 (field A, 2011). Significant effects in **bold**.

Effect	df	F	Pr>F
Sample date	**7, 105**	**22.11**	**<0.01**
Treatment	**2, 10**	**4.05**	**0.05**
Treatment x sample date	**14, 105**	**3.22**	**<0.01**[a]

[a] Significant treatment x sample date interaction signifies that the effect of treatment on potato leafhopper changes over time and the analysis proceeds by looking at dates individually as seen in figure 1.

Appendix II. Nutrient source treatment effects on potato leafhopper response through repeated measures analysis for trial 2 (field A, 2012). Significant effects in **bold**.

Effect	df	F	Pr>F
Sample date	**7, 105**	**21.60**	**<0.01**
Treatment	**2, 10**	**6.15**	**0.02**
Treatment x sample date	14, 105	1.08	0.38

Appendix III. Nutrient source treatment effects on potato leafhopper response through repeated measures analysis for trial 3 (field B, 2012). Significant effects in **bold**.

Effect	df	F	Pr>F
Sample date	**8, 72**	**24.71**	**<0.01**
Treatment	2, 6	0.20	0.82
Treatment x sample date	16, 72	0.32	0.99