IMPACTS OF THREE INSECT GROWTH REGULATORS AND THE PARTICLE BARRIER FILM, KAOLIN, ON ALFALFA WEEVIL, *HYPERA POSTICA* (GYLLENHAL), SECONDARY PEST, PEA APHID, *ACYRTHOSIPHON PISUM* (HARRIS) & NATURAL ENEMY COMPLEX

by

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ABSTRACT

Studies were conducted in Montana to evaluate the impacts of the insect growth regulators novaluron, diflubenzuron, azadirachtin and the particle barrier film, kaolin, on the primary pest, alfalfa weevil (AW, *Hypera Postica* [Gyllenhal)], natural enemies of alfalfa weevil and the secondary pest, pea aphid, *Acyrthosiphon pisum (Harris*). Kaolin, diflubenzuron and azadirachtin treatments caused low (<53%) AW mortality and did not prevent AW feeding damage across 5 field sites. Novaluron caused the highest mortality (74 ± 3% at one field site) while significantly reducing feeding damage across two of five field sites ($P < 0.05$) and two greenhouse trials. Plants treated with novaluron weighed significantly more than untreated plants at harvest in either greenhouse study with a final harvest weight of 2.7 ± 0.2 and 3.4 ± 0.3g / pot in the novaluron treated pots compared to 2.2 ± 0.1 and 2.4 ± 0.3 g / pot in the untreated; however harvest yields were not increased in field trials. All experimental treatments provided some pre-harvest benefits to the predator-alfalfa weevil and predator-pea aphid complex at various field sites; however novaluron treatments provided significantly higher predator-alfalfa weevil ratios consistently across four of five field sites when compared to the synthetic pyrethroid, lambda cyhalothrin ($P < 0.05$). At these four field sites, novaluron treated plots harbored an average predator-alfalfa weevil ratio of 0.15 ± 0.07 compared to 0.02 ± 0.02 in lambda cyahlothrin treated plots in the first harvest cycle. Parasitism rates were significantly higher when experimental treatments were used compared to the lambda cyhalothrin treated plots across five field sites ($P < 0.05$). The added benefit of conserving predators and parasitoids in combination with direct pesticide efficacy never reduced densities of AW or pea aphid to that of the synthetic pyrethroid treatment in the first or second harvest cycle. While novaluron had little benfit on reducing AW or pea aphid poulations to that of the synthetic pyrethroid treatment, it offers the best potential for developing a soft-chemical/biological system for protecting alfalfa from this key arthropod pest. Future studies taking advantage of novalurons mode of action as a feeding deterrent should be explored.

CHAPTER 1

INTRODUCTION

There are no registered pesticide chemistries for effective alfalfa weevil (AW, *Hypera postica* [G]) management that minimize non-target impacts. These would be useful tools for organic and conventional forage alfalfa (*Medicago sativa* [L.]) systems. There is a need for new, organically approved chemistries to support a growing organic hay market to supply the organic milk and beef industry (Fuerst et al. 2009; Guerena & Sullivan 2003). The fastest growing segment of organic agriculture in the U.S. is organic milk production, with a 25% increase in certified organic milk cows each year, from 2000 to 2005 (USDA 2012). Demand for certified organic beef is also increasing in the U.S. as evident by a 300% increase in certified organic beef livestock from 2001 – 2008 (USDA 2012). Organic hay in the U.S., predominantly pure alfalfa stands, has increased from 46,980 ha harvested in 2001 to 103,680 ha harvested in 2008; organic hay supports the growing organic milk and beef industries (Figure 1.1). Conventional pyrethroid, carbamate or organophosphate pesticides are unavailable for managing alfalfa weevils in these organic systems. In addition, previous investigations by Summers (1998) & Harper (1978) indicated that the use of conventional pesticide chemistries (eg. organophosphates, synthetic pyrethroid, and carbamate) in non-organic alfalfa disrupts natural enemy populations leading to secondary pest outbreaks. The study presented here was conducted to find alternative chemistries for managing AW that preserve natural enemies in conventional and/or organic alfalfa systems.

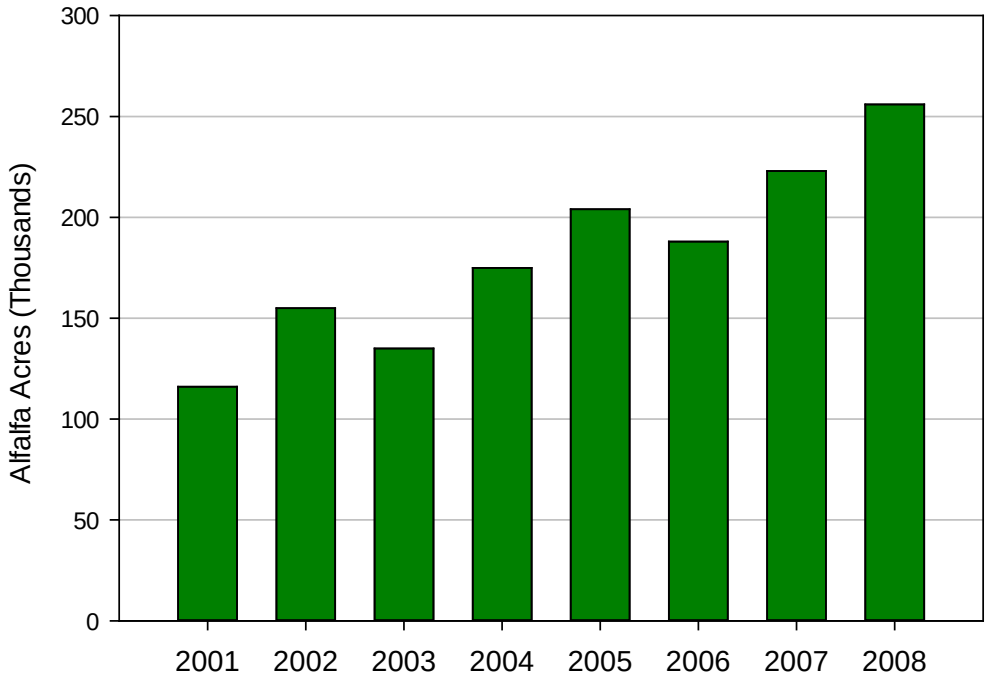


Figure. 1.1: Harvested acres of organic hay in the U.S. (USDA 2012)

The Importance of the Agronomic System

Alfalfa is a perennial plant that has been grown as a forage crop since the beginning of recorded history, originating in the vicinity of present day Iran and brought to North America in the early 1700's (Whyte et al. 1953; Wilsie 1962; Lacefield et al. 1997). It is the foremost forage crop in many semi-arid and temperate states in the US, with 58.9 million metric tons produced in 2011. In 2010, Montana farmers produced 4.06 million metric tons of alfalfa hay with a value of $363 million; Montana is ranked 3rd nationally in 2011 (NASS 2011). Alfalfa is a high quality feed for livestock that is easily digested, low in neutral detergent fibers and high in protein (Conrad and

Klopfenstein 1988). It is considered the most useful forage legume used as animal feed (Abdel Magid 1983), and a critical component to the dairy, beef (*Bos* spp.), sheep (*Ovis* spp.), horse (Equus spp.), swine (*Sus* spp.), and poultry (*Gallus* spp.) industries (Van Keuren and Matches 1988). Insecticide applications are used in approximately 34% of all alfalfa acres in the U.S., primarily targeting AW (Bailey 1994).

Alfalfa Weevil Significance and History

Alfalfa weevils are found throughout the contiguous 48 states (Hsaio 1993). Alfalfa weevil is the most damaging pest of forage alfalfa in the U.S. (USDA APHIS 1991). The AW is native to Europe but can be found in North America, North Africa, the Middle East, India, and western Asia (Radcliffe & Flanders 1998). Two distinct strains of weevils are known to occur in the U.S., including the western and eastern AW strains. The western strain was introduced in Utah in 1904 and has quickly spread since its introduction (Titus 1909), while the eastern AW strain originated in Maryland in 1952 (Poos and Bissell 1953). A closely related weevil, *Hypera brunneipennis*, was also discovered in Yuma, Arizona in 1939 (Wehrle 1940), and has historically been considered a separate species known as the Egyptian strain (Figure 1.2). The western and eastern AW overlap in at least nine states, while the Egyptian and western AW overlap in at least four states (Hsiao 1996; Radcliffe & Flanders 1998). Research by Hsaiao (1993) indicates the eastern AW is actually more closely related to the Egyptian AW than it is to the western AW.

Strains of AW differ biologically from each other (Davis 1967; Hsaio 1993; van den Bosch et al. 1982). The western strain pupates in ground litter, has an extended pre-oviposition period, a faster larval development rate, whereas the eastern and Egyption strains prefer pupating above ground, have a shorter pre-oviposition period, and a slower larval developmental rate (Rosenthal and Koehler 1968, Schroder and Steinhauer 1976). Egyption AW strains also prefer warmer environments while western and eastern strains are adapted to cooler climates.

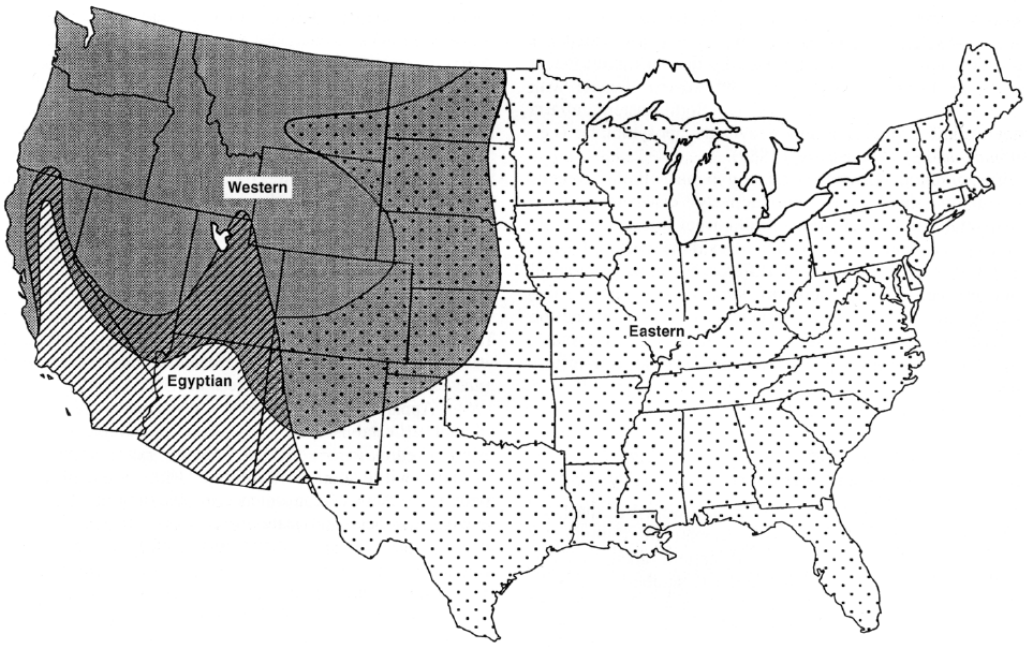


Figure 1.2: Distribution of alfalfa weevil strains across the U.S. (Adapted from Radcliffe & Flanders 1998).

Alfalfa Weevil Life Cycle

The western strain of the AW is present throughout most areas of Montana with intergrade populations of western / eastern AW present in southeastern regions (Fig. 1.2;

Radcliffe & Flanders 1998). The western and western / eastern intergrade populations in Montana are univoltine (Helgesen and Cooley 1976) with a majority of oviposition occurring in the spring (Blodgett 1996). A second generation is often present in locations across the U.S. below 40^0 latitude (White et al. 1969).

Western and western / eastern intergrade populations of AW in Montana hibernate during the adult stage and oviposit the following spring by chewing holes in alfalfa stems and depositing 5 to 15 eggs (Blodgett 1996). Females deposit up to 4000 eggs in a lifetime (Coles and Day 1977). Larvae emerge after 7 to 14 d of oviposition before feeding in developing plant terminals. As larvae mature they feed on fully developed leaves. Larvae pass throught four instars over three to four weeks prior to dropping to ground and forming a white cocoon for pupation (Blodgett 1996). Late summer adults may appear from pupae in 10 to 14 d prior to briefly feeding then entering aestivation (Summers et al. 1981). In late fall, adults feed for a short time before entering hibernation through the winter months.

Predicting timing of each life cycle event by using calendar dates is difficult to each aspect of life cycle being dependent on environmental conditions; however, prediction of AW life cycle events is possible using degree day calculations. Degree days accumulate when temperatures exceed the minimal threshold of 9^0 C (Harcourt 1981) and are below the maximum threshold of 31^0 C (Guppy and Mukerji 1974). Alfalfa weevil degree days can be calculated daily using the following formula: *Degree Days=(Minimum temperature+Maximum temperature)/2–48.*

Using degree days as a predictor of pest phenology in integrated pest management programs is widely accepted as equal to on-site sampling for AW (Brewer 2002). Online degree day calculators are available for calculating degree days using regional temperature data (Coop 2002). Degree day models can vary by latitude (Stilwell et al. 2010). This study noted AW emerging up to 19 d earlier in southern Nebraska compared to AW in northern Nebraska under similar degree days.

Economic Damage of Alfalfa Weevil

Alfalfa weevil adults and larvae cause feeding injury, however foliar feeding injury by adults is not significant. Larvae feed on buds and leaves of alfalfa, thus reducing yields and lowering nutritional value. Larvae damage plants indirectly through the removal of highly digestible, cell solute portion of alfalfa while leaving the less digestible structures (Summers 1998), and directly through removal of biomass. First and/or second instar larvae primarily damage growing tips, while 3rd and 4th instar larvae can defoliate entire plant (Landis & Haas 1990). Greater than 90% of feeding damage is caused by late instar larvae (Koehler & Pimentel 1973). Thirty larvae per 0.33 m^2 will cause about 190 kg / ha loss in hay at cutting. Higher densities have reported to cause losses of up to 2.2 metric tons / ha (Higgens et al. 1989), thus causing a significant loss in many first cuttings, and seriously lowering yields in the second cutting (DePew 1969).

Alfalfa weevil treatment thresholds are based on both stem-count or sweep net methods. Treatment is considered economical when larval populations average between 1.5 – 2.0 larvae / stem, or 20 larvae / sweep (Blodgett 1996). The sweep-net method is

used by taking ten sweeps at 5 sample sites with a 38 cm diameter sweep net. The stem-count method can be used by shaking larvae from ten alfalfa stems at 5 sites within field. Mean AW larvae / stem can be compared against stem height to decide whether insecticides are warranted (Higgins et al. 1991; Danielson et al. 1994).

Non-insecticidal Management of AW & Secondary Pest – Pea Aphid

Early Cutting

Harvesting has been identified as a valuable integrated pest management (IPM) tool for managing a variety of insects, including AW (Essig & Michelbacher 1933, Harper et al. 1990). Early cutting of alfalfa causes AW mortality directly, while limiting available food and increasing larval desiccation from direct sunlight while in windrows (Blodgett 1996). This technique is often ineffective if cutting occurs prior to peak oviposition (due to surviving AW in second crop regrowth), or if windrows are not baled soon after cutting. Delays in baling allow surviving larvae time to re-establish in the field to feed on tender regrowth, and warrant the use of chemical control (Blodgett et al. 2000). Blodgett et al. (2000) indicated raking soon after baling increases AW efficacy as much as 43% compared to early cutting alone.

The long term success of early cutting is dependent on the synchrony of AW populations with plant growth stage. As alfalfa matures, fiber content increases while protein content and digestibility decrease (Cash & Bowman 1993), with highest seasonal yields of alfalfa reported by harvesting when 10% of stems reach the bloom stage (Reynolds 1971). Repeated early cutting prior to the bloom stage within a growing

season may result in reduced dry matter yields and earlier stand declines (Nelson 1925). Allowing alfalfa stands to reach 1/10th bloom stage for at least one cutting / season helps maintain good plant stands (Cash & Bowman 1993). In addition, early cutting when root carbohydrates are reduced or the alfalfa stand has sustained winter injury will cause thinned stands susceptible to weed invasion (Blodgett et al. 2000).

Early cutting is a valuable tool in Montana for managing AW, however delays in baling, cutting prior to peak AW populations, and cutting when alfalfa has sustained winter injury can often lead to further losses from resurging AW populations or weed invasion. Insecticide applications are needed to protect alfalfa stands in these circumstances. In addition, chemical control is often the only option available in alfalfa stands intended for seed production.

Resistant Cultivars

Many cultivars including 'Team,' 'Arc,' 'Liberty,' 'Weevilchek,' and 'Cimmaron SR' tolerate moderate AW feeding and are considered partially tolerant (Sorenson et al. 1988). The search for alfalfa cultivars exhibiting strong resistance against AW has been unsuccessful (Zavaleta and Ruesink 1980). The mechanism of partially resistant cultivars is through compensative growth from axillary buds (Blodgett 1996), while glandular-haired alfalfa cultivars have shown resistance to other insect pests. Field studies by Dellinger (2006) indicated little resistance towards AW using glandular-haired alfalfa cultivars. Alfalfa weevil resistant alfalfa cultivars provide insufficient protection to validate their use (Blodgett et al. 2000).

Hymenopteran Parasitoids of Alfalfa Weevil

Fifteen natural enemies of the AW were found in Europe by 1912 (Chamberlain 1924). It was noted that AW may be kept under sustained control in the U.S. with the use of some of these beneficial parasites (Ayedh 1995). These include the larval parasitoids *Bathyplectes curculionis* (Thomson), *Bathyplectes anurus* (Thomson), *Bathyplectes stenostigma* (Thomson), *Oomyzus (=Tetrastichus) incertus* (Ratzeburg), *Microctonus colesi* (Drea); the egg parasitoid, *Patasson luna* (Girault); the pupal parasitoid, *Dibrachoides druso* (Walker); and the adult parasitoid, *Microctonus aethiopsdias* (Druso). Larval parasitoids are the most successful and significant parasitoids to AW populations in the U.S. (Ayedh 1995; Flanders 2000).

All three *Bathyplectes spp*. deposit eggs within AW larvae, but *B. curculionis* is by far the most prevalent species within the U.S.. *B. curculionis* was introduced from Italy into Utah in 1911 – 1913; *B. anurus* was first recovered from New Jersey and Pennsylvania in 1964, and *B. stenostigma* was first reported in New Jersey in 1961 (Dysart & Coles 1971). Releases of *B. curculionis* have been conducted at various locations across the U.S. since 1953 (Dysart & Day 1976). *B. anurus* and *B. stenostigma* only have one full generation / year, while *B. curculionis* has a first and partial second generation. *B. curculionis* and *B. anurus* prefer earlier instar larvae while *B. stenostigma* prefers later instar larvae. Developing parasitoid larvae form a cocoon inside the host cocoon and kill them within approximately 14 d (Chamberlain 1924). Dark brown, football-shaped cocoons of *B. curculionis* have an un-raised white band around the cocoon which can easily be identified, while *B. anurus* has a white equatorial band that is

not raised. Cocoons of *B. stenostigma* resemble a brown paper bag (Dysart & Day 1976). *B. curculionis* shows the highest parasitism rate of any AW parasitoid found in the U.S. (Ayedh 1995). To avoid hyperparasitism, *B. anurus* larvae may cause cocoons to jump from 5 – 7.5 cm high if disturbed or exposed to bright light (Weaver 1976). A study by Davis (1970) indicated carbofuran and phorate to have little impact on the parasitism rate of *B. curculionis*.

Oomyzus incertus parasitizes 3rd and 4th instar larvae. Dark brown to mahogany mummies are created after parasite kills AW larvae. Multiple parasites may be present within parasitized hosts. There are several generations of *O. incertus* / year (Weaver 1976).

Microctonus colesi was first found in the U.S. in 1962 in southeastern Pennsylvania. This univoltine parasitoid oviposits in 3rd to 4th instar larvae of AW (Dysart & Day 1976). The parasitoid larva completes development the following spring in the AW adults (Drea 1968). This species also reduces fertility of emerging spring adults that are infected (Coles & Puttler 1963).

Parasitism by the Hymenopterans *Microctonus aethiopoides* and *Bathyplectes spp*. raised AW mortality as high as 80% in Wisconsin and 60% in Minnesota (Flanders 2000). Due to these early successes, bio-control releases of adult and larval parasitoids were made from 1980 – 1990 by USDA-APHIS – PPQ personnel. This resulted in alfalfa farmers saving $8 million annually because of a 73% reduction in the number of hectares requiring insecticides by 1981 (Kingsley et al. 1993). Reduction in AW populations from western states have been marginal (Ayedh et al. 1996, Radcliffe & Flanders 1998), with

parasitism estimates of 0 – 20% in Montana (Blodgett 1996), and 2.9 to 7.1% reported in Colorado (Ayedh et al. 1996). Previous studies by Kingsley et al. (1993), Harcourt (1990) and Yeargan & Pass (1978) indicated *Bathyplectes curculionis* was not an effective biological control agent when AW densities were abundant, however percent parasitism increases as AW densities decrease (Schroder and Dodson 1985, Harcourt 1990, Kingsley et al. 1993). Parasitism rates in Montana and Colorado are not thought to keep high densities of AW from being a threat to the alfalfa crop, but may keep low densities of AW at non-economic levels.

Entomopathogenic Nematodes

Nematodes in the genera *Heterorhabditis* and *Steinernema* control a wide variety of important insect pests (Klein 1990; Shapiro et al. 2002). Infective juvenile nematodes (IJN) persist in soil and enter AW larvae through natural openings or the cuticle. Nematodes reproduce within the host, producing several hundred thousand IJN nematodes that emerge from the host to search out new hosts (Shapiro and Gaugler 2002). Microplots inoculated with one billion IJN/acre (including *S. carpocapsae* and *H. indica)* significantly lowered AW populations from 49-72% when compared to the untreated (Shah et al. 2011). Laboratory trials by Kim et al. (2007) found that *S. carpocapsae* and *H. indica* reduced populations of AW approximately 77.5 to 100% when infected with over 20 IJR / weevil. The use of nematodes to manage AW shows promise, however these parasites prey on a wide range of arthropod and plant species. Consequently, efficacy using nematodes can be reduced considerably if a wide range of prey species are available (Klein 1990).

Alfalfa Weevil Predators

Predators are generally considered inferior to parasitoids in biological control programs. Insect predators are often less specific than insect parasites that target a single pest species. This is often due to a predator's lack of synchrony with prey host dispersion and phenology when compared to parasites. Parasites also do not need to search for food as immatures compared to predators because the host provides their food source (Bohart et al. 1982). However, there are many examples of predators being used in successful biocontrol programs (Hagen et al. 1976; Huffaker et al. 1976; Messenger et al. 1976). This is especially true when a single prey species is available or if the predator exhibits selectivity towards only one prey species. Alfalfa weevil predators reported in the literature are spiders (Araneae), soft winged flour beetles (Melyridae), nabids (*Nabid* spp.), European earwigs (*Forficula auricularia* [Linnaeus]), bigeyed bugs, (*Georcis* spp.) assassin bugs (Reduviidae), lacewings (*Chrysopa* spp.), eumonid wasps (*Odynerus dilectus),* and coccinellids (Coccinellidae). These predators vary considerably as effective natural enemies for use in AW biocontrol programs (Yakhontov 1934; Ouaygode & Davis 1981; Bohart et al. 1982; Kalaskar & Evans 2001).

Irrigated alfalfa, which supports large and diverse insect populations, provides a favorable environment for coccinellids (Kajita & Evans 2010) which have been identified as the most valuable predator of AW in multiple investigations (Yakhontov 1934; Ouayogode & Davis 1981). Studies in Utah indicate lady beetle larvae occur later in the season, thus have less significance as AW predators compared to adult coccinellids (Ouayogode & Davis 1981). *Coccinella septumpunctata* (Linneaus) is the dominant

species in many alfalfa systems due to its high fitness and reproductive potential compared to other native coccinella species (Kajita & Evans 2010). This may be due to greater success compared with other coccinellids in adapting to AW as an alternative prey species to aphids (Evans & Toler 2007). Ouayogode & Davis (1981) identified coccinellids, nabids, and the goldeneyed lacewing, *Chrysopa oculata* (Say), as the most effective predators to AW, while spider species (Araneae), soft winged flower beetles (Melyridae), and European earwigs, *Forficula auricularia* (Linnaeus) as secondary, opportunistic predators.

Predaceous eumonid wasps are distributed across the western U.S., Canada, north to Alaska, and have been identified in some northeastern states. An investigation in Utah by Bohart et al. (1982) found eumonid wasp, *Odynerus dilectus*, nests to exclusively contain AW larvae, and further identified this species as a highly effective predator that may be used in biological control programs. This study found that O. dilectus can have a significant impact on AW when sufficiently abundant. This is due to high target specificity resulting in this species utilizing almost exclusively *Hypera* larvae as prey. They estimated *O. dilectus* to prey upon 200,000 AW larvae / 28 m^2 in plots near Logan, Utah (Bohart et al. 1982).

Even though the pea aphid, *Acyrthosiphon pisum*, is the primary prey of many predator species (Kalaskar 2001; Giles 1994), these predators could have an impact on AW populations if pea aphids are absent or in low numbers.

Secondary Pest - Pea Aphid

The pea aphid is found throughout North America and is a pest on legume crops including peas, clovers, and alfalfa. Adult aphids are approximately ¼" in length and range in color from green to yellow, to pale pink (Hodgson 2007). Adult pea aphids parthenogenetically produce from 50 to 100 nymphs at a rate of six to seven / day (Blodgett 2006). This pest is the most common aphid in Montana and Utah alfalfa production systems (Hodgson 2007); however populations seldom reach economic levels. The pea aphid can cause alfalfa to turn yellow and wilt under extremely high densities thus significantly decreasing cutting yield. Economic thresholds vary according to the maturity of alfalfa: 1) >20" stem length: 100 aphids / stem or sweep, 2) 10 – 20" stem length: 75 aphids / stem or sweep, 3) <10" stem length: 40 aphids / stem or sweep, and 4) 5" stem length: five aphids / stem or sweep (Hodgson 2007). Cuperus et al. (1982) indicated the economic threshold to be 75 pea aphids / sweep two weeks prior to harvest.

The importance of predators for controlling pea aphids has been recognized in multiple North American field investigations (Harper 1978). Elliot et al. (2002) identified coccinellids, common damsel bug, *Nabis rugosis* (Linneaus), and common lacewings, *Chrysoperia plorabunda* (Fitch), as primary predators to pea aphids. Coccinellids have been identified as the most valuable primary predator of pea aphids in multiple investigations (Evans & England 1996). The suppression of predators through broad-spectrum insecticide use often leads to secondary pest outbreaks of pea aphids. Evans & Karren (1993) demonstrated that applications of broad-spectrum carbofuran, dimethoate or parathion for managing early season AW caused an approximate six fold

increase in pea aphids two to three weeks later due to lack of predators. Linker et al. (1996) recommends treatment only if the ratio of beneficial insects (coccinelid larvae and adults) to the number of aphids / stem is less than or equal to 1:10. Pesticides which have high efficacy towards the AW with reduced impacts on predator / parasitoid complex should provide increased long term control of AW and pea aphids and lower input control costs.

Grazing

Fall and winter grazing of alfalfa has reduced spring AW populations by as much as 25% in grazed compared to non-grazed plots in Oklahoma (Dowdy et al. 1992). Winter and fall grazing has little impact in northern latitudes where multi-voltine life cycles don't exist, thereby eliminating fall-deposited eggs as the vulnerable overwintering stage susceptible to fall and winter grazing. Grazing impacts upon eggs oviposited from spring populations of alfalfa weevil were investigated by Goosey et al. (2004). This study indicated that spring grazing by sheep, *Ovis aries* (Linnaeus), reduced alfalfa weevil populations as much as 40 – 70% in grazed vs. non-grazed plots in Montana. Grazing is another option for managing AW, however constraints such as difficulty in obtaining livestock and costs of constructing adequate fencing creates barriers in the implementation of this IPM tactic.

Search for Alternative Insecticide Strategies

Insecticides are used to control AW in approximately 34% of the total alfalfa hectares across the U.S. (Bailey 1994). The primary products used in Montana as of

2011 are synthetic pyrethroid chemistries which have low mammalian toxicity, break down quickly in the environment, and are highly efficacious towards many insects. The broad-spectrum activity of synthetic pyrethroids often leads to a loss of beneficial predators and parasitoids due to broad-spectrum activity, while posing as a significant hazard towards fish and aquatic invertebrates (Mian & Mulla 1992). The loss of the AW predator / parasitoid complex with the use of broad-spectrum pesticides has been shown to increase future pest outbreaks (Evans & Karren 1993; Harper 1978). The organic hay market, which supports a growing organic milk market, limits the use of all conventional chemicals including synthetic pyrethroid, carbamate and organophosphate chemistries from the alfalfa pest control arsenal (Fuerst et al. 2009; Guerena & Sullivan 2003). New pest management tools are needed to manage AW in organic alfalfa systems, and conventional forage alfalfa systems.

Registering new pesticide products can be very costly due to data required and time needed to register new pesticide products. Toth (1996) reports it takes from six to nine years and costs an average of $50 million to pay for all expenses from the discovery, registration, to the final marketing of each active ingredient. The average time to register a conventional pesticide product through EPA was estimated to take 36 - 38 months (Toth 1996; EPA 2011). The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) as amended by the Food Quality Act of 1996 (FQPA) requires the Environmental Protection Agency (EPA) to allow for expedited review of pesticides designated as reduced risk since 1996. The EPA reduced-risk pesticide initiative and biopesticide and pollution prevention division was created to comply with the 1996

FQPA amendment to FIFRA. This initiative encourages the registration and use of low-risk pesticide products. Reduced risk pesticides and biopesticides can now be registered in as little as 14 to 11 months, respectively (EPA 1997).

Chemicals which qualify for expedited review must qualify as either a reduced-risk pesticide or biopesticide. A reduced-risk pesticide is defined by EPA as controlling pests without posing unreasonable risks to human health or the environment. Chemicals are classified as reduced-risk by their low impact on human health, low toxicity to non-target organisms, low potential for groundwater contamination, low use rates and low resistance potential (EPA 2011).

Biopesticides are naturally occurring chemicals (Ex. Naturally occurring semiochemical, hormones and insect growth regulators), microorganisms (microbial pesticide), and pesticide substances produced by plants containing plant incorporated protectants (PIP) that are effective in managing pests. A PIP is the genetic material inserted into a genetically modified organism (GMO) that produces a product to reduce a pest population (EPA 1997). Some biopesticides are labeled for use on organic systems by the Organic Materials Review Institute (OMRI). There are over 2,300 OMRI-approved products that are certified organic under the USDA National Organic Program (Organic Material Review Institute 2011), and can be used in the organic alfalfa market.

The OMRI approved active ingredient, azadirachtin**,** was registered as a reduced-risk biopesticide by the U.S. EPA in 1985, and was soon registered and approved for pest control in organic systems (Organic Material Review Institute 2011). It has low mammalian toxicity, degrades rapidly in the environment, and shows little harm to

beneficial insects (Lowery et al. 1993). Azadirachtin is the main active ingredient in neem oil, which is extracted from the neem tree *Azadirachta indica* 'A. Juss.' (Aerts 1997). Azadirachtin has shown activity on over 200 species of insects, with high acute toxicity against the European leafroller, *Archips rosana* (Linnaeus), desert locust, *Locusta migratoria* (Linnaeus), whiteflies (Aleyrodidae) and *aphis* spp. (Lowery et al. 1993; AliNiaZee et al. 1997; EPA 2012). Previous studies in Montana have indicated azadirachtin causes a significant reduction (65%) in AW under field conditions (Tharp et al. 2004). Yardim et al. (2001) found azadirachtin lowered populations of AW by 45 to 52% from 1998 to 1999. Azadirachtin has ecdysteroid and juvenile hormone properties with activity as an insect growth regulator (Aertz et al. 1997), while also acting as a stomach poison and feeding deterrent. Beneficials including minute pirate bugs (Anthocoridae), lacewings, coccinellids, nabids, and bees (Apoidea) were not affected by azadirachtin in previous trials (Yardim et al. 2001; Tharp et al. 2003; O'Neill et al. 2004; Tharp 2006). Studies by Oroumchi (1993) indicated that azadirachtin applied four times at weekly intervals interrupted AW larval development and increased alfalfa yields. For these reasons, azadirachtin would make an excellent candidate for further study as an alternative approach to AW management in conventional or organic systems.

Novaluron, registered by the EPA in 2001, is classified as a reduced-risk insect growth regulator (IGR). Novaluron inhibits the normal growth and development of the insect by inhibiting chitin formation, eventually causing death (Cutler 2005). IGR's are relatively safe to adult beneficial insects and the environment. This chemical has been found to be an effective tool used to control whiteflies (Aleyrodidae), thrips

(Thysanoptera) and the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), while having low impact on parasites, *Encarsia Formosa* (Gahen) and *Stratiolaelaps scimitus* (Womersley), a soil dwelling predatory mite (Cutler 2005). Previous studies in Montana alfalfa systems resulted in a low impact on beneficials including nabids, coccinellids and spiders, while reducing AW populations by 50 – 73% (Tharp et al. 2004; Tharp et al. 2005; Tharp 2006). However, Hodgson et al. (2010) found novaluron-treated seed alfalfa plots caused 84% mortality on alfalfa leaf cutting bees, *Megachile rotundata* (Fabricius), if females mated and nest 24 h after an application. Timely insecticide applications of novaluron when bees are not actively foraging could avert alfalfa leaf cutting bee mortality. This makes novaluron an excellent candidate for further study as an alternative to conventional chemicals in alfalfa systems.

A similar chemical, diflubenzuron, also acts as an IGR towards insects. This chemical has become an important tool in rangeland management of grasshoppers, providing effective long term control if applied at the proper insect growth stage. In addition, this chemical has toxicity against weevils, including citrus weevil, *Diaprepes abbreviates* (Linnaeus), rice water weevils, *Lissorhoptrus oryzophilus* (Kuschel), pepper weevils, *Anthonomus eugenii* (Cano), and boll weevils, *Anthonomus grandis,* Boheman (Villavaso et al. 1995; Liu 2002; Way 2003), while having minimal impact on natural enemies including bees, predaceous mites (Acari:Stigmaeidae), nabids, coccinellids, and lacewings (Villavaso et al. 1995; Schroeder et al. 1980; Keever 1977). Studies have found diflubenzuron is toxic to AW larvae, but had low mortality in field tests (Braithwaite et al. 1976; Chu 1981). It should be noted that applications were made

directly to AW larvae or adults in the field; many previous studies found the highest success by applications on egg-laying adults as an ovicide (Villavaso et al. 1995). Further study is needed to determine if diflubenzuron could be an alternative to managing AW populations in the field when applied in a proactive manner.

In recent years, the particle film kaolin has been used in integrated pest management programs against a variety of arthropod pests. It has been found to have efficacy against oblique-banded leafroller, *Choristoneura rosaceana* (Harris), potato leafhopper, *Empoasca fabae* (Harris), two spotted spider mite, *Tetranychus urticae* (Koch), pear rust mite, *Epitrimerus pyri* (Nalepa), codling moth, *Cydia pomonella* (Linnaeus), black pecan aphid, *Melanocallis caryaefoliae* (Davis), citrus root weevil, *Diaprepes abbreviates* (Linnaeus) and boll weevil (Cross et al. 1976; Showler 2002; Cottrell et al. 2002). Kaolin has been used for decades as a FDA-approved packaging ingredient in dried foods, and a carrier in cosmetics, toothpaste and antiperspirants. Therefore, this particulate is considered safe for humans and the environment and is registered as a biopesticide by the EPA. By 2000, kaolin was registered for pest control in organic systems by OMRI. Laboratory and field trials found kaolin may act by reducing ovipostion of pests, acting as a feeding detterant, blocking digestion and/or changing visual cues to protect crops from weevils (Showler 2002). Feeding on citrus leaves by root weevils. *D. abbreviates,* was reduced by 84%, and oviposition completely suppressed with the use of kaolin (Lapointe 2000). In addition, studies by Cross et al. (1976) found that other weevils in which kaolin was effective are attracted to certain colors for oviposition by adults, specifically in between the blue to green spectrum, with

a wavelength range of 500 -525nm. Kaolin suppressed root weevils and boll weevils and would be an excellent candidate for further study as an alternative low-risk approach for management of AW in conventional and organic forage alfalfa systems.

Summary

The studies presented in this thesis were designed to test whether azadirachtin, novaluron, diflubenzuron, and kaolin could be used as viable alternatives to traditional insecticides for management of AW. The primary use of these products would be for the alfalfa seed industry and growers wanting organically-approved or integrated management options for AW control. The objectives were to assess mortality, oviposition rates, growth rates, and repellency of AW as well as the response of secondary pest 'pea aphids', beneficial predators 'coccinellids, nabids, and lacewings' and larval parasites '*Bathyplectes* spp., *Oomyzus incertus, Microctonus colesi, Patasson luna*, and *Dibrachoides druso'*. Predator/prey relationships were tabulated to determine the most effective alternative based on not only efficacy towards primary and secondary pests, but also minimal impacts on non-targets. Results obtained from alternative treatment options were compared against the synthetic pyrethroid lambda cyhalothrin as a standard.

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CHAPTER 2

EFFICACY OF THREE INSECT GROWTH REGULATORS AND THE PARTICLE BARRIER FILM KAOLIN AGAINST ALFALFA WEEVIL (*HYPERA POSTICA* GYLLENHAL)

Abstract

This study was conducted to evaluate the insect growth regulators novaluron, diflubenzuron, azadirachtin, and the particle barrier film, kaolin, for managing alfalfa weevil (AW, *Hypera postica* Gyllenhal). Diflubenzuron, azadirachtin and kaolin reduced AW densities across three field sites by as much as 23.6 ± 2.7%, 25.0 ± 9.0% and 30.3 ± 10.9%, respectively; however reductions were low and not statistically comparable to the lambda cyhalothrin application that reduced densities by 97.7 ± 1.3% across field sites. The most promising chemical evaluated was novaluron due to AW mortality reaching as high as 74% and reductions in AW feeding damage equal to that of the lambda cyhalothrin treatment when AW densities exceeded the economic threshold at one field site (LSD Test; $P<0.0001$). At this site novaluron and lambda cyhalothrin treated plots had a leaf defoliation index (LDI) of 1.0 ± 0.1 and 0.7 ± 0.3, respectively, while untreated plots had an LDI of 2.7 ± 0.3 (LDI range: 0 – 3). Novaluron significantly reduced AW damage when compared to the untreated plots immediately prior to harvest in two of three field trials and two greenhouse studies. Feeding reductions from applictions of novaluron were likely due to direct mortality, while acting as a feeding deterrant towards surviving larvae. Protection from feeding seems to be temporary as novaluron treatments no longer protected plants 14 DAT resulting in an average LDI of 2.8 compared to

untreated plants that had an LDI of 3.0 in two greenhouse trials ($P > 0.05$). Plants treated with novaluron weighed significantly more than untreated plants at harvest in both greenhouse trials; however harvest yields were not increased in field trials ($P = 0.05$). Future studies may wish to evaluate yield improvements by combining novaluron with early harvest strategies to take full advantage of novaluron's temporary AW feeding deterrence on alfalfa.

Introduction

Insecticides are used to control alfalfa weevil (AW, *Hypera postic*a [G]) in approximately 25% of the alfalfa (*Medicago sativa* [L]) hectares across the U.S. (Hower et al. 1999). The primary products used in Montana are synthetic pyrethroids which have low mammalian toxicity, break down quickly in the environment, and are highly efficacious towards many insects. The broad-spectrum activity of synthetic pyrethroid, carbamate, or organophosphate chemistries often leads to a loss of beneficial predators and parasitoids and secondary pest outbreaks of aphids (Harper 1978; Summers 1998). Synthetic insecticides also pose a significant hazard towards fish and aquatic invertebrates (Mian & Mulla 1992). There are no registered insecticide chemistries for effective AW management that minimize non-target impacts. Furthermore, there is a need for new, organically approved chemistries to support the growing organic hay market to supply the organic milk and beef industry (Guerena & Sullivan 2003; Fuerst et al. 2009). Organic hay in the U.S., predominantly pure alfalfa stands, has increased from 46,980 ha harvested in 2001 to 103,680 ha harvested in 2008 (USDA 2012). Few

organically approved alternatives are available but are needed to protect yields from damaging key pests, AW.

Alfalfa is a perennial plant that has been grown as a forage crop since the beginning of recorded history, originating in the vicinity of present day Iran and brought to North America in the early 1700's (Whyte et al. 1953; Wilsie 1962; Lacefield et al. 1997). It is the foremost crop in many semi-arid and temperate states in the US, with 51.8 metric tons produced in 2013. In 2013, Montana farmers produced 3.56 million metric tons of alfalfa hay with a value of $558 million; Montana is ranked 3rd nationally in 2013 (NASS 2014). Alfalfa is a high quality feed for livestock that is easily digested, low in neutral fibers and high in protein (Conrad and Klopfenstein 1988). It is considered the most useful forage legume used as animal feed (Abdel Magid 1983), and a critical component to the dairy, beef (*Bos* spp.), sheep (*Ovis* spp.), horse (*Equus* spp.), swine (*Sus* spp.), and poultry (*Gallus* spp.) industries (Van Keuren & Matches 1988).

Alfalfa weevil is the most damaging pest of forage alfalfa in the U.S., and is found throughout the contiguous 48 states (USDA APHIS 1991; Hsaio 1993). The AW is native to Europe but can be found in North America, North Africa, the Middle East, India, and western Asia (Radcliffe & Flanders 1998). Larve feed on buds and leaves of alfalfa, thus reducing yields and lowering nutritional value. Thirty larvae / 0.33 m^2 will cause approximately 190 kg / ha loss in hay at cutting. Higher densities of AW have been reported to cause a complete loss in many first cuttings, with carryover damage to the second cutting (Higgens et al. 1989).). Alfalfa weevils are found throughout the contiguous 48 states (Hsaio 1993).

Many non-insecticidal alternatives exist for managing AW including resistant varieties, early cutting, parasitoids, predators, and grazing, however each option has limitations. Cultivars including 'Team,' 'Arc,' 'Liberty,' 'Weevilchek,' and 'Cimmaron SR' tolerate moderate AW feeding and are considered partially tolerant (Sorenson et al. 1988). The search for alfalfa cultivars exhibiting strong resistance against AW has been unsuccessful (Zavaleta and Ruesink 1980; Blodgett et al. 2000; Dellinger et al. 2006). Early cutting is a valuable tool for managing a variety of insects, including AW (Essig & Michelbacher 1933, Harper et al. 1990), however delays in baling, cutting prior to peak AW populations, and cutting when alfalfa has sustained winter injury can often lead to further losses from resurging AW and weed populations (Blodgett et al. 2000). Grazing can reduce AW by 40 – 70% in Montana (Goosey et al. 2004), however constraints such as difficulty in obtaining livestock and costs of constructing adequate fencing creates fundamental problems in the implementation of this IPM tactic. Alfalfa weevil in many northeastern and some mid-western states has been managed successfully with the use of Hymenopteran parasitoids (Flanders & Radcliffe 2000), however impacts on AW from western states has been low (Ayedh et al. 1996, Radcliffe & Flanders 1998, Flanders 2000), with a 0 – 20% parasitism rate reported in Montana (Blodgett 1996) and a 2.9 – 7.1% parasitism rate reported in Colorado (Ayedh et al. 1996). Parasitism rates in Montana and Colorado are not thought to keep high densities of AW from being a threat to the alfalfa crop, but may keep low densities of AW at non-economic levels (Ayedh et al. 1996). Irrigated alfalfa, which supports large and diverse insect populations, provides a favorable environment for many AW predators (Kajita & Evans 2010). Ouayogode &

Davis (1981) identified coccinellids (Coccinellidae), nabids (Nabidae), and the goldeneyed lacewing, *Chrysopa oculata* (Say), as the most effective predators to AW, while spider species (Araneae), soft winged flower beetles (Melyridae), and European earwigs, *Forficula auricularia* (Linnaeus) as secondary, opportunistic predators. Using highly efficacious insecticide chemistries that reduce impacts on beneficial natural enemy complex may provide longer AW control as compared to conventional broad spectrum insecticides.

Search for Alternative Insecticide Strategies

Registering new pesticide products can be very costly due to data required and time needed to register pesticide products. Toth (1996) reports it takes from six to nine years and costs an average of $50 million to pay for all expenses from the discovery, registration, to the final marketing of each active ingredient. The average time to register a conventional pesticide product through EPA was in itself from 36 - 38 months (Toth 1996; EPA 2011). The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) as amended by the Food Quality Act of 1996 (FQPA) requires the Environmental Protection Agency (EPA) to allow for expedited review of certain pesticides since 1996. The EPA reduced-risk pesticide initiative and biopesticide and pollution prevention division was created to comply with the 1996 FQPA amendment to FIFRA. This initiative encourages the registration and use of reduced-risk pesticide products. Reduced risk pesticides and biopesticides can now be registered in as little as 14 to 11 months, respectively (EPA 1997). Some reduced risk pesticides are also labeled for use on organic systems by the Organic Materials Review Institute (OMRI). There are over 2,300 OMRI-approved

products that are certified organic under the USDA National Organic Program (Organic Material Review Institute 2011), and can be used in the growing organic alfalfa market.

The OMRI approved active ingredient, azadirachtin, was registered as a reduced-risk biopesticide by the U.S. EPA in 1985, and was soon registered and approved for pest control in organic systems (Organic Material Review Institute 2011). As an insect growth regulator azadirachtin has ecdysteroid and juvenile hormone properties (Aertz et al. 1997), while also acting as a stomach poison and feeding deterrent. It has low mammalian toxicity, degrades rapidly in the environment, and shows little harm to beneficial insects (Lowery et al. 1993; Yardim et al. 2001). Azadirachtin is the main active ingredient in neem oil, which is extracted from *Azadirachta indica* (A. Juss.), neem tree (Aerts 1997). Azadirachtin has shown activity on over 200 species of insects, with high acute toxicity on the European leafroller, *Archips rosana* (Linnaeus), desert locust, *Locusta migratoria* (Linnaeus), whiteflies (Aleyrodidae), and *Aphis* spp., (Lowery et al. 1993; AliNiaZee et al. 1997; EPA 2012). Previous studies in Montana have indicated azadirachtin causes a significant reduction (65%) in AW under field conditions (Tharp et al. 2004). Yardim et al. (2001) found azadirachtin lowered populations of AW by 45 to 52% at field sites from 1998 to 1999, while studies by Oroumchi (1993) show that azadirachtin applied four times at weekly intervals interrupted AW larval development and increased alfalfa yields. Azadirachtin makes an excellent candidate for further study as an alternative approach to AW management in conventional or organic alfalfa systems.

Novaluron, registered by the EPA in 2001, is a pesticide that is also classified as a reduced-risk insect growth regulator (IGR). Novaluron inhibits the normal growth and development of the insect by inhibiting chitin formation, eventually causing insect death (Cutler 2005). IGR's are relatively safe on adult beneficial insects while having low toxicity to mammals and being non-toxic towards birds, earthworms, and soil microflora (Kostyukovsky and Trostanetsky 2006). This chemical has been found to be an effective tool against whiteflies, Aleyrodidae, thrips, Thysanoptera and the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), while having low impact on parasites, *Encarsia Formosa* (Gahen) and *Stratiolaelaps scimitus* (Womersley), a soil dwelling predatory mite (Ishaaya et al. 2001; Cutler 2005). This makes novaluron an excellent candidate for further study as an alternative to conventional chemicals in forage or seed alfalfa systems.

Diflubenzuron also acts as an IGR, specifically, a chitin synthesis inhibitor towards insects. This chemical has become an important tool in rangeland management of grasshoppers, providing effective long term control if applied at the proper insect growth state (Latchininsky 2004). In addition, this chemical has toxicity against weevils, including citrus weevil, *Diaprepes abbreviates* (Linneaus, rice water weevils *Lissorhoptrus oryzophilus* (Kuschel), pepper weevils, *Anthonomus eugenii* (Cano) and *Anthonomus grandis (*Boheman), the boll weevil (Villavaso et al. 1995; Liu 2002; Way 2003), while having minimal impact on natural enemies including damsel bugs, Nabidae, coccinellids, Coccinelidae and lace wings, Chrysopidae (Keever 1977; Schroeder et al. 1980; Villavaso et al. 1995). Studies have found diflubenzuron is toxic to AW larvae, but had low mortality in field tests (Chu 1981; Braithwaite et al. 1976). It should be noted

that Braithwaite et al. (1976) reported a possible leaf deterrence effect from applications of diflubenzuron, while Villavaso et al. (1995) has shown that diflubenzuron has ovicidal properties against boll weevil. Further study is needed to determine if diflubenzuron could be an alternative to managing AW populations in the field when applied on ovipositing adults or could be used to deter AW larval feeding.

The particle film kaolin has been used in integrated pest management programs against a wide variety of arthropod pests. It has been found to have efficacy against oblique-banded leafrollers, *Choristoneura rosaceana* (Harris), potato leafhoppers *Empoasca fabae* (Harris), two spotted spider mites, *Tetranychus urticae* (Koch), pear rust mite, *Epitrimerus pyri* (Nalepa), codling moth, *Cydia pomonella* (L.),curculio, *Diaprepes*, black pecan aphids, *Melanocallis caryaefoliae* (Davis), citrus root weevil, *Diaprepes abbreviates* (Linnaeus) and boll weevil (Cross et al. 1976; Lapointe 2000; Showler 2002; Cottrell et al. 2002). Kaolin has been used for decades as a FDA-approved packaging ingredient in dried foods, and a carrier in cosmetics, toothpaste and antiperspirants. Therefore, this particulate is considered safe for humans and the environment and is registered as a biopesticide by the EPA. By 2000, kaolin was registered for pest control in organic systems by OMRI. Laboratory and field trials indicate kaolin acts as a feeding deterrent, blocks digestion, reduces oviposition as well as directly influencing insects migrating into the field through color (Cross et al. 1976; Showler 2002). Cross et al. (1976) indicated boll weevil ovipositing adults are attracted to colors in between the blue to green spectrum, with a wavelength range of 500 -525nm. Kaolin suppressed root weevils and boll weevils (Lapointe 2000; Showler 2002; Cross et

al. 1976) and would be an excellent candidate for further study as an alternative low-risk approach for management of AW in conventional and organic forage alfalfa systems.

Summary

This study was designed to test whether azadirachtin, novaluron, diflubenzuron, and kaolin could be used as viable alternatives to traditional insecticides for management of AW. The primary use of these products would be for the alfalfa seed industry and growers wanting organically-approved or integrated management options for AW control. The objectives were to assess mortality, growth rates, and repellency of AW under field and greenhouse conditions. Results obtained from alternative treatment options were compared against lambda cyhalothrin as a standard.

Materials & Methods

Chemical treatments included in this study were the insect growth regulators novaluron (Rimon 10EC, Chemtura Corp., Middlebury, CT), diflubenzuron (Dimilin 2L, Crompton, Middlebury, CT) and azadirachtin (Neemix 4.5, Certis USA, Columbia, MD); the particle barrier film, kaolin (Surround WP, Engelhard Corp., Iselin, NJ) and the synthetic pyethroid, lambda cyhalothrin (Warrior 1E, Syngenta Crop Protection, Greensboro, NC). All chemical applications were made with a CO_2 powered backpack sprayer with a 2 m wide boom for field trials (Spraying Systems, Wheaton, IL) and a single hand wand (Spraying Systems, Wheaton, IL) for laboratory trials. All applications except kaolin were applied using Teejet model XR8001VS nozzles (Spraying Systems, Wheaton, IL) which delivered an output of 83.3 liters/ha at 30 PSI. Kaolin applications

used Teejet XR8010 nozzles (Spraying Systems, Wheaton, IL) which delivered an output of 378 liters / ha at 30 PSI. Foliar applications of kaolin (6,544 g [AI] / ha), azadirachtin (7.8 g [AI] / ha), novaluron (31.0 g [AI] / ha), diflubenzuron (22.6 g [AI] / ha), and lambda cyhalurothrin (5.5 g [AI] / ha) were compared to the untreated control in each field and greenhouse trial.

Seasonal growth and development of AW was predicted using AW degree day (DD) calculations using a minimum developmental threshold of 9^0C beginning on first March of each year (Blodgett 1996). The online phenology and DD calculator Version 4.51 (Oregon State University & WRIPM Center 2012) was used to calculate DD using the sine wave method (Stilwell et al. 2010). The sine wave method is more accurate than other methods when minimum temperatures fall below the developmental minimum temperature of the insect (Herms 2006). Temperature and RH was calculated on an hourly basis using HOBO H8 Pro Series (Onset, Pocasset, MA) Temp/RH logger set 0.5 m above the soil surface.

Field Trials

Insecticide Application Timing, 2006. Synchronizing an insecticide application with vulnerable AW developmental stages is critical when evaluating a pesticide's effectiveness. The 2006 field trial was conducted to determine the best timing of each pesticide application, and corresponding vulnerable AW developmental stages to target when evaluating each pesticides efficacy. The study was conducted on a fifth year commercial forage alfalfa (cv. 'Shaw') production field 6.4 km northwest of Bozeman,

Gallatin County, MT. Plots measuring 6.6 by 8.3 m were arranged as a RCB design with 14 treatments replicated four times against a wheel line sprinkler irrigation system delivering 5 cm of precipitation every seven d. Six chemical treatments were further divided into different application windows for a total of 14 treatments. Application windows targeted various life stages of AW, including pre-ovipositing adults, ovipositing adults, early larvae and late larvae. Pre-ovipositing applications targeted adult AW when initially detected in plots, ovipositing adult applications targeted peak adult AW, early larval applications targeted AW at first to second instar, late larvae applications targeted second to third instar larvae. Kaolin applications consisted of four different application treatments with consecutive applications occurring within the same plots: 1) pre-oviposition (JD Date [JD] 129), 2) pre-oviposition & ovipositing adults (JD 129, 143), 3) weekly (JD 129, 143, 157, and 164), and 4) early larvae & late larvae (JD 157 and 164). Novaluron and azadirachtin were applied on two different schedules which included: 1) early larvae (JD 157) and 2) late larvae (JD: 164). Diflubenzuron was applied on four different schedules which included: 1) pre-ovipositing adults (JD 129), 2) ovipositing adults (JD 143), 3) early larvae (JD 157), and 4) late larvae (JD 164). Lambda cyhalothrin was applied on the late larvae stage (JD 164) only (Figure 2.1). Life stages targeted were correlated with AW DD (Blodgett 1996) to serve as a guideline for proper date of foliar applications. All foliar applications were made on days with temperatures ranging from 16 to 24 degrees C and 0 – 10 mph winds.

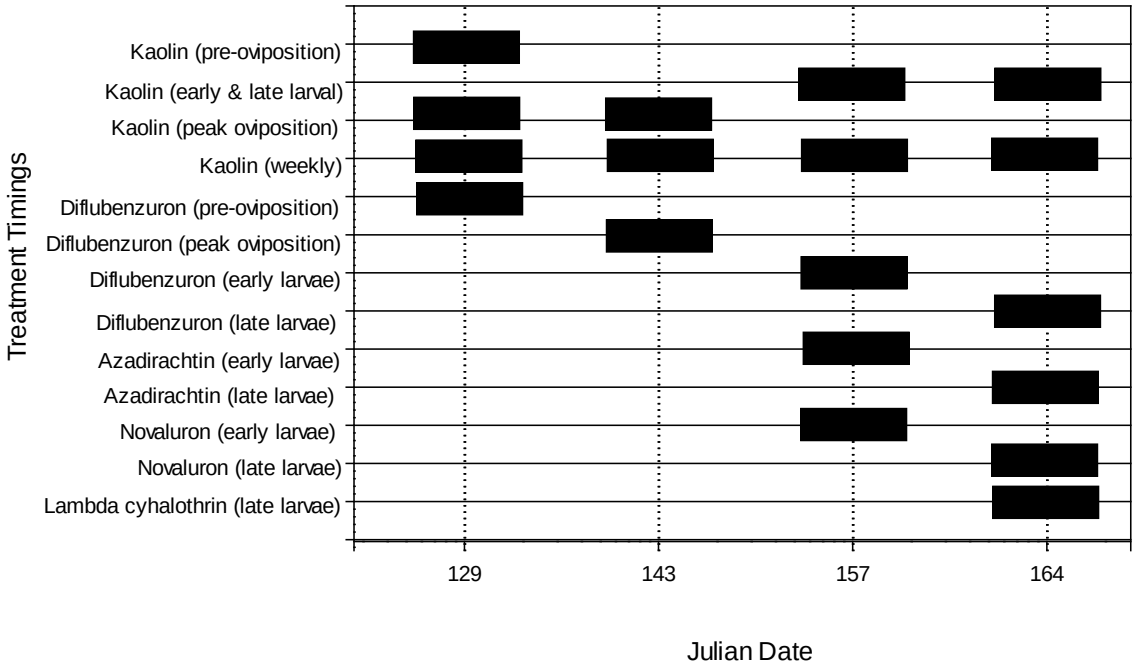


Figure 2.1: Application timings for kaolin, diflubenzuron, azadirachtin, novaluron and lambda cyhalothrin at various Julian Dates in Bozeman, 2006.

Timing of foliar applications for future studies were based on analyses of surviving AW, alfalfa leaf defoliation ratings, eggs / stem, harvest stem height and harvest weight from the 2006 study. Methods for measurement of these variables are described below.

Insecticide Efficacy. Best treatment and timing determined from the 2006 study were further evaluated in plots in two forage alfalfa fields in 2009. The Bozeman site was conducted eight km SW of Bozeman, MT, in a sixth year forage alfalfa (cv. 'Shaw') stand. The Huntley trial was conducted in a 5th year forage alfalfa (cv 'Shaw') stand at the Southern Agricultural Research Center 7 km east of Huntley, MT. Each field was

watered bi-weekly with a wheel-move sprinkler irrigation system delivering 5 cm of precipitation every 7 d.

Plots measuring 6.6 by 8.3 m were arranged as a RCB design with six treatments replicated four times against the irrigation systems at the Bozeman 2009 site, and replicated three times at the Huntley 2009 site. Kaolin was applied at early larval emergence and late larvae (JD 142 and 147 in Huntley; JD 162 and 169 in Bozeman, respectively), novaluron and diflubenzuron were applied at early larval emergence (JD 142 in Huntley and JD 162 in Bozeman), and lambda cyhalothrin and azadirachtin were applied at late larval emergence (JD 147 in Huntley and JD 169 in Bozeman).

Alfalfa Weevil Population Estimates. Alfalfa weevil larvae and AW eggs were assessed in efficacy trials. Alfalfa weevil larvae were collected by taking ten 180^0 sweeps with a 38 cm sweep net in one of six quadrats within each plot. Quadrat sampling rotated systematically in a clockwise fashion to avoid any biased sampling effects on insects across consecutive sample dates. Sweep sampling was initiated immediately prior to each spray application (pre-treatment) and continued weekly until first cutting. Sample dates for the Bozeman 2006 site were JD 157, 164, 170, and 177; for the Huntley site were JD 142, 147, 155, and 162; and for the Bozeman 2009 site were JD 162, 169, 176, and 182. All sweep samples were placed in 3.8 l plastic zip-lock bags prior to transport and 4°C storage in walk in coolers at Marsh Laboratory, MSU-Bozeman. Alfalfa weevil larvae were later counted prior to being categorized to growth stage (instar 1 – 4) by measuring head capsule width (Bartell & Roberts 1974). An instar index was created by summing the instar of each larvae and dividing by the total number of larvae.

Alfalfa weevil eggs were assessed by randomly collecting 20 alfalfa stems on a weekly basis from each plot, starting when peak ovipositing adults were detected at approximately 226 AW DD (JD 143, JD 128 and JD 148) and continuing weekly until peak larvae at approximately 425 AW DD (JD 177, JD 162 and JD 182) at the Bozeman 2006, Huntley 2009 and Bozeman 2009 field sites, respectively (Blodgett 1996). All stem samples were immediately placed in 90 by 60 cm paper bags, placed in a cooler, and returned to the laboratory and frozen for later analysis. Stems were later split and examined for AW eggs.

Agronomic Measurements. A total of 30 stems (ten stems at three random locations within each plot) were evaluated for AW feeding damage, height and alfalfa stage of development. Visual assessments of insect damage using a categorical leaf defoliation index provided relative crop loss estimates (Tharp et al. 2000; Olfert et al. 1995). Alfalfa weevil leaf defoliation was assessed visually with a leaf defoliation index (LDI) that used a numerical rating from 0 – 3, where 0 = no leaf defoliation, 1 = 1 – 25%, 2 = 26-75%, 3 = >75%. Stem height was assessed by measuring the length of each alfalfa stem (cm) from alfalfa crown to tips. Alfalfa stage of development was assessed by using the mean stage by count (MSC) method described by Kalu-Fick (1983). Average stem height, defoliation ratings, and alfalfa growth stage ratings were obtained.

Yield was assessed by clipping forage within two 0.33 m^2 aluminum rings / plot on JD 177 (developmental stage: MSC 5.8), 174 (MSC 5.3) and 182 (MSC 5.7), at the Bozeman 2006, Huntley and Bozeman 2009 sites, respectively. Alfalfa was transferred to 90 by 60 cm paper bags prior and oven dried for 72 h at 37.8^o C prior to weighing.

Statistical Analysis. Each field site was analyzed separately due to unequal sample dates between sites. Scatter plots of residuals versus the independent variables, as well as the Shapiro-Wilk test for normality indicated a normal distribution ($P > 0.05$) of cutting weight, eggs / stem, AW growth stage, alfalfa growth stage, and stem height. The Shapiro-Wilk test ($P<0.05$) indicated lack of normality of surviving AW / sweep and LDI ratings. Residual scatter plots indicated a Poisson distribution of these variables, square root + 0.5 transformation was used to normalize these data (Draper & Smith 1981; Zar 1984). Alfalfa weevils / sweep were converted to percent reduction in AW using Abbott's formula (Abbott 1925). Shapiro-Wilk ($P<0.05$) test and scatter plots indicated a binomial distribution of percent reduction in alfalfa weevil, percentages were arcsine-square root transformed to normalize data (Zar 1984).

Only post application data (14 and 21 DAT) from AW / sweep, LDI ratings and biomass at harvest were used to evaluate best application treatment timings in the 2006 study. All factors and sample dates were evaluated for the 2006 and 2009 insecticide efficacy trials

Treatment effects over time were analyzed using PROC general linear models (GLM) with time as a repeated measures ($P = 0.05$). If treatment or interaction effects were significant, treatment effects for each time period were analyzed using the Fisher protected (LSD) multiple comparison test using SAS (SAS Institute 2001).

Linear regression was used to quantify the influence of AW growth stage versus sampling date and alfalfa growth stage (MSC) versus AW DD by using PROC REG on

SAS (SAS Institute 2001). Confidence intervals were used to assess significant differences in treatment slopes and y intercepts.

Greenhouse Studies

Responses of AW populations to the top performing insecticide active ingredient, novaluron, were further tested under greenhouse conditions. Alfalfa plants for greenhouse trials were obtained from a second year commercial production alfalfa (cv 'Imperial') field in Broadwater County, MT. On JD 115 (2011), 150 early vegetative (MSC 1.4) alfalfa plants were obtained. These plants were placed in 90 by 60 cm paper bags, placed in walk in coolers (4°C) at the Montana State University Plant Growth Center, Bozeman, MT. Plants were transferred to growth chambers with a photoperiod of 16:8 (L:D) h and temperatures of 28:24 °C, RH = 30%.

Second instar AW larvae were collected on JD 166 from the same field using a 38 cm diameter sweep net. Alfalfa weevils were transferred to 22 by 30 cm paper bags with ten alfalfa stems, and transferred to coolers (4° C). Alfalfa weevils were later staged (1 – 4) by measuring head capsule width and placed into petri dishes for use in greenhouse experiments (Bartell & Roberts 1974).

Two greenhouse trials were initiated on JD 167 and JD 181 by arranging 36, 15 cm diameter pots in a randomized complete block design, with three treatments, six replicates and two subsamples / treatment- replicate. The treatments were novaluron, lambda cyhalothrin and an untreated control with identical application equipment & rates described earlier. Each trial was conducted with previously collected forage alfalfa (cv. Imperial), one plant / pot, with four stems / pot, trimmed to ten cm height. A 2.5 cm layer

of quartz sand was deposited over the soil in each pot to provide a seal when cages were later inserted into the sand. Twelve second instar AW larvae were deposited on each plant, with three deposited / stem using a fine camel hair paint brush 24 h prior to foliar insecticide applications. Acetate cages measuring 12 by 90 cm were placed over each pot. Cages were constructed to provide adequate ventilation through screening on top of the cage and on both sides of cage using 2 x 2 mm gauge nylon screen.

Sampling Procedure for Greenhouse Trials. Total live AW larvae on plant, displaced live AW larvae (off plant roaming) and leaf defoliation were assessed at 1, 2, 3, 7, and 14 days after treatment (DAT). Visual assessments of insect damage using a categorical index provide relative crop loss estimates (Tharp et al. 2000; Olfert et al. 1995). Leaf defoliation was assessed visually using a numerical rating from 0 – 3, where 0 = no leaf defoliation, 1 = 1 – 25%, 2 = 26-75%, 3 = >75%. An average AW defoliation rating was obtained by rating each stem for the entire cage.

Stem height was assessed at the last sample date prior to cutting alfalfa stems and placing in 90 by 60 cm paper bags and placing in drying oven (38 °C) for 72 h. Alfalfa was removed from dryers and weighed.

Scatter plots of residuals versus the independent variables, as well as the Shapiro-Wilk test for normality indicated a normal distribution ($P > 0.05$) of leaf defoliation, stem height, and biomass at harvest, thus transformations were not needed for these variables in either greeenhouse trial. Number of AW on plant was converted to corrected mortality using Abbott's formula (Abbott 1925). Number of displaced roaming AW larvae (off plant) was converted to percent displaced larvae. Shapiro-Wilk ($P<0.05$) test and scatter

plots indicated a binomial distribution of percent reduction in AW and percent displaced AW larvae, thus an arc sine of the square root transformation was used to normalize the data (Zar 1984).

Treatment effects over time were analyzed using PROC analysis of variance (ANOVA) with time as a repeated measure in all enclosures ($P = 0.05$; SAS Institute 2002). If treatment or interaction effects were significant, treatment effects for each period were analyzed using the Fisher protected (LSD) multiple comparison test (SAS Institute 2002*)*.

Results

Field Trials

Evaluation of Insecticide Application Timing , 2006. The impact of novaluron application timings upon AW leaf defoliation and surviving larvae were evaluated by date due to significant date by application timing interactions ($P < 0.05$). All other application timings/treatment combinations were evaluated over all time periods.

Significant differences in the number of AW larvae were present between application timings in only the kaolin and novaluron treatments ($P < 0.05$). Significantly more AW larvae were found after kaolin plots were treated at the adult pre-ovipositing or adult ovipositing stage, compared to applications targeting AW larvae or weekly applications ($F = 16.86$, $df = 3$, $P < 0.001$). Applications of kaolin in synchrony with the early or late AW larval growth stages provided significantly better control than adult AW

applications; AW populations were reduced by 58% with larval applications. On JD 170, significantly more AW were present in novaluron plots treated at the late larval stage compared with novaluron plots treated at the early larval stage ($F = 9.72$, $df = 1$, $P = 0.05$). On JD 177 ($F = 31.21$, $df = 1$, $P = 0.01$). On the next sample date significantly more AW were present in novaluron plots treated at the early larval stages compared to the peak larval application. Early larval applications of novaluron caused a 59% reduction compared to the late larval applications on the first sample date, while late applications caused a 65% reduction in AW compared to the early application on the second sample date.

There were no significant differences in LDI's present between any application timing in plots treated with kaolin, azadirachtin or diflubenzuron ($P > 0.05$), however plots treated with an early larval application of novaluron significantly reduced AW feeding damage on JD 177 when compared to the peak larvae application ($F=11.00$, $df = 1$, $P = 0.04$). On this date, alfalfa stems within the early treated plots had a mean index of 1.0, while alfalfa stems within the late treatment had a mean index of 1.75 (Figure 2.2). Alfalfa weevil feeding damage in the untreated plots increased throughout the trial until peaking with an LDI of 2.2 on JD 177.

Significant differences between treatments in cutting weight and eggs / stem were not observed among timings of applications regardless of treatment. Yield averaged between 7,120 and 10,309 kg/ha across all application timing/treatments ($P > 0.05$).

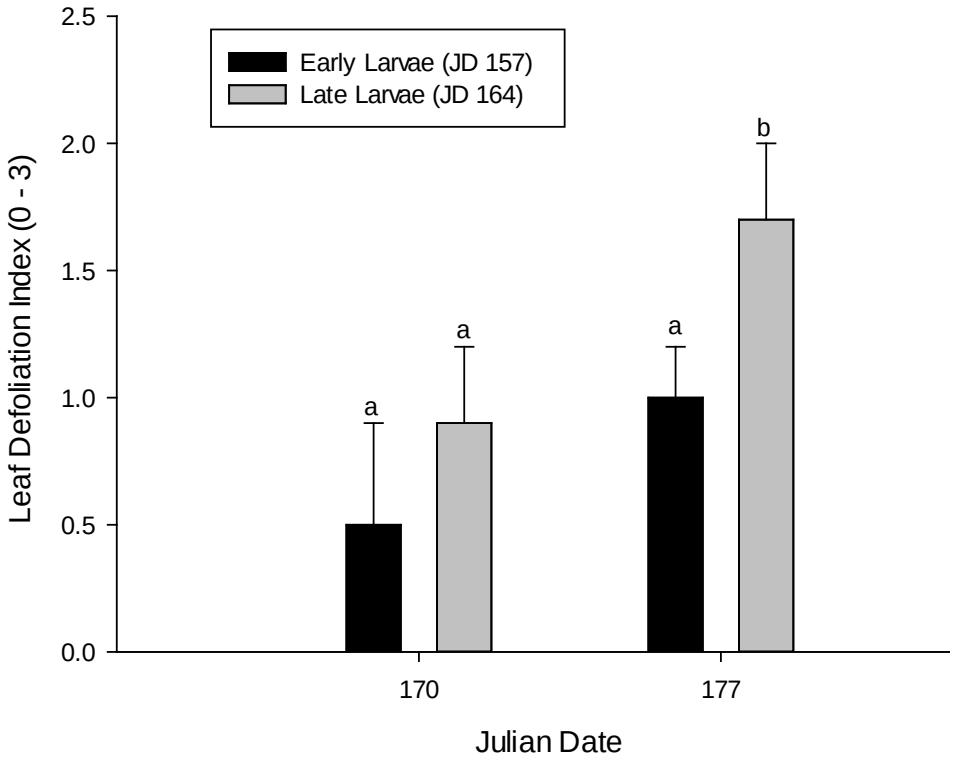


Figure 2.2: Comparison of application timings of novaluron to suppress feeding damage (0 = no leaf defoliation, 1 = 1 – 25%, 2 = 26 – 75%, 3 > 75% leaf defoliation) of alfalfa weevils at various Julian Dates in Bozeman, 2006. Early larvae applications target 1st to 2nd instar larvae (JD 157) while late larvae applications target 2nd – 3rd instar larvae (JD 164). Different letters within columns represent significantly differences (LSD Test; P = 0.05)

Efficacy Field Trials – Alfalfa Weevil Population Estimates**.** The effects of insecticide treatments on percent reduction in AW, eggs / stem, AW larvae developmental stage were measured in three field sites in 2006 and 2009. The impact of insecticide treatments upon all response variables were evaluated by sample date at all field sites due to significant date by treatment interactions ($P < 0.05$).

The 2006 and 2009 Bozeman sites had a peak AW density of 7.8 and 13.9 larvae / sweep on JD 177 and JD 182, respectively. This was well below the economic threshold of 20 larvae / sweep (Blodgett 1996), however AW densities in untreated plots at the

Huntley 2009 site increased past the economic threshold on JD 155 and JD 162, with 23.0 and 28.3 larvae / sweep, respectively.

Significant differences in percent reduction of AW larvae were found among treatments in post application sample dates at all field sites ($P < 0.05$). Diflubenzuron, azadirachtin and kaolin reduced AW densities across three field sites by as much as 23.6 ± 2.7%, 25.0 ± 9.0% and 30.3 ± 10.9%, respectively; however reductions were below lambda cyhalothrin applications that reduced densities by as much as 99.7 ± 1.3% across field sites. Kaolin, diflubenzuron and azadirachtin treatments caused low AW mortality and never reduced AW larvae densities to that of the lambda cyhalothrin treatment at any field site or year. Novaluron applications significantly reduced AW larval densities by 44 ± 15.0% across field sites, with reductions equaling that of the lambda cyhalothrin treatment at the Bozeman 2006 field site ($P < 0.0001$). Novaluron treated plots had the highest AW mortality at the Bozeman 2006 and Huntley 2009 sites, with a peak of 74% on JD 170 (13 d post) at the Bozeman 2006 site, and peak of 27% at the Huntley site on JD 162 (20 d post). At the Bozeman 2009 site, azadirachtin treated plots had the highest AW larval mortality at 13 DAT, with a mean of 42% at JD 182 ($F = 31.52$; df = 8, 15; $P < 0.0001$). No experimental insecticide application increased mortality > 90%, as was observed in the lambda cyhalothrin treated plots (Table 2.1).

Alfalfa weevil larvae developed at different rates between pesticide treatments at the Huntley site on JD 155 ($F = 7.39$, df = 7, 10, $P = 0.003$) and JD 162 (9.15, df = 7, 10, $P = 0.002$). On JD 155 and JD 162 novaluron treated plots contained larvae that were significantly less developed (instar index = 2.3 and 3.2, respectively) when compared to

Table 2.1: Percent reduction in alfalfa weevil larvae / sweep ± SE after treatment with various pesticides at various Julian Dates (JD).

Field	Treatment	Rate (gai/ha)	% AW Reduction			
2006 Bozeman			JD 157[a]	JD 164[a]	JD 170	JD 177
	Diflubenzuron	22.7	26 ± 12	26 ± 16	29 ± 14*	21 ± 8*
	Azadirachtin	7.8	-	0 ± 0	16 ± 16	22 ± 16
	Novaluron	31.0	20 ± 17	51 ± 14	74 ± 3*	62 ± 8*
	Kaolin	6,544.6	3 ± 1	24 ± 10	48 ± 12*	52 ± 4*
	λ cyhalothrin	5.5	-	0 ± 0	92 ± 2*	95 ± 4*
	F- Statistic		1.00	1.65	18.72	16.59
	df(model, error)		6, 9	8, 15	8, 15	8, 15
	P – value		NS	NS	0.0001	0.0001
2009 Huntley			JD 142	JD 147[a]	JD 155	JD 162
	Diflubenzuron	22.7	0 ± 0	32 ± 16	10 ± 8	21 ± 12*
	Azadirachtin	7.8	-	0 ± 0	8 ± 8	11 ± 5
	Novaluron	31.0	0 ± 0	5 ± 4	22 ± 20	27 ± 16*
	Kaolin	6,544.6	0 ± 0	0 ± 0	0 ± 0	18 ± 12
	λ cyhalothrin	5.5	-	0 ± 0	87 ± 3*	99 ± 2*
	F - Statistic		0.87	3.09	9.34	19.3
	df(model, error)		5, 6	7, 10	7, 10	7, 10
	P – value		NS	NS	0.001	<0.0001
2009 Bozeman			JD 162	JD 169[a]	JD 176	JD 182
	Diflubenzuron	22.7	27 ± 18	0 ± 0	21 ± 8*	12 ± 7
	Azadirachtin	7.8	-	0 ± 0	39 ± 9*	42 ± 4*
	Novaluron	31.0	27 ± 16	14 ± 7	21 ± 8*	31 ± 10*
	Kaolin	6,544.6	12 ± 5	11 ± 11	14 ± 10*	21 ± 9*
	λ cyhalothrin	5.5	-	0 ± 0	99 ± 2*	98 ± 3*
	F - Statistic		0.97	1.45	19.87	31.52
	df(model, error)		8, 15	8, 15	8, 15	8, 15
	P – value		NS	NS	<0.0001	<0.0001

*Means within columns followed by * are significantly different than the untreated (LSD Test after square root arc-sine transformation; *P=0.05*).
[a] Shaded areas represent date of applications.

larvae within untreated plots (instar index = 2.6 and 3.8, respectively). Azadirachtin treated plots also contained larvae which matured slower than larvae from the untreated plots on JD 162 (15 DAT) in Huntley, 2009. On this date azadirachtin treated plots contained larvae with a mean instar index of 3.2 compared to untreated plots which

contained larvae with a mean instar index of 3.8 (Figure 2.3). Significant differences in eggs / stem were not present between insecticidal treatments at any field site (P>0.05).

The Bozeman 2006 site had peak 0.8 ± 0.3 eggs / stem on JD 143, Huntley 2009 site had a peak 0.4 ± 0.1 eggs / stem on JD 147 and the Bozeman 2009 site had a peak 0.4 ± 0.3 eggs / stem on JD 162.

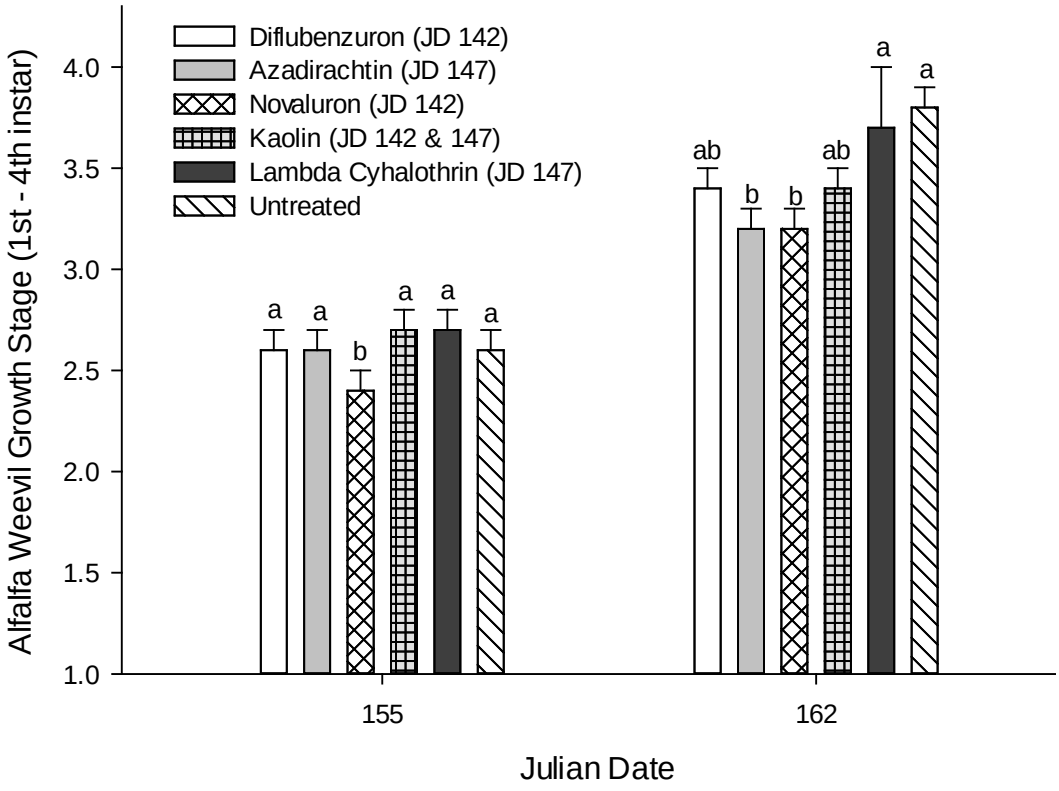


Figure 2.3: Alfalfa weevil growth stage ± SE (1^{st} – 4^{th} instar) at various Julian Dates after insecticide applications in forage alfalfa in Huntley, 2009 (LSD Test; *P = 0.05*). Application dates are shown in parenthesis in legend.

Field Comparison of Larval Development & Crop Development**.** Larvae matured at an earlier alfalfa developmental stage in untreated plots at the Huntley site (MSC of 1.0) compared to the Bozeman 2006 and Bozeman 2009 site (MSC 2.0 – 3.0) at the early

larvae application dates of JD 157, JD 142 and JD 162, respectively. On this date, alfalfa development varied by site, from a range of 46 and 54 cm / stem in untreated plots at the Bozeman 2006 and Bozeman 2009 sites, respectively, to 24 cm at the Huntley site.

By JD 162 at the Huntley 2009 site, AW matured to an instar index of 3.8 compared to an instar index of 2.8 at either Bozeman site at JD 177 and JD 182. Regressions of alfalfa rate of maturity by AW degree days indicate significantly more developed alfalfa when compared to AW DD development within either Bozeman site when compared to the Huntley 2009 site (Figure 2.4; 95% CI). The slower rate of alfalfa development in the Huntley site versus degree days enabled later instar AW larvae more time to damage alfalfa plots prior to the ideal cutting stage (for the beef cattle industry in Montana) at early flowering, MSC 5.0 (Cash et al. 1995).

Efficacy Trials – Agronomic Measurements. The effects of insecticide treatments on AW leaf defoliation, alfalfa stem height, and cutting weight were measured at three field sites in 2006 and 2009. The impact of insecticide treatments upon all response variables were evaluated by sampling date at all field sites due to significant date by treatment interactions ($P < 0.05$).

Alfalfa weevil leaf defoliation ratings (0-3) at the Bozeman 2006 and Huntley 2009 sites rose steadily from an LDI of 0.0 ± 0.0 initially in the untreated plots to a mean LDI of 2.2 ± 0.2 and 2.7 ± 0.3 by the JD 177 and JD 162 sample dates, respectively. Untreated alfalfa at the Bozeman 2009 site had little AW damage by the final sample date (LDI of 0.3 ± 0.3). There were significant differences in LDI between pesticide

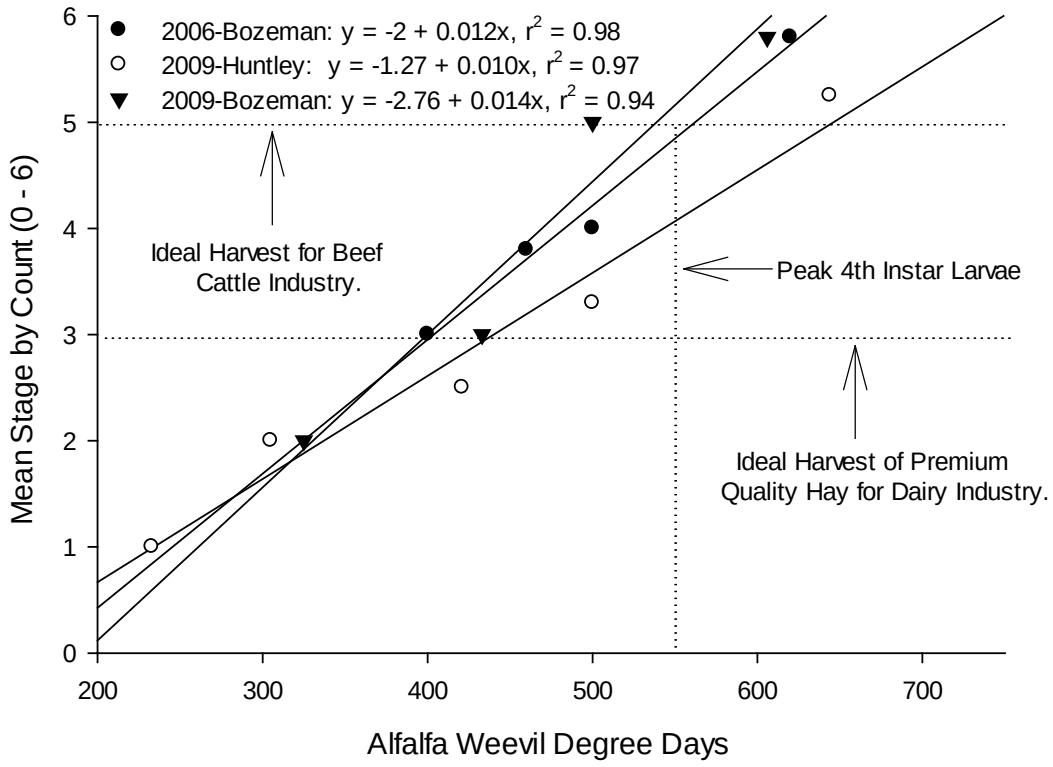


Figure 2.4: Regressions of forage alfalfa growth stage (MSC) versus alfalfa weevil degree days in untreated plots across three fields from 2006 – 2009.

treatments on the last two sample dates at the Bozeman 2006 and Huntley 2009 sites ($P <$ 0.0003). At the Bozeman 2006 site, plots treated with novaluron had significantly reduced AW leaf defoliation from an LDI of 2.2 in the untreated plots compared to an LDI of 1.1 in the novaluron plots on JD 177 ($F = 41.66$, df = 8, 15, $P < 0.0001$). At the Huntley site, plots treated with novaluron and diflubenzuron significantly reduced AW leaf defoliation compared to that of the untreated on JD 162 ($F = 22.42$, df = 7, 10, $P <$ 0.0001). At this site on this date, novaluron treated plots had an LDI of 1.0 ± 0.1; diflubenzuron treated plots had an LDI of 1.7 ± 0.3; while untreated plots had an LDI of 2.5 ± 0.3 (Figure 2.5). Leaf defoliation within the novaluron treated plots was not significantly different than the lambda cyhalothrin treated plots at the Huntley 2009 field

site on JD 162. Significant differences in leaf defoliation between treated plots were not present at any sample date at the Bozeman 2009 site ($P > 0.05$).

In Montana, producers may wish to harvest at the early bud stage (MSC 3.0) to optimize hay quality (>20% crude protein, <30% ADF, <40% NDF; Cash et al. 1995) for marketing premium quality hay to the dairy market. The regression of alfalfa stage of development versus AW growth rate shows early harvest (MSC 3.0) to correlate with the first sample date (JD 157) at the Bozeman 2006 site; the second sample date (JD 169) at the Bozeman 2009 site; and between the 3rd and 4th sample date (JD 155 – 162) at the Huntley 2009 site (Figure 2.4). Our studies demonstrate novaluron to protect fields from AW damage equal to that of the lambda cyhalothrin treatment across field sites if harvested at the early bud stage sample dates (Figure 2.5). Harvesting at this stage will either precede AW damage (Bozeman 2006 and 2009 field sites), or reduce AW damage significantly from the untreated plots and equal to the lambda cyhalothrin treatment if combined with a novaluron application (Huntley 2009 field site).

Significant differences in cutting weight (kg/ha) were not present between insecticidal treatments at any field site with a yield range of 6,381 – 8,252 kg/ha within untreated plots across field sites ($P > 0.05$). However there were significant differences in alfalfa stem height present between pesticide treatments on JD 162 in the Huntley 2009 study ($F = 9.99$, df = 7, 10, $P = 0.001$). On this date, alfalfa stems in the novaluron and lambda cyhalothrin treated plots were longer (83 – 87 cm) than stems within the untreated plots (70 cm). Lengths of stems within novaluron treated plots were not significantly different than length of stems within lambda cyhalothrin treated plots.

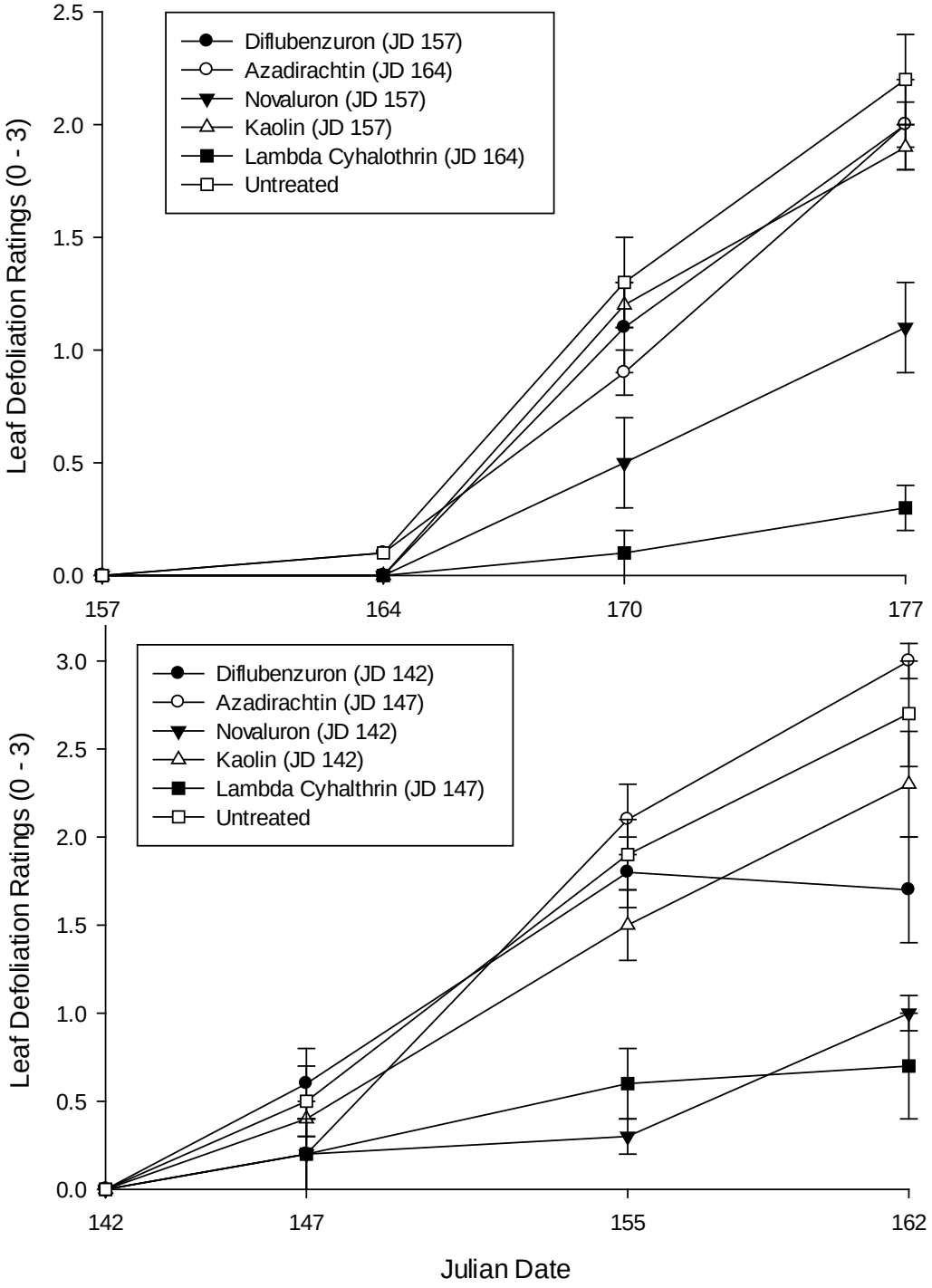


Figure 2.5: Alfalfa weevil leaf defoliation ratings where 0 = no leaf defoliation, 1 = 1 – 25%, 2 = 26 – 75% and 3 is > 75% leaf defoliation. Forage alfalfa was treated with various pesticide formulations under field conditions. **Top.** Bozeman 2006 field site. **Bottom.** Huntley, 2009 field site. Application dates are shown in parenthesis in legend.

Greenhouse Investigation of Top Performing Insecticide

The effects of insecticide treatments on AW mortality, leaf defoliation, percent displaced AW larvae, alfalfa stem height and cutting weight were measured in two greenhouse trials in 2010. The impact of insecticide treatments upon all response variables were evaluated by sample date in each greenhouse trial due to significant date by treatment interactions in either greenhouse trial ($P < 0.05$).

Significant differences in AW mortality were present ($P < 0.05$) among novaluron treatments at seven and 14 d after treatment (DAT) in two greenhouse trials. Alfalfa weevil mortality on novaluron treated plants on 7 DAT, although low in greenhouse trial #1 (23 ± 8) and greenhouse trial #2 (14 ± 7%), was significantly different than AW mortality compared to the untreated control in trial #1 ($F = 48.04$, df = 7, 10, $P < 0.0001$) and trial #2 ($F = 121.71$, df = 7, 10, $P < 0.0001$). These significant differences extended to 14 DAT in trial #1 ($F = 44.12$, df = 7, 10, $P < 0.0001$), but not in trial #2.
In trials #2, untreated AW larvae mortality increased to 70% by 14 DAT due to lack of adequate biomass for AW larvae to feed. Leaf defoliation on untreated plants quickly rose to 1.0 by one DAT, and peaked at 3.0 by 14 DAT in either trial (Figure 2.6).

On 14 DAT, some biomass remained within trial #1 untreated pots, while 100% defoliation was observed in untreated pots in trial #2. Leaf defoliation in enclosures was significantly different between treatments ($P < 0.05$) at all post application dates in either greenhouse trial. Novaluron treated plants had significantly less feeding damage when compared to the untreated in either greenhouse trial (LDI = 1.8, 2.7, respectively, for trial #1 on 7 DAT; LDI = 1.4, 2.1, respectively, for trial #2 on 3 DAT; Figure 2.6).

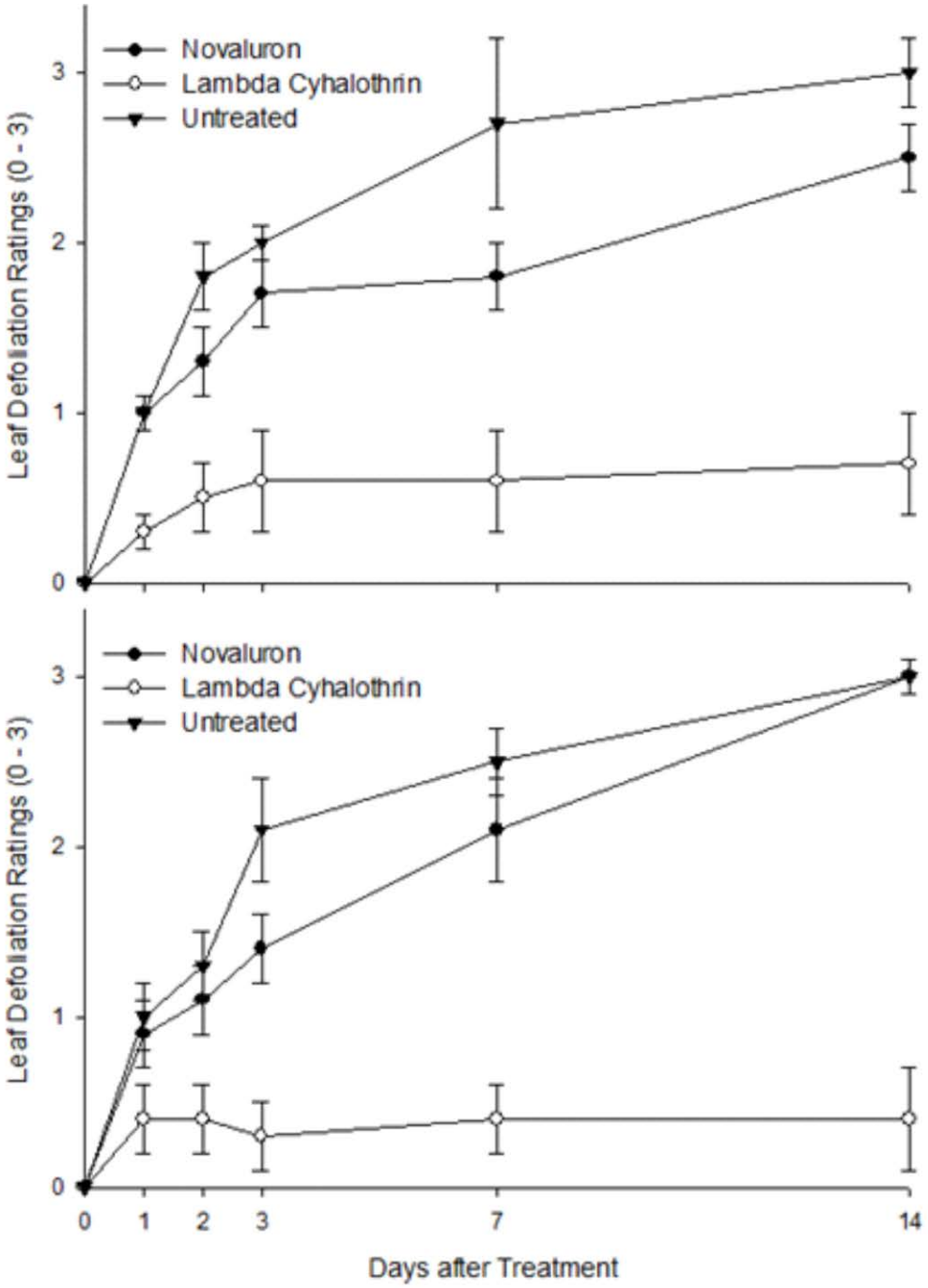


Figure 2.6: Alfalfa weevil leaf defoliation index (LDI) ratings where 0 = no leaf defoliation, 1 = 1 – 25%, 2 = 26 – 75% and 3 is > 75% leaf defoliation. Forage alfalfa was treated with novaluron and lambda cyhalothrin at Montana State University, Bozeman, Montana. **Top**. Greenhouse trial #1. **Bottom.** Greenhouse trial #2.

Novaluron treatments contained a significantly higher proportion of AW larvae wandering off plant in enclosures compared to the untreated from 2 to 14 DAT ($P < 0.05$). Novaluron treatments resulted in 17% and 19% displaced larvae by 2 DAT in greenhouse trial #1 and #2, respectively, compared to significantly less AW larvae in the untreated with 1% and 4% mortality, respectively. These statistical trends continued through the duration of each greenhouse trial (Table 2.2).

Table 2.2: Percent alfalfa weevil (AW) larvae wandering off alfalfa stems ± SE at various days after treatment (DAT) after forage alfalfa was treated with insecticidal treatments under greenhouse conditions at MSU, 2010.

Trial	Treatment	Rate	% AW Larvae Wandering Off Plant				
		gai/ha	1 DAT	2 DAT	3 DAT	7 DAT	14 DAT
Trial #1	Novaluron	31.0	6 ± 3a	19 ± 3b	25 ± 2b	30 ± 5b	25 ± 4b
	λ cyhalothrin	5.5	7 ± 3a	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a
	Untreated		1 ± 1a	1 ± 1a	0 ± 0a	0 ± 0a	0 ± 0a
	F- Statistic		1.96	59.34	428.82	132.24	114.39
	df(model, error)		7, 10	7, 10	7, 10	7, 10	7, 10
	P - value		NS	<0.0001	<0.0001	<0.0001	<0.0001
Trial #2	Novaluron	31.0	4 ± 2a	17 ± 4b	25 ± 3b	29 ± 3b	14 ± 5b
	λ cyhalothrin	5.5	0 ± 0a	1 ± 1a	1 ± 1a	0 ± 0a	1 ± 1a
	Untreated		4 ± 2a	4 ± 3a	4 ± 3a	0 ± 0a	0 ± 0a
	F - Statistic		2.14	6.17	16.07	346.30	11.87
	df(model, error)		7, 10	7, 10	7, 10	7, 10	7, 10
	P - value		NS	0.02	0.0008	<0.0001	0.002

*Means within columns followed by * are significantly different than the untreated (LSD Test after arc-sine, square root transformation; *P=0.05;* Data presented is not transformed).

There were significant differences in final stem length and final cutting weight among treatments in greenhouse trial #2 while there were significant differences in only final cutting weight among treatments in greenhouse trial #1 (Table 2.3). In greenhouse trial #2 lambda cyhalothrin treated stems were significantly longer than untreated stems, however novaluron treated stems were not significantly different than the untreated.

Plants treated with novaluron weighed significantly more than untreated plants at 14 DAT in either greenhouse trial. On this date novaluron treated pots contained 2.7 to 3.4 g of biomass / pot, while untreated pots contained 2.2 to 2.4 g / pot in greenhouse trial #1 and #2, respectively (Table 2.3).

Table 2.3: Biomass (grams) ± SE and final plant height ± SE 14 d post application after forage alfalfa was treated with novaluron and lambda cyhalothrin in two greenhouse trials, MSU, Bozeman, MT.

		Greenhouse Trial #1		Greenhouse Trial #2	
Treatment	Rate (gai/ha)	Plant Ht (cm)	Biomass (g)	Plant Ht (cm)	Biomass (g)
Novaluron	31.0	34.4 ± 1.5a	3.4 ± 0.3b	28.9 ± 2.0ab	2.7 ± 0.2b
λ cyhalothrin	5.5	36.0 ± 2.0a	3.5 ± 0.2b	31.9 ± 2.0b	2.9 ± 0.2b
Untreated		29.4 ± 1.7a	2.4 ± 0.3a	22.7 ± 1.8a	2.2 ± 0.1a
F – Statistic		3.44	5.52	5.35	12.88
DF (model, error)		7, 10	7, 10	7, 10	7, 10
P-value		NS	0.02	0.02	0.001

*Treatments with similar letters within columns are not significantly different (LSD Test; *P = 0.05*).

Discussion

Evaluations of Optimum Timing of Application

The most effective application timed to conincide with vulnerable life stages of a pest was considered. Timing of application of three insect growth regulators and the particle barrier film, kaolin, were assessed in field trials in 2006.

The timing of kaolin and novaluron applications effected AW larvae densities in field trials. Alfalfa weevil larvae were decreased by approximately 58% when kaolin was applied weekly or applied on larval stages compared to application that targeted only adult AW. The decrease in AW from this inert particle barrier film may be due to

mortality as a result of larval starvation. Observations of AW larvae dropping to ground after struggling to move on kaolin treated foliage were noted numerous times in this investigation. Previous studies have demonstrated kaolin to cause larval starvation due to inhibiting movement, limiting olfactory cues or through blocking of hind-gut after ingestion in many insect species (Knight et al. 2000; Showler 2003; Barker et al. 2006). This likely resulted in AW larvae dropping to the ground as a result of starvation or in an attempt to search for a more preferred food source.

Although weekly applications of kaolin performed equally well when compared to applications targeting only AW larvae, weekly applications of kaolin particle film are often undesirable because of labor and fuel costs as well as soil compaction from vehicle traffic (Showler 2002). Two consecutive applications of kaolin targeting only early and late AW larvae are superior to more costly weekly applications or applications targeting AW larvae and adults.

Early applications of novaluron targeting emerging larvae are superior to later applications. This study demonstrated that novaluron applications targeting second to third instar AW larvae resulted in increases in AW leaf defoliation when compared to earlier applications targeting emerging AW larvae. This may be due to reductions in AW larvae noted only after two weeks of application with either application timing. The delayed action of insect growth regulators, including novaluron, has been noted in many previous investigations (Ishaaya et al. 2003; Hodgson et al. 2010; Kamal & Khater 2010). The two week delay of novaluron in reducing AW larval densities indicates earlier treatment timings are superior due to cumulative increases in AW larval feeding damage

with later applications. Lopez et al. (2008) demonstrated greater mortality towards early instar nymphs of southern green stink bug, *Nezara viridula* (Linnaeus), while Hodgson et al. (2010) reported lower rates of survival when early instar leaf cutting bees, *Megachile rotundata* (Fabricius), were exposed to novaluron compared to later instars.

The timing of azadirachtin and diflubenzuron applications caused little effect on performance. This was likely due to a minimal reduction in AW larval densities, egg deposition, or leaf defoliation regardless of timing of application in this study. The low efficacy observed in this trial by azadirachtin and diflubenzuron makes it difficult to choose optimum application timings. Diflubenzuron has significant ovicidal and insect growth regulating properties against a wide range of insect pests, while azadirachtin acts as a feeding deterrent, ecdysone disruptor and causes direct histopathological effects (Aerts & Mordue 1997; Villavoso et al. 1995). Many investigations have shown azadirachtin and diflubenzuron to be more toxic towards earlier instar larvae than later instar larvae when targeting a variety of other insect pests including semilooper, *Achaea Janata* (Linnaeus), tobacco leaf eating caterpillar, *Spodoptera litura* (Fabricius), sweetpotato whitefly, *Bemisia tabaci* (Gennadius) and root weevil, *Diaprepes abbreviatus* (Kadam et al. 1995; Mule & Patil 2000; Weathersbee & Tang 2002; Kumar et al. 2005). Slight increases in performance may be possible with an early larvae or adult application; however this study indicated no realistic advantage of using any timing over the other when managing AW larvae.

Evaluations of Insecticide Efficacy

Insects may be affected by pesticides in a variety of ways. Insects may be directly killed by pesticide exposure or undergo a variety of sub-lethal effects including but not limited to behavioral changes, growth development delay or feeding deterrence. We have evaluated the toxicity, rate of development and feeding deterrence of three insect growth regulators and the particle barrier film, kaolin, against AW.

Kaolin. Foliar applied treatments of kaolin provided little protection from AW larvae. An 18 to 52% reduction in AW was observed across field sites by 21 DAT with little reduction in AW damage. This reduction seems largely due to AW larvae finding it difficult to move through this inert particle barrier; larvae were hindered by kaolin particles attaching to body in each of our experiments. Kaolin is thought to function largely as a physical barrier or irritant (Glenn et al. 1999). A previous study by Sackett et al. (2005) indicated little direct mortality from *Choristoneura rosaceana* (Lepidoptera: Tortricidae) larvae feeding on kaolin, however 120 of 200 larvae fell off plants within one hr of exposure to kaolin treated plants. Although Sackett et al. (2005) indicated a high reduction of AW immediately, they also noticed many of the roaming larvae re-establishing on untreated plants nearby. It is likely that AW reductions in our study are a result of AW larvae unable to re-establish on alfalfa plants after dropping off plants.

Kaolin seemed to offer little protection from AW, however efficacy may be improved. We observed a reduction in the kaolin clay particulate residue underneath the alfalfa canopy when compared to the residue on more exposed portions of the alfalfa plant. This was due to a dense alfalfa canopy which is difficult for pesticides to penetrate,

especially for pesticides such as kaolin that must be applied with nozzles delivering large droplets (Bach 1985; Gohlich 1985). Although kaolin seems to irritate AW larvae and force larvae to search for more palatable food sources, they may easily re-establish beneath the protected plant canopy and continue to damage relatively untreated alfalfa plants. Previous studies have indicated higher spray volumes may increase penetration of spray applications into dense plant canopies (Bach 1985; Gohlich 1985). We did increase output of our applications to 378 liters/ha, however spray output recommendations for kaolin range as high as 934 liters/ha (Surround WP Engelhard Corporation). Equipment that can deliver much higher spray outputs may be desirable to penetrate a dense alfalfa canopy. More research is needed to indicate whether higher spray volumes of kaolin may manage AW larvae populations.

Azadirachtin. Foliar applications of azadirachtin caused little mortality towards AW, and offered little protection from AW larval feeding damage. A peak 11 – 42% reduction in AW larvae was observed across field sites in our investigations. This was comparable to a study by Yardim et al. (2001) that demonstrated applications of azadirachtin to reduce AW by only 45.2 to 50.2%. Even with little reduction in AW, Yardim et al. (2001) indicated azadirachtin to be of potential value in an IPM program if economic benefits in yield could be obtained. This may be due to azadirachtin's activity as a feeding deterrent in many insects at sub-lethal doses (Aerts & Mordue 1997; Aliniazee et al. 1997). Our study indicated that applications of azadirachtin caused no reduction in AW leaf defoliation, or any net gain in yield. Lack of azadirachtin's activity

as a feeding deterrent has also been noted by Cowles (2004) on the closely related vine weevil, *Otiorhynchus sulcatus* (Fabricius).

Azadirachtin treated insects did show a notable delay in development, however little larval mortality was observed. Azadirachtin causes growth disruption through its effect on ecdysteroid and juvenile hormone titers (Aerts & Mordue 1997) that may result in growth delay without mortality, or mortality from molting aberrations at the larval or pupal stages. A delay in development was also noted by Aerts & Mordue (1997) when *Spodoptera* larvae treated with azadirachtin entered pupation later than untreated larvae. They indicated that azadirachtin treatments later resulted in blockage of development in the pupal stage, death during molt into the adult phase or emergence of adults with deformations. Many investigations have found azadirachtin to cause toxicity at the pupal stage of development even when applications are upon early instar larvae (Medina et al. 2003; Aerts & Mordue 1997). In our investigation, either azadirachtin is causing delays in development without causing any mortality, or azadirachtin is delaying development and will cause mortality at the pupal stage of development. Pupal mortality, if present, wouldn't protect an alfalfa crop from the most damaging larval developmental stage. The lack of activity as a feeding deterrent combined with low toxicity at the larval stage make azadirachtin a poor choice for IPM programs targeting AW.

Diflubenzuron. Diflubenzuron treated plots caused a slight reduction in AW (21 to 29%) in all field sites, and a feeding deterrent effect was noted. Alfalfa weevil feeding deterrent effect was also noted by Braithwaite et al. (1976). Braithwaite et al.'s (1976) investigation never showed a drastic reduction in AW larvae populations but did show a

protective effect from AW larvae feeding damage with applications of diflubenzuron. They further discussed the possibility of an anti-feeding mechanism. Our study confirms these findings but indicates that feeding reductions may vary from site to site. Leaf defoliation reductions were observed only when AW densities exceeded the economic threshold in one of our field sites, thus allowing for clear comparisons between treatments. Although leaf defoliation reductions were noted with the application of diflubenzuron when densities exceeded economic thresholds, yield was not different than the untreated. This contrasts with results by Braithwaite et al (1976), when they demonstrated applications of diflubenzuron to significantly increase alfalfa yields from that of the untreated. This may be due to Braithwaite (1976) evaluating diflubenzuron applications that were repeated three times every ten days, while our study evaluated only one application of diflubenzuron. Repeated applications of diflubenzuron may provide additional protection of alfalfa from feeding damage, however increases in soil compaction and application costs may further limit the practical use of this pesticide for managing AW. Due to only slight reductions in AW larvae and leaf defoliation, diflubenzuron may be of limited use in an IPM program targeting AW larvae.

Novaluron. Foliar applied treatments of novaluron provided increased AW control compared to the untreated and other alternative treatment strategies. This was due to novaluron reducing AW larval populations by as much as 74% while also reducing feeding damage significantly in two of three field sites. Alfalfa weevil feeding damage within novaluron treated plots was reduced to levels equal to the standard, lambda cyhalothrin, in one of the three sites. Feeding reductions in field studies were likely due

to direct reductions in AW and acting as a feeding deterrent towards surviving larvae. Feeding reductions and other behavioral changes have been noted with a similar insect growth regulator, diflubenzuron (Braithwaite et al 1976; Villavosa et al. 1995). Applications of diflubenzuron resulted in decreased flight activity in *Anthonomus grandis grandis* (Boheman), the boll weevil (Villavaso et al. 1995), while Braithwaite et al. (1976) noted protection from AW feeding damage from applications of diflubenzuron. Diflubenzuron and novaluron both act as novel benzoyl phenyl urea compounds that act as chitin synthesis inhibitors. Though the literature describes novaluron to have only ovicidal and larvicidal properties, our study suggests that novaluron may be further acting as a feeding deterrent similar to diflubenzuron.

This feeding deterrence was further verified in greenhouse studies. Leaf defoliation was reduced on novaluron treated plants from 3 to 7 DAT, however this was temporary. By 14 DAT plants were no longer protected by applications of novaluron. Leaf defoliation was likely reduced by 30% in these trials due to reduced AW densities from direct mortality and remaining larvae observed not feeding while searching for a more palatable food source. This was verified on 7 DAT in greenhouse trials when 29% and 30% (greenhouse trial #1 and #2, respectively) of AW larvae were observed wandering off plant searching for an alternative food source. The difficulty in AW larvae allocating an acceptable food source may be due to novaluron inducing an unpalatable food source, antagonism with chemoreceptors from incomplete cuticle formation or an indirect effect upon host search patterns induced from morbidity. Low levels of mortality

combined with a significant leaf feeding deterrence increased cutting weight from that of the untreated in greenhouse trials.

Novaluron may be an effective management tool if combined with early harvest strategies due to the temporary action as a feeding deterrent. Our plots were harvested at approximately early bloom (MSC 5.0) as this optimizes forage yield and quality for beef production; however harvest upon earlier growth stages of alfalfa may maximize the temporary benefits of novaluron as a feeding deterrent. Cash & Bowman (2002) indicated that established stands can withstand one cutting at the mid-bud stage (MSC 3.5) with little loss in seasonal yield or quality. Producers with consistent AW populations may consider the advantage of novalurons temporary feeding detterant effect by harvesting premium quality hay (>20% crude protein, <30% ADF, <40% NDF) at the early bud stage (MSC 3.0) in Montana as recommended by Cash et al. (1995). Our field studies demonstrate novaluron to protect alfalfa from AW larvae damage equal to that of the lambda cyhalothrin treatment if alfalfa was harvested at the early bud stage. Our results demonstrate that harvesting at this stage will either precede AW damage (Bozeman 2006 and 2009 field sites), or reduce AW damage significantly from the untreated plots and equal to the lambda cyhalothrin treatment if combined with a novaluron application (Huntley 2009 field site). Using novaluron with early cutting may be a preferred tool for managing AW due to its potential for reducing impacts on predators and parasitoids compared to conventional broad-spectrum pesticide treatments. At a field site in 2010, Tharp et al. (Chapter 3) observed minimal impact towards lady bird beetles, damsel bugs and parasitoids in the first harvest cycle in addition to a significant but low reduction in

AW larvae ($P < 0.05$; 22% reductions). Our studies demonstrate applications of novaluron may protect fields even when low reductions of AW larvae are noted. Future research should focus on novaluron in combination with early harvest strategies; and if the preservation of natural enemies will reduce second generation AW larvae in areas with multi-voltine populations.

Summary

All foliar applied treatments reduced AW larval densities; reductions were not equal to the standard, lambda cyhalothrin. The most promising chemical evaluated was novaluron due to a significant feeding deterrent effect noted repeatedly in field and greenhouse trials. Novaluron may be an excellent alternative for managing AW larvae due to low toxicity towards mammals (LD50 > 5000 mg/kg) while being non-toxic towards birds, earthworms, most beneficial arthropod predators/parasitoids, and soil microflora (Ishaaya et al. 2001; Ishaaya and Horowitz 2002; Kostyukovsky & Trostanetsky 2006). It should be noted that Hodgson et al. (2010) reported 84% of alfalfa leaf cutting bee (*Megachile rotundata*) progeny died when females were allowed to mate and nest 24 h after a novaluron application. This may limit novaluron's use in the alfalfa seed industry; however it may still be a useful pre-bloom management tool in forage alfalfa systems. Future studies may wish to evaluate novaluron in combination with early harvest to maximize benefits.

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CHAPTER 3

IMPACTS OF THREE INSECT GROWTH REGULATORS AND THE PARTICLE BARRIER FILM, KAOLIN, ON NATURAL ENEMIES OF ALFALFA WEEVIL, *HYPERA POSTICA* (GYLLENHAL) AND SECONDARY PEST, PEA APHID, ACYRTHOSIPHON PISUM (HARRIS)

Abstract

Field investigations were conducted in Montana to evaluate the impacts of the insect growth regulators novaluron, diflubenzuron, azadirachtin and the particle barrier film, kaolin, on natural enemies of alfalfa weevil, *Hypera postica* (Gyllenhal), and the secondary pest, pea aphid, *Acyrthosiphon pisum (Harris*). All chemistries provided some pre-harvest benefits to the predator-alfalfa weevil and predator-pea aphid complex at various field sites; novaluron treatments provided significantly higher predator-alfalfa weevil ratios consistently across four of five field sites when compared to the synthetic pyrethroid, lambda cyhalothrin ($P < 0.05$). At these four field sites, novaluron treated plots harbored an average predator-alfalfa weevil ratio of 0.15 ± 0.07 compared to 0.02 ± 0.02 in lambda cyahlothrin treated plots in the first harvest cycle. In two larger scale studies novaluron applications resulted in statisticaly significant but low reductions in alfalfa weevil at the first harvest cycle (22.0 ± 1.0%); however alfalfa weevil densities were not suppressed in the second harvest cycle *(P* = 0.05). Novaluron application reduced pea aphid populations by only 3.0 ± 0.2% across five field sites; but conserved lady beetles (Coccinellidae) and damsel bugs (Nabidae) compared to the synthetic pyrethroid treatment. Parasitism rates were also decreased to 2.4 ± 1.1% with the

application of lambda cyhalothrin compared to 16.6 ± 2.2% with the application of novaluron ($P < 0.05$). By preserving parasitoids and benefiting the predator-alfalfa weevil ratio novaluron applications may limit pest outbreaks of pea aphids while and second generation alfalfa weevil populations in subsequent harvest cycles.

Introduction

Alfalfa (*Medico sativa* [L.]) harbors a wide array of beneficial insects that are negatively impacted by broad spectrum insecticide applications used to manage alfalfa weevil (AW, *Hypera postica* [Gyllenhal]; Harper 1978; Summers 1998). Reductions in predator / parasitoid complex from broad spectrum insecticide applications can lead to secondary pest outbreaks of pea aphids, *Acyrthosiphon pisum* (Harris), or recurring AW outbreaks that increase long term management costs (Evans & Karren 1993; Summers 1998). Selective chemistries to manage AW that reduce impacts on beneficial insects are needed to replace broad-spectrum insecticides that currently are used on 34% of the total alfalfa acres sprayed annually across the U.S. (Bailey 1994).

Reduced risk chemistries that are organically approved may also be used for the growing organic hay market that supplies the dairy and beef industries (Guerena & Sullivan 2003; Fuerst et al. 2009). Organic hay in the U.S., predominantly pure alfalfa stands, has increased from 46,980 ha harvested in 2001 to 103,680 ha harvested in 2008 (USDA 2012).

Alfalfa is a perennial plant that has been grown as a forage crop since the beginning of recorded history, originating in the vicinity of present day Iran and brought to North America in the early 1700's (Whyte et al. 1953; Wilsie 1962; Lacefield et al.

1997). It is the foremost crop in many semi-arid and temperate states in the US, with 51.8 metric tons produced in 2013. In 2013, Montana farmers produced 3.56 million metric tons of alfalfa hay with a value of $558 million; Montana is ranked 3rd nationally in 2013 (NASS 2014). Alfalfa is a high quality feed for livestock that is easily digested, low in neutral fibers and high in protein (Conrad and Klopfenstein 1988). It is considered the most useful forage legume used as animal feed (Abdel Magid 1983), and a critical component to the dairy, beef (*Bos* spp.), sheep (*Ovis* spp.), horse (Equus spp.), swine (*Sus* spp.), and poultry (*Gallus* spp.) industries (Van Keuren & Matches 1988).

Alfalfa weevil is the most damaging pest of forage alfalfa in the U.S. (USDA APHIS 1991). Larvae feed on buds, stems, and leaves of alfalfa, thus stunting the plant, reducing yields, and lowering nutritional value. Thirty larvae / 0.33 m^2 will cause approximately 31 kg / ha loss in hay at cutting. Higher densities have been reported to cause losses of up to 367 kg / ha, thus causing a complete loss in many first cuttings, and seriously lowering yields in the second cutting (Higgens et al. 1989).

The pea aphid is found throughout North America and is a pest on legume crops including peas, clovers, and alfalfa. This pest is the most common aphid in Montana and Utah alfalfa production systems (Hodgson 2007) with infestations causing alfalfa to turn yellow and wilt under extremely high densities thus significantly decreasing cutting yield. Cuperus et al. (1982) indicated the economic threshold to be 75 pea aphids / sweep two weeks prior to harvest.

The preservation of natural enemies in conjunction with reduced risk pesticides shows promise as a more sustainable approach to pest management. Success has been

reported in the literature regarding the use of Hymenopteran parasitoids in managing AW. Flanders (2000) reported parasitism by the Hymenopterans, *Microctonus aethiopoides* (Druso) and *Bathyplectes spp*. to raise AW mortality as high as 80% in Wisconsin and 60% in Minnesota. Parasite releases by the USDA APHIS resulted in alfalfa farmers saving eight million dollars annually due to a 73% reduction in the number of hectares requiring insecticides by 1981 (Kingsley et al. 1993). Reductions in AW from western states has been marginal (Ayedh et al. 1996, Radcliffe & Flanders 1998), with 0 – 20% parasitism reported in Montana (Blodgett 1996), and 2.9 - 7.1% parasitism reported in Colorado (Ayedh et al. 1996). Parasitism rates in Montana and Colorado are not thought to keep high densities of AW from being a threat to the alfalfa crop, but may keep low densities of AW at non-economic levels if used in conjunction with pesticides that pose little risk to AW natural enemies.

In states where parasitoids are not known to manage AW populations, predators are of increasing importance. There are many examples of predators being used in successful biocontrol programs (Hagen et al. 1976; Huffaker et al. 1976; Messenger et al. 1976). Ouayogode & Davis (1981) and Elliot et al. (2002) identified lady beetles (Coccinellids), damsel bugs (Nabidae), and golden-eyed and common lacewings, *Chrysopa oculata* (Say) *& Chrysoperia plorabunda* (Fitch), respectively, as primary predators to AW and pea aphids. Coccinellids have been identified as the most valuable primary predator of either pest in multiple investigations (Yakhontov 1934; Ouayogode & Davis 1981; Elliot et al. 2002). Kalaskar and Evans (2001) demonstrated that many coccinellid species including, *Coccinella septempunctata* (Linnaeus) will target AW

populations only when pea aphid populations are reduced or absent. A predator / prey analysis that considers AW and pea aphids as co-interactive prey is needed to determine a suitable low risk pesticide for managing AW.

Selection of Alternative Insecticides

The EPA reduced-risk pesticide initiative and bio-pesticide and pollution prevention division was created to comply with the 1996 FQPA amendment to FIFRA. This initiative encourages the registration and use of reduced-risk pesticide products (EPA 1997). A reduced-risk pesticide is defined by EPA as controlling pests without posing unreasonable risks to human health or the environment. These chemicals are classified as reduced-risk due to sharing many qualities such as low impact on human health, low toxicity to non-target organisms, low potential for groundwater contamination, low use rates and low resistance potential (EPA 2011).

Some reduced risk pesticides are also labeled for use on organic systems by the Organic Materials Review Institute (OMRI). There are over 2,300 OMRI approved products that are certified organic under the USDA National Organic Program (Organic Material Review Institute 2011), and can be used in the organic alfalfa market.

The OMRI approved active ingredient, azadirachtin**,** was registered as a reduced-risk pesticide by the U.S. EPA in 1985, and was soon registered and approved for pest control in organic systems (Organic Material Review Institute 2011). Azadirachtin has ecdysteroid and juvenile hormone properties as an insect growth regulator (Aertz et al. 1997), while also acting as a stomach poison and feeding deterrent. It has low mammalian toxicity, degrades rapidly in the environment, and shows little harm to

beneficial insects (Lowery et al. 1993). Azadirachtin has shown activity on over 200 species of insects, with high acute toxicity on the European leafroller, *Archips rosana* (Linnaeus), desert locust, *Locusta migratoria* (Linnaeus), whiteflies (Aleyrodidae) and *Aphis* spp., aphids (Lowery et al. 1993; AliNiaZee et al. 1997; Ulrichs et el. 2001; EPA 2012). Studies by Oroumchi (1993) indicated that azadirachtin applied four times at weekly intervals interrupted AW larval development and increased alfalfa yields. Yardim et al. (2001) found azadirachtin lowered populations of AW by 45 to 52% from 1998 to 1999, while studies in Chapter 2 found azadirachtin to reduce AW larvae populations by 25% across three field sites from 2006 to 2009. Beneficials including minute pirate bugs (Anthocoridae), lacewings (Chrysopidae), lady beetles (Coccinellidae), damsel bugs (Nabidae) and bees (Apoidea) were not affected by azadirachtin in previous trials (Yardim et al. 2001; Tharp et al. 2003).

Novaluron, registered by the EPA in 2001, is classified as a reduced-risk pesticide that is also classified as an insect growth regulator (IGR). Novaluron inhibits the normal growth and development of the insect by inhibiting chitin formation, eventually causing death (Cutler 2005). IGR's are relatively safe on adult beneficial insects and the environment. This chemical has been found to be an effective tool used to control whiteflies (Aleyrodidae), thrips (Thysanoptera) and the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), while having low impact on parasites, *Encarsia Formosa* (Gahen) and *Stratiolaelaps scimitus* (Womersley), a soil dwelling predatory mite (Cutler 2005). Previous studies in Chapter 2 found novaluron to reduce AW larvae

populations by as much as 74% at one field site, while significantly reducing feeding damage in two of three field sites and all greenhouse trials.

A similar chemical, diflubenzuron, also acts as an IGR towards insects, specifically as a chitin synthesis inhibitor. This chemical is an important tool in rangeland management of grasshoppers, providing effective long term control if applied at the proper insect growth stage. In addition, this chemical has toxicity against weevils, including citrus weevil, *Diaprepes abbreviates* (Linnaeus), rice water weevils, *Lissorhoptrus oryzophilus* (Kuschel), pepper weevils, *Anthonomus eugenii* (Cano) and *Anthonomus grandis (*Boheman), the boll weevil (Villavaso et al. 1995; Liu 2002; Way 2003), while having minimal impact on natural enemies including bees, predaceous mites, nabids, lady beetles, and damsel bugs (Villavaso et al. 1995; Schroeder et al. 1980; Keever 1977). Studies have indicated diflubenzuron is toxic to AW larvae, but had low mortality in field tests (Chu 1981; Braithwaite et al. 1976). Further study in chapter 2 found diflbenzuron to reduce AW densities by 23.6% in three field trials from 2006 to 2009.

The particle barrier film, kaolin, is considered safe for humans and the environment and is registered as a biopesticide by the EPA. By 2000, kaolin was registered for pest control in organic systems by OMRI. In recent years, the particle film kaolin has been used in integrated pest management programs against a variety of arthropod pests. It has been found to have efficacy against oblique-banded leafrollers, *Choristoneura rosaceana* (Harris), potato leafhoppers, *Empoasca fabae* (Harris), two spotted spider mites, *Tetranychus urticae* (Koch), pear rust mite, *Epitrimerus pyri*

(Nalepa), codling moth, *Cydia pomonella* (Linnaeus), curculio, *Diaprepes*, black pecan aphids, *Melanocallis caryaefoliae* (Davis), citrus root weevil, *Diaprepes abbreviates* (Linnaeus) and boll weevil (Cross et al. 1976; Cottrell et al. 2002; Showler 2002). Studies in chapter 2 found kaolin to reduce AW larvae populations by 30.3% across three field trials from 2006 to 2009.

These pesticides are excellent candidates for further study as alternative approaches for managing AW and secondary pest, pea aphid, while preserving natural enemies in conventional and organic forage alfalfa systems.

Summary

The studies presented were designed to test the impacts of azadirachtin, novaluron, diflubenzuron and kaolin on AW natural enemies and resulting predator / prey relationships of AW and the secondary pest, pea aphid, to determine a viable alternative to traditional insecticides for management of AW. The primary use of these products would be for the alfalfa seed industry and growers wanting organically-approved or integrated management options for AW control. The objectives were to assess survival of prey (AW, pea aphids), survival of predator/parasitoid complex, and resulting predator / prey relationships at various seasonal intervals at multiple field sites. Results obtained from alternative treatment options were compared against lambda cyhalothrin as a standard.

Materials & Methods

On each sample date a total of 30 stems (ten stems at three random locations within each plot) were evaluated for alfalfa stage of development. Alfalfa stage of development was assessed by using the mean stage by count (MSC) method described by Kalu-Fick (1983).

Pesticide Screening Trials

Field trials were conducted to select top performing chemicals to be further tested in larger scale studies. Chemical treatments were novaluron (Rimon 10EC; 9.3% [AI], Chemtura Corp., Middlebury, CT), diflubenzuron (Dimilin 2L, Crompton, Middlebury, CT), azadirachtin (Neemix 4.5, Certis USA, Columbia, MD), kaolin (Surround WP, Engelhard Corp., Iselin, NJ), and lambda cyhalothrin (Warrior 1E, Syngenta Crop Protection, Greensboro, NC). All chemicals were applied with a CO_2 powered backpack sprayer and a 2 m wide boom (Spraying Systems, Wheaton, IL). All applications except the kaolin used Teejet model XR8001VS nozzles (Spraying Systems, Wheaton, IL) which delivered an output of 83.3 liters/ha at 30 PSI. Kaolin applications used Teejet XR8010 nozzles (Spraying Systems, Wheaton, IL) which delivered an output of 378 liters / ha at 30 PSI. Foliar applications of kaolin (6,544 g [AI] / ha), azadirachtin (7.8 g [AI] / ha), novaluron (30.9 g [AI] / ha), diflubenzuron (22.6 g [AI] / ha), and lambda cyhalurothrin (5.5 g [AI] / ha) were compared to the untreated control in each field trial.

Screening field trials were conducted at three field sites in 2006 and 2009. In 2006, a field site was located 6.4 km northwest of Bozeman, MT in a fifth year

commercial forage alfalfa (cv. 'Shaw') production field. In 2009, trials were conducted 8 km SW of Bozeman, MT in a 6th year forage alfalfa (cv. 'Shaw') stand, and in a 5th year forage alfalfa (cv 'Shaw') stand at the Southern Agricultural Research Center 7 km east of Huntley, MT. Each field was watered bi-weekly with a wheel-move sprinkler irrigation system delivering 5 cm of precipitation every 7 D.

Plots measuring 6.6 by 8.3 m were arranged as a RCB design with six treatments replicated four times against the irrigation system at the Bozeman 2006, 2009 sites, and replicated three times against the irrigation system at the Huntley 2009 site. Timing of application for each insecticide treatment was in synchrony with most susceptible AW larval stages as determined by results in Chapter 2. Kaolin was applied at AW early larval emergence (first and second instar) and late larval (second to third instar) growth stages (Julian Date [JD] 157 and 164 in Bozeman, 2006; JD 142 and 147 in Huntley, 2006; JD 162 and 169 in Bozeman, 2009), novaluron and diflubenzuron were applied at AW early larval emergence (JD 142 in Huntley, 2006; JD 157 in Bozeman, 2006; and JD 162 in Bozeman, 2009), and lambda cyhalothrin and azadirachtin were applied at AW late larval emergence (JD 147 in Huntley; JD 164 in Bozeman, 2006; and JD 169 in Bozeman, 2009). All foliar applications were made on days with temperatures ranging from 16 to 24 degrees C and 0 – 10 mph winds.

Top Performing Insecticide Trials

The top performing chemical selected from screening trials was evaluated over multiple harvest cycles in two large scale field trials. These trials investigated novaluron and lambda cyhalothrin (same application rates and equipment as used in screening trials)

versus the untreated control. Field trials were conducted at two field sites in 2010. One field site was located 1.7 km north of Toston, MT in a 4th year commercial forage alfalfa (cv. 'Shaw') production field, while a second site was located in a second year forage alfalfa (cv 'Shaw') stand at the Southern Agricultural Research Center 7 km east of Huntley, MT. Each field was watered bi-weekly with a 2.3 diameter wheel-move sprinkler irrigation system delivering 5 cm of precipitation every 7 d.

Plots measuring 8.7 by 15 m were arranged as a RCB design with six treatments replicated four times against a grass border. Novaluron and lambda cyhalothrin were applied at AW early larval emergence (JD 152 and JD 153 in Toston and Huntley, respectively). All foliar applications were made on days with temperatures ranging from 16 to 24°C and 0 – 10 mph winds.

Predator, Prey and Predator/Prey Estimates

Sweep sampling was initiated prior to treatment and continued weekly until first cutting at all field sites. Sample dates for the Bozeman 2006 site were JD 157, 164, 170, and 177 (MSC 3.0, 3.8, 4.0, and 5.8, respectively); for the Huntley 2009 site were JD 142, 147, 155 and 162 (MSC 1.0, 2.0, 2.5, and 3.3, respectively); for the Bozeman 2009 site were JD 162, 169, 176, and 182 (MSC 2.0, 3.0, 5.0, and 5.8, respectively); for the Toston 2010 site were JD 152, 158, 165 and 174 (MSC 2.0, 3.8, 3.8, and 5.5, respectively); for the Huntley 2010 site were JD 153, 159, 166, and 173 (MSC 2.0, 3.8, 3.9, and 5.3, respectively).

Post-harvest data were only collected from the larger scale 2010 field sites. At these sites sweep sampling was initiated approximately three weeks after first harvest

cycle cutting and continued weekly until second cutting. Second harvest cycle sample dates for the Toston 2010 site were JD 200, 209 and 215 (MSC 3.0, 3.8 and 5.0, respectively); for the Huntley 2010 site were JD 194, 200 and 207 (MSC 2.5, 4.0 and 5.0 respectively). Quadrat sampling rotated systematically in a clockwise fashion to avoid any bias from previous sampling removal. All sweep samples were placed in 3.8 l plastic zip-lock bags prior to transport and 4°C storage.

To interpret the impact of pesticides upon predator / prey assemblages; AW larvae, AW primary predators and the primary lady beetle prey, pea aphid, were evaluated (Kalaskar and Evans 2001). Primary AW and pea aphid predators were designated by Elliot et al. (2002) and Ouayogode & Davis (1981) as lady beetles, damsel bugs and lacewings. These insects were assessed by taking ten 180^0 sweeps with a 38 cm sweep net in one of six quadrats within each plot. Lady beetle, damsel bug and lacewing species assemblages were further identified to the taxonomic level. Alfalfa weevil larvae were later counted prior to being categorized to growth stage (instar 1 – 4) by measuring head capsule width (Bartell & Roberts 1974). Alfalfa weevil larvae densities were only reported for the 2010 studies since AW larval densities from the 2006 to 2009 screening trials can be obtained from the chapter 2 results.

Parasite Assessments

Alfalfa weevil parasitism rates were assessed according to methods of Ayedh et al. (1996). Immediately prior to first cutting (JD 177 at the Bozeman 2006 site; JD 174 at the Huntley 2009 site; JD 182 at the Bozeman 2009 site; JD 174 at the Toston 2010 site; JD 173 at the Huntley 2010 site) 100 sweeps were taken from plots. Due to extremely

low AW numbers, 300 sweeps were taken from the lambda cyhalothrin treated plots. Contents of sweep samples were placed in large paper bags measuring 60 cm by 90 cm and transported to Marsh Laboratory, Montana State University, Bozeman, Montana. Larvae (15 at Bozeman 2006 site; 50 at all other sites) were separated from swept arthropods and placed into 30 cm by 45 cm paper bags for rearing of parasitoids. Bags were kept at 25°C (RH = 30%) and fresh alfalfa was added daily until AW pupation. Parasitized pupae, emerged adult parasitoids and dead larvae / pupae were counted. Total parasitized pupae were further adjusted for mortality by dividing parasitized pupae by the adjusted total (total reared – non-parasitized dead larvae) to obtain percent parasitism. Parasitized pupae were identified to species and counted according to descriptions of Ayedh et al. (1996) and Weaver (1976).

Statistical Analysis

Each field site was analyzed separately due to unequal sample dates between sites. Dates were analyzed separately at the 2006 and 2009 field investigations due to a significant date by treatment interaction ($P < 0.05$), however at the 2010 field sites, data were grouped by three post application dates in the first harvest cycle termed ‘first harvest cycle’ (JD 159, 166, and 173 in Huntley; JD 158, 165 and 174 in Toston), and grouped by three sample dates in the second harvest cycle now termed ‘second harvest cycles’ (JD 194, 200 and 207 in Huntley; JD 200, and 209 and 215 in Toston).

Scatter plots of residuals versus the independent variables, as well as the Shapiro-Wilk test for normality indicated a Poisson distribution of lady beetle (*spp.*), damsel bugs and total predators; square root ± 0.5 transformation was used (Draper & Smith 1981; Zar

1984) while AW larval counts and pea aphid counts were analyzed following a log + 1 transformation to stabilize variance (Snedecor and Cochran 1982). All proportional data including predator/prey ratios and parasitism rates were arc sine square root + 0.5 transformed to normalize a binomial distribution (Zar 1984). For reporting purposes AW / ten sweeps were converted to percent reductions in AW using Abbott's formula (Abbott 1925).

Predator prey relationships were tabulated according to methods of Denys & Tscharntke (2001). AW and pea aphid were each analyzed as prey, while coccinellids, lacewings and damsel bugs were summed as predators for either species. The predator / prey ratio was calculated by dividing the total number of predators by the total number of prey.

Treatment effects over time were analyzed using PROC general linear models (GLM) with time as a repeated measures ($P = 0.05$; SAS 2002). If treatment or interaction effects were significant, treatment effects for each time period were analyzed using the Fisher protected (LSD) multiple comparison test (SAS 2002).

Results

Pesticide Screening Trials

Evaluation of Prey. Treatment effects of insecticide treatments on pea aphids were measured in three field sites in 2006 and 2009. Impacts of insecticide treatments on AW larvae were reported in chapter 2.

The 2009 Huntley and 2009 Bozeman sites had a pea aphid density of 47.3 ± 4.4 and 39.0 ± 4.5 / 10 sweeps immediately prior to harvest (JD 162 and 182, respectively). This was well below the economic threshold of 750 – 1,000 pea aphids / 10 sweeps (Hodgson 2007; Cuperus et al. 1982). However, pea aphid densities in untreated plots at the Bozeman 2006 site increased past the economic threshold by the last sample date (JD 177) immediately prior to harvest, with 1,037 ± 177.8 pea aphids / 10 sweeps.

Lambda cyhalothrin significantly reduced reduced pea aphid densities ($P < 0.05$) by 65, 60 and 97% at the Bozeman 2006, Huntley 2009 and Bozeman 2009 sites, respectively. The experimental treatments did not reduce pea aphid populations ($P > 0.05$).

Evaluation of Predators**.** The effects of insecticide treatments on total lady beetles, each lady beetle species, damsel bugs and total predators were evaluated in three field sites in 2006 and 2009. Lacewings were not found at any of our field sites.

Lady Beetles. The Bozeman 2006 and 2009 sites had similar lady beetle species distributions, while the Huntley site had fewer total species. At the Bozeman sites, the seven spotted lady beetle, *Coccinella septempunctata* (Linnaeus), comprised over 74% of the lady beetle species composition followed by 10% transverse, *Coccinella transversoguttata* (Brown) and 10% convergent, *Hippodamia convergens* (Guerin). Many other species were found at lower numbers at the Bozeman sites including the three-banded lady beetle, *Coccinella trifasciata* (Linnaeus), spider mite destroyer, *Stethorus punctum* (LeConte), and parenthesis lady beetle, *Hippodamia parenthesis*

(Say). The Huntley 2009 site consisted of only two species of coccinellids, with 97% identified as *C. septempuncta* and 3% as *H. parenthesis.*

Higher populations of *C. septempunctata* were recorded in plots treated with the experimental products at the Bozeman 2009 field site (JD 176; F = 7.16, df = 8, 15, *P* = 0.001). However, at the Bozeman 2006 field site only novaluron and diflubenzuron treated plots resulted in significantly higher populations of *C. septempunctata* when compared to the lambda cyhalothrin treatment (JD 170; F = 5.26, df = 8, 15, *P* = 0.005). Lambda cyhalothrin treatments eliminated lady beetle populations. Novaluron and diflubenzuron treated plots resulted in an average 23% and 19% reduction in *C. septempunctata*, respectively. Novaluron treated plots had significantly higher populations of *C. septempunctata* on JD 177 at the Bozeman 2006 site resulting in a 65% reduction in *C. septempunctata* within lambda cyhalothrin treated plots and no reduction in novaluron treated plots (F = 4.78, df = 8, 15, *P* = 0.008). Novaluron treatments consistently conserved *C. septempunctata* when compared to the lambda cyahlothrin treatment at the Bozeman sites; however differences were absent at the Huntley 2009 site.

The only experimental product that conserved *H. convergens* populations was novaluron at the Bozeman 2006 site. At the Bozeman 2006 field site, novaluron treated plots had 1.0 ± 0.4 *H. convergens* compared to 0.0 ± 0.0 in the lambda cyhalothrin treated plots (F = 3.75, df = 8, 15, *P* = 0.02).

Significantly higher densities of total lady beetles were captured within all experimental treatments when compared to the standard, lambda cyhalothrin at the Bozeman 2009 site (JD 176; F = 6.46, df = 8, 15, *P* = 0.002). Diflubenzuron and

novaluron plots also harbored significantly higher levels of lady beetles on JD 177 at the Bozeman 2006 site ($F = 8.73$, df = 8, 15, $P = 0.005$). No significant differences in total lady beetle densities were observed at the Huntley site ($P > 0.05$). When averaged across all field sites (JD 177 Bozeman 2006; JD 162 Huntley 2009; JD 176 Bozeman 2009) novaluron and diflubenzuron treatments contained 2.2 and 3.2 lady beetles, respectively, while the lambda cyhalothrin treated plots had an average 0.26 lady beetles resulting in 21%, 0% and 91% reductions, respectively. Novaluron and diflubenzuron never significantly reduced lady bird beetles from untreated plots across all field sites on any post application date ($P > 0.05$).

Damsel Bugs. Significant differences in densities of damsel bugs were observed between treatments at the Bozeman 2006 site. All other sites contained very low densities of damsel bugs. At this site, approximately 95% of damsel bugs collected were the common damsel bug, *Nabis americoferus* (Carayon). On JD 177, significantly higher numbers of damsel bugs were found in diflubenzuron, azadirachtin, novaluron, kaolin and untreated plots compared to plots treated with lambda cyhalothrin, with 3.5 ± 0.9, 2.5 ± 0.3, 1.8 ± 0.3, 2.3 ± 0.3, 2.3 ± 1.0, and 0.3 ± 0.3 damsel bugs / 10 sweeps, respectively ($F = 4.06$, df = 8, 15, $P = 0.01$).

Total Predators. Total predators were analyzed by summing all lady beetle (Coccinellidae) and damsel bugs (Nabidae). Significant differences in total predators were observed at all field sites at various post application time intervals. The predominant predator species represented in untreated control plots represented more than 65% of total predator numbers across field sites.

Novaluron treated plots conserved predator densities from that of the standard, lambda cyhalothrin, more frequently than any other experimental treatment. This was observed at the Bozeman 2006 site on JD 170 when novaluron treated plots contained significantly higher densities of predators (2.0 ± 1.1 predators / 10 sweeps) compared to no predator species found in the lambda cyhalothrin treated plots (0.0 ± 0.0 / 10 sweeps; $F = 4.74$, df = 8, 15, $P = 0.008$). No other treatment strategy harbored significantly more predators from that of the standard, lambda cyahlothrin. On JD 170 at the Bozeman 2006 site, novaluron reduced predators by 13% compared to a 100% in lambda cyhalothrin treated plots when mortality was adjusted by the untreated control.

Significantly higher levels of predators were observed in any experimental treatment when compared to lambda cyhalothrin treated plots across all field sites on the following sample dates: 1) on JD 177 at the Bozeman 2006 field site ($F = 5.74$, df = 8, 15, $P = 0.004$); 2) on JD 162 at the Huntley 2009 field site ($F = 3.01$, df = 7, 10, $P = 0.05$), and 3) on JD 176 at the Bozeman 2009 field site ($F = 7.63$, df = 8, 15, $P = 0.001$). Diflubenzuron, azadirachtin, novaluron, kaolin and lambda cyhalothrin reduced predators by 17, 10, 7, 10, and 93%, respectively, when averaged by post application date and sites. In ascending order, novaluron had the lowest reduction in predators, followed by azadirachtin and kaolin, diflubenzuron and finally, lambda cyhalothrin.

Evaluation of Predator / Prey Relationships. Significant differences in the predator-AW ratio were observed between treatments at all field sites ($P < 0.05$), however differences in the predator-pea aphid ratio were only observed at the Bozeman

field sites. The Huntley 2009 site had similar though not significantly different predator-pea aphid ratio trends when compared to the Bozeman field sites ($P > 0.05$).

Novaluron was the only experimental treatment that consistently had higher predator-AW ratios when compared to ratios within the lambda cyhalothrin treated plots across all field sites. Across field sites, novaluron treated plots had an average predator-AW ratio of 0.15 compared to 0.02 predators / AW in the lambda cyhalothrin treated plots. On the final sample date at the Bozeman 2006 and Huntley 2009 field sites, only novaluron treated plots significantly increased the predator-AW ratio when compared to the lambda cyhalothrin treated plots (Figure 3.1). Novaluron treated plots had a predator-AW ratio of 0.31 ± 0.06 (Bozeman 2006; F = 2.97, df = 8, 15, $P = 0.05$) and 0.03 ± 0.01 (Huntley 2009; F = 3.69, df = 7, 10, $P = 0.04$) while lambda cyhalothrin treated plots had no predators detected in either field site. All alternative treatments had a significantly higher predator-AW ratio when compared to the lambda cyhalothrin treated plots on JD 176 at the Bozeman 2009 site (Figure 3.1).

Plots treated with any experimental treatment had a significantly higher predator-pea aphid ratio at two of three field sites. However, novaluron consistently had greater ratios at the Bozeman 2006 site. On the final sample date the predator-pea aphid ratio was 0.04, 0.16, 0.08 and 0.07 in the diflubenzuron, azadirachtin, novaluron and kaolin treated plots, respectively, compared to <0.001 predators / pea aphid in the lambda cyhalothrin treated plots (Figure 3.1).

At the Bozeman 2006 field site, on JD 170, only novaluron treated plots had significantly higher predator-pea aphid ratios when compared to the lambda

cyhalothrin treated plots (F = 5.08, df = 8, 15, *P* = 0.006). On the next consecutive sample date (JD 177) all experimental treatments had significantly higher predator-pea aphid ratios compared to the lambda cyhalothrin treated plots (Figure 3.1). These trends also existed at the Bozeman 2009 field site where all experimental treatments had significantly higher predator-pea aphid ratios when compared to the lambda cyhalothrin treated plots on JD 176 (*P* = 0.01; Figure 3.1). At the Huntley 2009 site there were no significant differences in predator/prey ratios between any of the treatments (Figure 3.1).

Assessments of Parasitism. Significant differences in parasitized pupae and dead larvae/pupae were observed at all three field sites ($P < 0.05$). Two ichneumonid parasitoids, *Oomyzus incertus* (Ratzeburg), and *Bathyplectes curculionis* (Thomson) were reared from spring generations of AW larvae. Of cocoons parasitized, 21% were identified as *O. incertus* while 79% were identified as *B. curculionis*.

Alfalfa weevil larval parasitism rates from the novaluron and kaolin plots were consistently higher ($P < 0.05$) than from the lambda cyhalothrin treated plots at all field sites. At the Bozeman 2006 field site, larval parasitism rates were significantly increased when larvae were collected from the untreated, novaluron and kaolin plots. Parasitism in novaluron treated plots averaged 20 ± 8%, kaolin treated plots averaged 17 ± 4% and lambda cyahlothrin treated plots averaged 4 ± 2% (F = 3.12, df = 8, 15, *P* = 0.04). At the Bozeman and Huntley 2009 field sites, AW larvae reared from diflubenzuron, azadirachtin, novaluron, kaolin and untreated plots had a significantly higher parasitism rate when compared to the lambda cyhalothrin treated plots: 9 ± 2%, 19 ± 4%, 18 ± 4%,

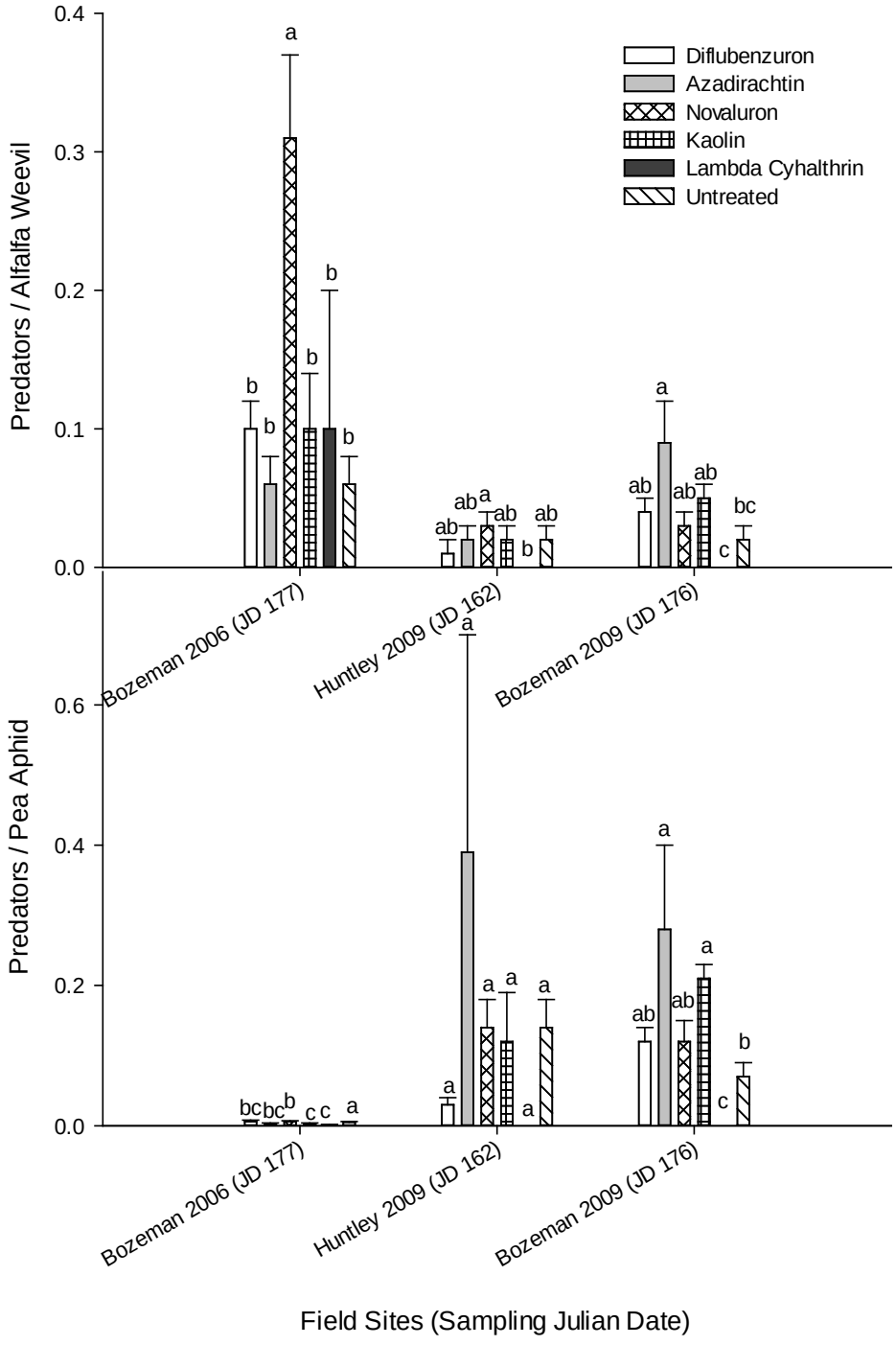


Figure 3.1: Predator-alfalfa weevil and predator-pea aphid ratios ± SE after application of various pesticides. Applications of novaluron, azadirachtin and kaolin were on Julian Date (JD) 157, 142 and 162 at the Bozeman 2006, Huntley 2009 and Bozeman 2009 sites, respectively. Applications of lambda cyhalothrin and azadirachtin were approximately 7 d after early application dates. Means within columns followed by similar letters are not significantly different (LSD Test after arc sine, square root + 0.5 transformation; *P=0.05;* Data presented is untransformed).

15 ± 1%, and 17 ± 1% parasitism rate at the Huntley 2009 site; and 7 ± 3%, 10 ± 2%, 9 ± 6%, 10 ± 4%, and 12 ± 3% parasitism rate at the Bozeman 2009 site compared to an average 0.5% parasitism in the lambda cyhalothrin treated plots (F = 7.66, df = 7, 10, *P* = 0.003; 3.12, df = 8,12, *P* = 0.04 at the Huntley and Bozeman 2009 sites, respectively). Parasitism rates of AW larvae collected from any experimental treatment were never significantly different than the untreated control plots at any field site (*P* > 0.05) with the exception of azadirachtin treated plots at the Bozeman 2006 field site. Parasitism rates in descending order are: untreated plots (17.3%), novaluron (15.7%), azadirachtin (10.7%), kaolin (10.5%), diflubenzuron (10.0%) and lambda cyhalothrin (1.7%).

When rearing parasitoids, AW larval mortality (when there was a lack of parasitoid cocoon) was significantly higher when larvae were collected from the lambda cyhalothrin treated plots (12 ± 3, 23 ± 5, and 45 ± 5% at the Bozeman 2006, Huntley 2009 and Bozeman 2009 field sites, respectively) compared to experimental treatments. Mortality from reared AW larvae collected from the lambda cyhalothrin treated plots was significantly higher when compared to all other treatments including the untreated in the Bozeman 2006 and 2009 field sites (F = 3.00, df = 8, 15, *P* = 0.04; F = 10.57, df = 8, 12; *P* =0.009, respectively). However in the Huntley 2009 site, AW collected from the lambda cyhalothrin plots had significantly higher mortality when compared to the diflubenzuron and untreated plots (F = 3.22, df = 7, 10, *P* = 0.05). Mortality of reared AW larvae collected from plots sprayed with experimental treatments never significantly was different than the untreated at any field site (*P* > 0.05).

Top Performing Insecticide Trials

Evaluation of Prey. Alfalfa weevil larvae and pea aphid densities were quite low at each field site, with the exception of pea aphids in the first harvest cycle at the Huntley site. Though AW larvae and pea aphid densities were quite low, significant differences between treatments were observed in the first harvest cycle ($P < 0.05$), although differences were absent in the second harvest cycle (Figure 3.2).

Alfalfa Weevil Larvae. Densities of AW larvae within untreated plots averaged 26.6 ± 1.8 / 10 sweeps at the Toston site and 41.4 ± 3.1 AW / 10 sweeps at the Huntley site within the first harvest cycle. AW densities in untreated plots decreased significantly by the second harvest cycle with 9.1 ± 2.4 / 10 sweeps at the Toston site and 2.9 ± 0.6 AW / 10 sweeps at the Huntley site.

The application of novaluron significantly reduced populations of AW larvae from that of the untreated control within the first harvest cycle at either field site ($P < 0.05$). However AW populations within novaluron plots were never significantly higher than populations within lambda cyhalothrin treated plots ($P < 0.05$). Averaged across sites, novaluron reduced AW larval populations from 34.0 / 10 sweeps in untreated plots to 26.5 / 10 sweeps in novaluron treated plots, while lambda cyhalothrin reduced populations to 4.5 AW / 10 sweeps (Figure 3.2).

Pea Aphids. Densities of pea aphids never exceeded the economic threshold of 1,000 aphids / 10 sweeps (> 20” stems) within untreated plots in either the first or second harvest cycle at either field site. Pea aphid populations averaged 39.6 ± 3.2 / 10 sweeps at the Toston site in the first harvest cycle prior to rising to an average 57.5 ± 9.8 / 10

sweeps within the second harvest cycle. This contrasted with the Huntley 2009 site where pea aphids averaged 239.4 ± 42.1 pea aphids / 10 sweeps within the first harvest cycle prior to decreasing to an average 32.4 ± 6.4 / 10 sweeps within the second harvest cycle (Figure 3.2).

The application of novaluron didn't significantly reduce populations of pea aphids from that of the untreated within any harvest cycle in either field site ($P > 0.05$). Applications of lambda cyhalothrin reduced populations significantly from that of the untreated in the first harvest cycle at either field site. Significant differences in pea aphid populations were not observed between any treatments in the second harvest cycle at either field site (Figure 3.2).

Evaluation of Predators**.** The effects of insecticide treatments on total lady beetles, each lady beetle species, damsel bugs and total predators were measured in two field sites in 2010. Damsel bugs and lady beetles were found across field sites. The most prevalent predators were lady beetles at the Toston site (74 and 77% in the first and second harvest cycle, respectively) and Huntley site (51% and 71% lady beetles in the first and second harvest cycle, respectively). Significant differences between treatments in total lady beetles, lady beetle species, damsel bugs and total predators were not observed between treatments in the second harvest cycle. However significant differences between treatments were observed in the first harvest cycle ($P < 0.05$).

Lady Beetles. More lady beetle species were observed at the Toston site when compared to the Huntley site within either harvest cycle. At the Toston site within the first harvest cycle the seven spotted lady beetle, *Coccinella septempunctata* consisted of

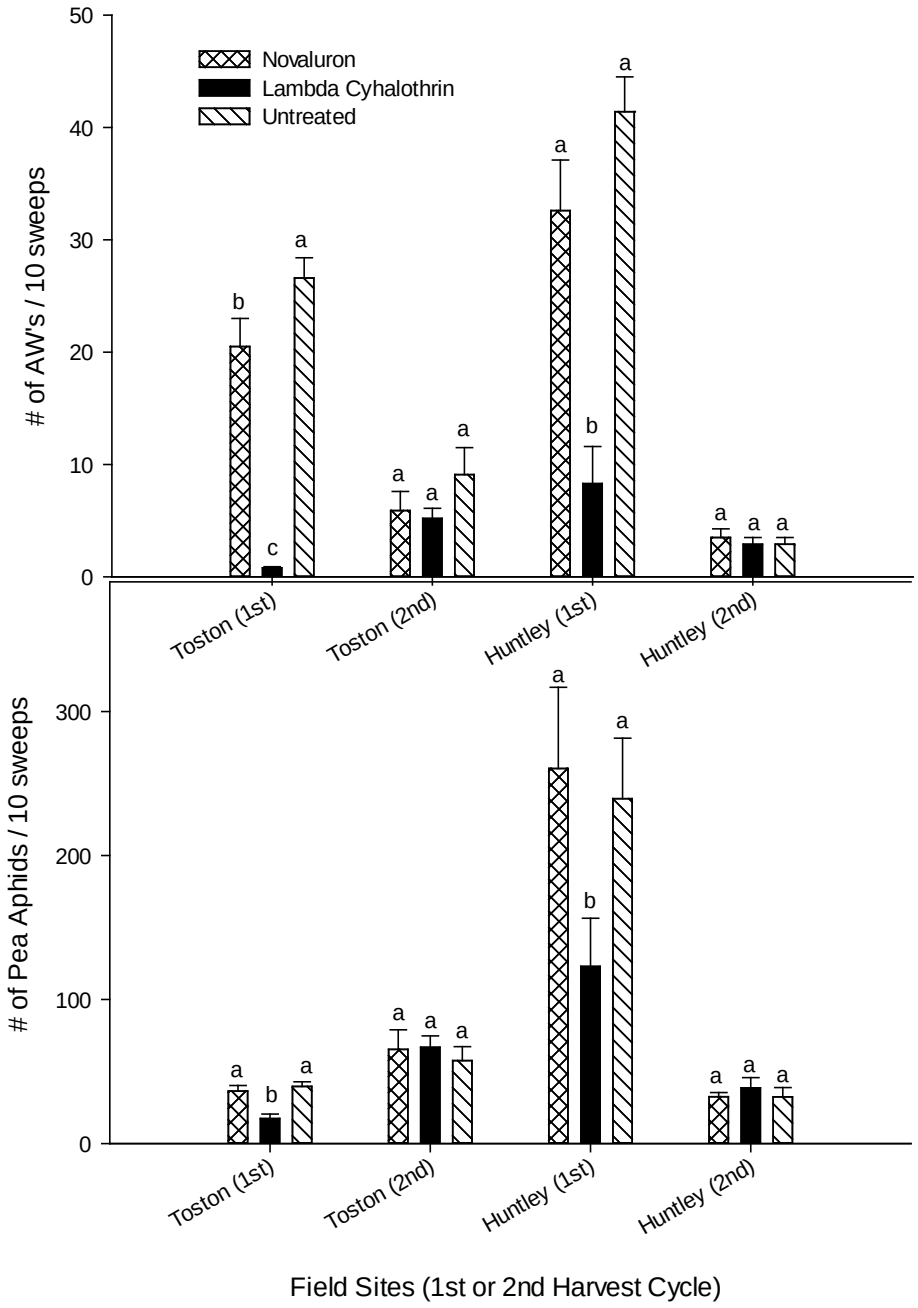


Figure 3.2: Average first & second harvest cycle alfalfa weevils (AW) and pea aphids / 10 sweeps ± SE over three first harvest and second harvest cycle dates after applications of lambda cyhalothrin and novaluron at multiple field sites (Means within columns followed by similar letters are not significantly different; LSD Test after log + 1 transformation; *P=0.05;* Data presented is untransformed). First harvest cycle sample dates were averaged over Julian Date 159, 166 and 173 in Huntley & Julian Date 158, 165 and 174 in Toston. Second harvest cycle sample dates were averaged over Julian Date 194, 200 and 207 in Huntley & Julian Date 200, 209 and 215 in Toston. Applications were made on Julian Date 153 and 152 in Huntley and Toston, respectively.

86% of the lady beetle species composition followed by the convergent lady beetle, *Hippodamia convergens*, transverse lady beetle *Coccinella transversoguttata*, caseys lady beetle, *Hippodamia caseyi* (Johnson), 13-spotted lady beetle, *Hippodamia tredecimpunctata* (Linneaus) and parenthesis lady beetle, *Hippodamia parenthesis* with 6, 3, 2, 2, and 1% of the species composition, respectively. Species composition at the Toston site dropped to four species within the second harvest cycle that included 95% *Coccinlla septempunctata*, 2% *Coccinella transversoguttata*, 2% *Hippodamia caseyi* and 1% *Hippodamia convergens*. The Huntley 2010 site consisted of only two species of lady beetles within the first harvest cycle, with 79% of them identified as *Coccinella septempuncta* and 21% identified as *Hippodamia convergens.* The number of species at the Huntley site stayed consistent into the second harvest cycle with 90% of the lady beetles identified as *C. septempunctata* and 10% *H. convergens.*

Significantly higher ($P < 0.05$) densities of lady beetles were collected from the novaluron and untreated plots when compared to the standard, lambda cyhalothrin in the first harvest cycle at the Toston site ($F = 31.24$, df = 5, 30, $P < 0.0001$). At this site lady beetles within the novaluron, untreated and lambda cyhalothrin treated plots averaged 3.2 ± 0.6, 4.0 ± 0.5 and 0.0 ± 0.0 lady beetles / 10 sweeps, respectively.

Damsel Bugs. Significant differences in damsel bugs were present between treatments at the Toston site in 2010. Significantly higher densities of common damsel bugs, *Nabis americoferus*, were found within the novaluron and untreated plots when compared to the standard, lambda cyhalothrin, in the first harvest cycle at the Toston site ($F = 7.05$, df = 5, 30, $P < 0.003$). Within the first harvest cycle populations of damsel

bugs within the novaluron, untreated and lambda cyhalothrin treated plots averaged 1.1 ± 0.3, 0.0 ± 0.0 and 1.2 ± 0.3 damsel bugs / 10 sweeps, respectively.

Total Predators. Significant differences in total predators were observed only within the first harvest cycle at the Toston site (F = 28.19; df = 5, 30; $P < 0.0001$). At this site, untreated and novaluron plots harbored higher predator densities (5.4 ± 0.9; 4.3 ± 0.9 predators / 10 sweeps, respectively) compared to densities in the lambda cyhalothrin treated plots (0.0 ± 0.0 / 10 sweeps). Significant differences weren't observed between treatments in the second harvest cycle as predator densities within the lambda cyhalothrin treated plots increased to 5.8 ± 1.8 / 10 sweeps while densities in other treatments remained relatively consistent ($P < 0.05$).

Evaluation of Predator / Prey Relationships. Significant differences in the predator-AW and predator-aphid ratio were observed between treatments in the first harvest cycle at the Toston site ($P < 0.05$); however differences were not observed between treatments at the Huntley 2009 site or in the second harvest cycle. At the Toston site, lambda cyhalothrin treated plots had a significantly lower predator-AW and predator-pea aphid ratio than either the untreated or novaluron treated plots ($P < 0.05$). A predator-AW ratio of 0.00 ± 0.00 was observed in the lambda cyhalothrin treated plots compared to 0.21 ± 0.04 and 0.23 ± 0.06 percent predators / AW in the untreated and novaluron treated plots, respectively ($P = 0.0005$; Table 3.1). In the first harvest cycle, the predator-pea aphid ratio was also significantly reduced in plots treated with lambda cyhalothrin when compared to either the untreated or novaluron treated plots at the Toston site (Table 3.1).

Table 3.1: Average first & second harvest cycle predators / alfalfa weevil (AW) & predators / pea aphid ± SE after forage alfalfa was treated with novaluron and lambda cyhalothrin at field sites near Toston and Huntley, MT in 2010.

Field	Treatment [c]	Rate gai/ha	Toston		Huntley	
Predators / AW			1[s] Harvest Cycle[a]	2[nd] Harvest Cycle[b]	1[st] Harvest Cycle[a]	2[nd] Harvest Cycle[b]
	Novaluron	31.0	0.23 ± 0.06a	0.74 ± 0.24	0.08 ± 0.02	1.97 ± 0.77
	λ Cyhalothrin	5.5	0.00 ± 0.00b	1.13 ± 0.29	0.26 ± 0.04	1.73 ± 0.36
	Untreated		0.21 ± 0.04a	0.64 ± 0.16	0.04 ± 0.01	2.54 ± 1.00
	F - Statistic		34.35	1.02	2.06	0.39
	df(model, error)		5, 6	5, 6	5, 6	5, 6
	P – value		0.0005	NS	NS	NS
Predators / aphid			1[st] Harvest Cycle[a]	2[nd] Harvest Cycle[b]	1[st] Harvest Cycle[a]	2[nd] Harvest Cycle[b]
	Novaluron	31.0	0.12 ± 0.06a	0.05 ± 0.01	0.01 ± 0.01	0.18 ± 0.06
	λ Cyhalothrin	5.5	0.00 ± 0.00b	0.08 ± 0.02	0.04 ± 0.03	0.12 ± 0.01
	Untreated		0.14 ± 0.03a	0.11 ± 0.04	0.01 ± 0.01	0.20 ± 0.07
	F - Statistic		46.38	1.42	0.88	0.87
	df(model, error)		5, 6	5, 6	5, 6	5, 6
	P – value		0.0002	NS	NS	NS

*Means within columns followed by similar letters are not significantly different (Data from the 1[st] harvest cycle was analyzed using arc sine, square root transformation; *P=0.05;* All data presented is untransformed).

[a] First harvest cycle sample dates were averaged over Julian Date 159, 166 and 173 in Huntley & Julian Date 158, 165 and 174 in Toston.

[b] Second harvest cycle sample dates were averaged over Julian Date 194, 200 and 207 in Huntley & Julian Date 200, 209 and 215 in Toston.

[c] Applications were made on Julian Date 153 and 152 in Huntley and Toston, respectively.

Assessments of Parasitism. Two ichneumonid parasitoids, *Oomyzus incertus* and *Bathyplectes curculionis* were reared from spring generations of AW larvae. Thirty three percent of parasitized cocoons were identified as *Oomyzus incertus* while 67% were identified as *Bathyplectes curculionis*.

Significant differences in parasitized pupae and dead larvae were observed at either field site ($P < 0.05$). *Bathyplectes curculionis* parasitism rates were significantly higher in the novaluron and untreated plots ($P < 0.05$) when compared to the lambda

cyhalothrin treated plots at either field site, however differences in overall parasitism rates and *Oomyzus incertus* parasitism were present only at the Toston 2010 site (Table 3.2).

Table 3.2: Larval mortality, adult emergence and parasitism rates ± SE after rearing 50 larvae from plots after application of novaluron and lambda cyhalothrin in 2010.

Field	Treatment	Rate gai/ha	Larval Mortality, Percent Parasitism and Adult Emergence*			
2010 Toston			Mortality	*Oomyzus incertus (%)*	*Bathyplectes curculionis (%)*	Parasitism Rate
	Novaluron	31.0	4 ± 2b	6 ± 2a	9 ± 1a	15 ± 3a
	Lambda Cyhalothrin	5.5	36 ± 4a	0 ± 0b	1 ± 1b	1 ± 1b
	Untreated		6 ± 3b	3 ± 1a	19 ± 2a	21 ± 2a
	F - Statistic		20.61	7.22	68.17	72.55
	df(model, error)		5, 6	5, 6	5, 6	5, 6
	P – value		0.002	0.02	<0.0001	<0.0001
2010 Huntley			Mortality	*Oomyzus incertus (%)*	*Bathyplectes curculionis (%)*	Parasitism Rate
	Novaluron	31.0	6 ± 1b	3 ± 1a	17 ± 3a	21 ± 4a
	Lambda Cyhalothrin	5.5	30 ± 6a	1 ± 1a	5 ± 4b	6 ± 5a
	Untreated		2 ± 1b	3 ± 2a	13 ± 1a	16 ± 2a
	F – Statistic		17.29	0.65	5.71	4.35
	df(model, error)		5, 6	5, 6	5, 6	5, 6
	P – value		0.003	NS	0.04	NS

*Means within columns followed by similar letters are not significantly different (LSD Test after arc sine, square root transformation; *P=0.05;* Data presented is untransformed).

AW larval mortality was significantly higher when reared AW larvae were collected from the lambda cyhalothrin treated plots compared to mortality from AW larvae collected from untreated and novaluron treated plots at either field site ($P < 0.05$). Mortality of AW reared from the lambda cyhalothrin treated plots was 36 ± 4 and 30 ± 6 from Toston and Huntley, respectively, while averaging between a range of two to six across field sites within the untreated and novaluron treated plots (Table 3.2).

Discussion

Evaluation of Pests

The effects of alternative insecticide treatments on AW and pea aphid were evaluated at five field sites over 2006, 2009 and 2010. Significant reductions in AW densities were observed at all field sites after application of many of the chemistries investigated. However experimental treatments failed to reduce pea aphid populations from that of the untreated.

Alfalfa Weevils. Novaluron consistently reduced AW populations from that of the untreated; however reductions were low (21 – 23%) at either field site in 2010 ($P < 0.05$). Studies in chapter 2 (that assessed AW larvae in the 2006 and 2009 field sites) also found novaluron to significantly reduce AW larval populations to levels comparable to that of the untreated control more consistently than diflubenzuron, azadirachtin and kaolin. This study and the study cited in chapter 2 found that novaluron never reduced AW larvae populations to that of the synthetic pyrethroid, lambda cyhalothrin. A delay in activity noted in chapter 2 may make this chemical conducive for managing AW in the second harvest cycle, however this wasn't the case. AW densities weren't reduced in the second harvest cycle from pre-harvest applications of novaluron. This was likely due to a large proportion of AW entering pupation by the beginning of the second harvest cycle at our field sites combined with the cutting of alfalfa causing AW mortality directly, while limiting available food and increasing larval desiccation from direct sunlight while in windrows (Blodgett 1996). An evaluation of the impacts of early season novaluron

applications on second generation (multi-voltine) AW populations may be beneficial; however this isn't possible in Montana due to the presence of a single generation of western and western / eastern intergrade populations (Helgesen and Cooley 1976).

Pea Aphids. No experimental treatment significantly reduced pea aphid populations from that of the untreated in the first or second harvest cycle. These chemistries offer little promise for implementation in an IPM program for managing pea aphids in forage alfalfa.

Novaluron, azadirachtin, and diflubenzuron showed little efficacy towards pea aphids possibly due to each insecticides' primary mode of action as insect growth regulators. Pea aphid populations in alfalfa stands are mixed with adults and immature nymphs present simultaneously. Nymphal mortality from the application of an insect growth regulator (IGR) could be quickly negated by surviving aphid adults which have a high reproductive rate. In addition, chitin synthesis inhibitors such as novaluron and diflubenzuron have shown success primarily targeting larval stages of holometabolous insects not hemi-metabolous nymphs (Cutler et al. 2005; Villavaso et al. 1995). Several studies have indicated neem extracts can provide adequate control of many aphid species under field conditions (Shauer 1987; Stark & Rangus 1994; Lowery & Isman 1995; Ulrichs et al. 2001); however studies demonstrating effectiveness in managing aphids in alfalfa are lacking. Yardim et al. (2001) observed a marginal 11.1% to 41.0% reduction in aphids using low and high rates of neem in alfalfa, while Stark & Rangus (1994) demonstrated reductions in pea aphids in beans but not in forage alfalfa. Our study

further demonstrates that azadirachtin would make a poor candidate for managing pea aphids in a forage alfalfa system.

Kaolin seemed to offer little protection from pea aphid, however efficacy may be improved. Reductions in efficacy may be due to a dense alfalfa canopy which is difficult for pesticides to penetrate, especially for pesticides such as kaolin that must be applied with nozzles delivering large droplets (Bach 1985; Gohlich 1985). Our pesticide application equipment delivered an output of 378 liters/ha, however spray output recommendations for kaolin range as high as 934 liters/ha (Surround WP Engelhard Corporation). Previous studies have indicated higher spray volumes may increase penetration of spray applications into dense plant canopies (Bach 1985; Gohlich 1985). Equipment that can deliver much higher spray outputs may be desirable to penetrate a dense alfalfa canopy. More research is needed to indicate whether higher spray volumes of kaolin may manage pea aphid populations.

Evaluation of Predators

The impacts of alternative pesticides upon AW predators including lady bird beetles, damsel bugs and lacewings were evaluated at multiple field sites in 2006, 2009 and 2010. Sufficient numbers of lady bird beetles and damsel bugs were present in these studies, however few lacewings were observed at any field site.

Lady Beetles. Determining species composition of lady beetles is critical when determining impacts of pesticides studied. Some species are known to be superior predators towards pea aphids and AW when compared to other lady beetle species. Our

studies indicate far fewer lady beetle species at the Huntley sites compared to all other sites investigated, however all sites were dominated by *Coccinella septempunctata*. *Coccinella septempunctata* consisted of 74 – 97% of the species composition across all field sites. The abundance of *Coccinella septempunctata* across field sites was beneficial to this study as this species has higher success than many other species in adapting to AW as an alternative prey species (Evans & Toler 2007; Evans 2004).

First harvest cycle lady beetles were more abundant in plots sprayed with any of the experimental treatments when compared to densities within the synthetic pyrethroid treated plots; however success was variable between field sites. Azadirachtin and kaolin were less consistent across field sites than novaluron and diflubenzuron at conserving lady bird beetle densities to that of the untreated plots. Novaluron and diflubenzuron applications never significantly reduced populations of coccinellids from that of the untreated plots. The consistency of novaluron and diflubenzuron versus kaolin and azadirachtin is probably due to the variable modes of action and the prevalence of adult lady beetles in our studies. The mode of action of kaolin and azadirachtin doesn't discriminate between immature and adult insects. Kaolin acts as a barrier film against a wide array of immature and adult insects while azadirachtin acts not only as an insect growth regulator but also as a stomach poison and feeding deterrent on many adult insects (Aertz et al. 1997). Contrastingly, novaluron and diflubenzuron are both chitin synthesis inhibitors that primarily show success targeting immature larval stages of insects (Cutler et al. 2005; Villavaso et al. 1995). The benefit of conserving lady beetles in novaluron and diflubenzuron treated plots in the first harvest cycle likely added to

reductions in AW or aphids prior to harvest, however it should be noted that this benefit never reduced pea aphid densities from that of the untreated at any time.

Lady bird beetles seemed unaffected by pre-harvest applications of lambda cyhalothrin in the second harvest cycle. This was likely due to a degradation of lambda cyhalothrin occurring simultaneous to the migration of adult lady beetles from adjacent untreated areas over a 42 d period. Consequently, the benefits of using early season alternative treatment strategies to increase lady beetle densities in the second harvest cycle are difficult to extrapolate without much larger field scale studies.

Damsel Bugs. All experimental treatments conserved significantly more damsel bugs than the synthetic pyrethroid treatment at only two of five field sites. The lack of significant treatment effects in densities of damsel bugs at three field sites were likely due to very low densities of damsel bugs, predominantly *Nabis americoferus*, found at these field sites. The decrease in damsel bug numbers in pyrethroid treated plots was temporary, with no differences between the pyrethroid treated plots and untreated plots in the second harvest cycle at any time. Previous studies have also indicated minimal impact towards damsel bugs with azadirachtin (Yardim et al. 2001); diflubenzuron (Keever 1977); kaolin and novaluron (Tharp et al. 2004; Tharp et al. 2005).

Total Predators. Lady beetles were the most prevalent predator at every field site, averaging 67% of species composition over 5 field sites in the first harvest cycle and 71% of total species composition in the second harvest cycle in 2010. Lacewings were not found and damsel bugs were found in lower numbers compared to lady beetles. This may

be due to our studies being conducted in pure alfalfa stands as opposed to a mixed grass alfalfa stand. A previous study by Barney et al. (1984) found that damsel bugs were more abundant in a mixed grass stand than pure alfalfa.

The benefit of using experimental treatments for maximizing predator populations was evident in our studies, however temporary. Plots treated with azadirachtin, kaolin, diflubenzuron or novaluron conserved more total predators in the first harvest cycle than plots sprayed with lambda cyhalothrin ($P < 0.05$) in four out of five field sites. The Huntley 2010 site had very few total predators in the first harvest cycle, likely making an accurate analysis of predators difficult. In screening trials across three field sites in 2006 and 2009, diflubenzuron, azadirachtin, novaluron, kaolin and lambda cyhalothrin reduced predators by 17, 10, 7, and 10%, respectively compared to 93% in the lambda cyhalothrin treated plots. Larger scale trials in 2010 further verified an average 10% reduction in predators when novaluron was applied at two field locations. Though all experimental treatments offered higher numbers of predators from that of the synthetic pyrethroid treatment, novaluron treated plots offered the most consistency across sample dates and field locations. The added benefit of using experimental treatments to maintain higher predator numbers within the first harvest cycle are likely due to decreasing AW larvae densities in synergy with the insecticide application itself. This benefit, even when combined with insecticide efficacy, never reduced AW larvae populations by over 73% at any field site within the first harvest cycle.

The long term implications on predators from using these experimental treatments are difficult to extrapolate. This study was unable to show a long term increase in

predator densities from that of the synthetic pyrethroid treatment in the second harvest cycle. Second harvest cycle predator numbers were sufficient; however the lack of differences suggests degradation of lambda cyhalothrin in the second harvest cycle and/or a significant movement of adult damsel bugs and/or lady beetles from bordering untreated areas. Much larger scale field studies would be helpful in determining whether the use of these experimental chemistries could have a sustainable impact on predator populations.

Evaluation of Predator / Prey Complex

Evaluations of the predator / prey of either pea aphids or AW larvae are superior to a narrow focus on either predators or pests when evaluating any pesticides potential as a sustainable management alternative. Investigations by Linker et al. (1996) have determined ratios that would be beneficial for minimizing secondary pest outbreaks, while other studies have deduced that predator removal often leads to increased levels of herbivorous insects resulting in higher levels of plant damage (Halaj and Wise 2001).

Evaluation of Predator / AW Relationships. Azadirachtin, kaolin and diflubenzuron were ineffective in consistently increasing the predator-AW ratio; however novaluron applications consistently benefited the predator -AW ratio when compared to the synthetic pyrethroid treatment in the first harvest cycle at all field sites in screening trials. When assessed over multiple harvest cycles in 2010, novaluron applications once again increased the predator-AW ratio from that of the synthetic pyrethroid treatment at the Toston site (0.23 ± 0.06 predators / AW, 0.00 ± 0.00, respectively); however

differences were not observed at the Huntley site. This was likely due to low predator densities. Benefits to the predator-AW relationship through the use of novaluron, although short term, may result in a beneficial ratio for reducing AW populations if pea aphids are a limiting factor.

Few AW larvae were present in the second harvest cycle resulting in difficulty extrapolating a longer term benefit to the predator-AW ratio over a longer period of time. Studies investigating novalurons' impact on multi-voltine AW may be beneficial; however future studies may also wish to investigate predator-AW relationships on a field scale. This would maximize AW numbers while lowering movement of predators from untreated areas.

Evaluation of Predator / Pea Aphid Relationships. All experimental treatments studied temporarily increased the predator-pea aphid ratio from that of the lambda cyhalothrin treatment in two of three field sites in screening trials, although supportive trends existed at the 3rd field site. This benefit could be observed by the final sample date across field sites with an average 0.04, 0.16, 0.08 and 0.06 predators-pea aphid in the diflubenzuron, azadirachtin, novaluron and kaolin plots, respectively, compared to <0.001 predators / pea aphid in the lambda cyhalothrin treated plots. Benefits to the predator-pea aphid ratio were primarily from conservation of natural enemies and not through significant reductions in pea aphids (0 – 17% reductions across field sites).

Linker et al. (1996) indicated spraying only if predator-pea aphid densities are lower than 0.1 to minimize secondary pest outbreaks from reduced predation from broad-spectrum sprays. Two of three field sites in screening trials had densities that exceeded

this beneficial ratio when insecticides were applied. This beneficial ratio of predators-pea aphids was maintained for the duration of the study only when experimental treatments were used. Lambda cyhalothrin treated plots decreased the predator to pea aphid ratio to near zero on every post application sample date within the first harvest cycle. This indicates the susceptibility of lambda cyhalothrin treated plots to secondary pest outbreaks of pea aphids if environmental conditions were ideal. Another study by Evans & Karren (1993) has shown a decrease in the predator-pea aphid ratio from broad-spectrum synthetic pyrethroid applications that lead to secondary pest outbreaks in later harvest cycles.

When only the top performing chemistry (novaluron) was evaluated over multiple harvest cycles, impacts to the predator-pea aphid complex were once again observed in the first harvest cycle in one of two field sites. A lack of differences within the second harvest cycle suggests the limits of using novaluron to expect post-harvest benefits to the predator-pea aphid ratio in alfalfa stands. An increase in the predator-pea aphid ratio driven only by a preservation of predators is of little value to managing AW larvae or pea aphids. This is partly due to pea aphids' reproductive capability to produce from 50 to 100 nymphs at a rate of six to seven / day (Blodgett 2006) in combination with the pea aphid being the primary prey of AW predators (Kalaskar & Evans 2001; Giles et al. 1994). Since novaluron doesn't eliminate the primary prey of AW larval predators, predator impacts would often be minimal towards AW larvae due to persistent pea aphid populations.

Evaluation of Contrasting Results in Predator / Prey Relationships in 2010. We observed contrasting results between field sites in 2010 when evaluating predator-prey relationships after applications of novaluron. This is likely due to the population dynamics at the Huntley 2010 site; consequently how this impacts the predator-prey ratio. Novaluron applications resulted in unremarkable reductions in AW and pea aphid; however novaluron applications also preserve predators when compared to the synthetic pyrethroid treatment. By preserving predators and offering some mortality, novaluron applications benefit the predator-prey complex; however high densities of predators need to be present. Low to moderate AW larvae and pea aphid mortality combined with low predator densities resulted in little benefit to the predator-prey ratio when using novaluron at the Huntley 2010 site.

This demonstrates the usefulness of predator prey ratios when choosing an insecticide for managing a pest. Results indicate the added benefit of using an experimental chemistry that preserves natural enemies selectively when natural enemy populations are high, not when few predators are present. Few predators found at the Huntley 2010 site may be due to a younger two year old stand compared to a five or six year old stand at other field sites investigated. Age of habitats has been shown by Denys and Tscharntke (2002) to significantly increase predator- prey ratios. Denys and Tsharntke found a 300% increase in the predator-prey ratio in a six year mixed weed and grass stand versus a one year weed and grass stand.

Parasitoids

Only three parasitoid wasps were detected in Montana by Byran et al. (1993) from 1980 – 1989 with detections primarily consisting of *Bathyplectes curculionis* and in much lower numbers *Bathyplectes stenostigma* (Thomson) and *Microctonus aethiopoides* (Loan). Our studies detected only two species including the ichneumonid larval parasitoid, *B. curculionis* and the Eulopid larval parasitoid, *Oomyzus incertus*. *Bathyplectes curculionis* has been verified by multiple studies to be the most widely established and successful larval parasitoid of western strains of AW (Maund & Hsiao 1991; Ayedh et al. 1996), and *Oomyzus incertus* releases were made by the USDA-APHIS in Montana with little success. This is likely due to the minimal success of *Oomyzus incertus* when parasitizing western strains of AW (Volker 1975).

The detection of *Oomyzus incertus* in our investigation may suggest an eastern strain of AW that has migrated to new areas of Montana, or the presence of an introduced strain of *Oomyzus incertus* that can successfully parasitize western strains of AW. The presence of the eastern strain of AW as far west as Toston, Montana would indicate only a slight migration (170 – 250 miles) of the western / eastern intergrade populations from boundaries suggested by Radcliffe & Flanders (1998). Eastern strain AW or hybrid populations may be present in low densities at our field sites. This may also be the result of the introduction of a more effective strain of *Oomyzus incertus* that would result in much higher parasitism rates on western strains. This situation occurred in California when an Iranian strain of *Oomyzus incertus* released in 1978 was shown to be widely

successful in parasitizing western and Egyption strains of AW (Radcliffe & Flanders 1998).

AW parasitism rates were unaffected by applications of diflubenzuron, novaluron and kaolin when compared to the untreated; however lambda cyhalothrin applications significantly lowered parasitism rates from that of the untreated across all field sites. Azadirachtin applications significantly decreased parasitism rates at one field site in 2006, however this detrimental impact was not observed in repeated trials. The highest parasitism rates were in the untreated plots (17.3%) followed by novaluron treated plots (15.7%), azadirachtin (10.7%), kaolin (10.5%), diflubenzuron (10.0%) and finally lambda cyhalothrin with only 1.7% AW larval parasitism. Novaluron and diflubenzuron's selective impact as chitin synthesis inhibitors would likely have little impact on adult parasitoid wasps; while kaolin action as a particle barrier film likely wouldn't inhibit adult parasitoids from accessing AW larvae.

These statistical trends were further supported by studies in 2010 which assessed parasitism rates of novaluron and lambda cyhalothrin. In this study, parasitism rates by *Bathyplectes curculionis* were significantly reduced by lambda cyhalothrin applications across field sites. There were no significant differences in *Oomyzus incertus* parasitism rates at the 2010 sites due to a low parasitism rate across field sites (3%) and applications targeting early instar AW larvae, not 3rd and 4th instar larvae that are preferred by *Oomyzus incertus* (Kingsley et al. 1993).

Parasitism rates found in our study may be helpful in managing low levels of AW larvae; however they would be ineffective at managing high populations. These results agree with a previous study in Colorado by Ayedh et al. (1996) which found *Bathyplectes curculionis* to be the most abundant parasitoid targeting western strain AW larvae; however with parasitism rates too low to successfully manage AW larvae. Yeargan & Pass (1978) also confirmed that *Bathyplectes curculionis* isn't effective at managing high AW populations.

AW larvae reared from lambda cyhalothrin treated plots had higher mortality when compared to all other treatments. Mortality from reared AW were unusually high whether lambda cyhalothrin was applied 14 d prior (three field sites in 2006 and 2009; 26%) or 21 d prior (two field sites in 2010; 33%) from the day of collection. This is likely due to the collection of morbid larvae caused from the residual activity of lambda cyhalothrin on surviving larvae. Either the Hymenopteran parasites were reduced directly by insecticide applications or parasitized larvae were killed more readily by the lambda cyhalothrin treatment, thus biasing our parasitism rates. A previous study by Davis (1970) has shown that when longer residual pesticides including carbofuran were applied two to three weeks prior to harvest, significant reductions in parasitism were noted; however treatments did not act differentially on parasitized larvae. In addition, all of our applications targeted approximately second instar (mean 2.0 instar across five field sites) AW larvae which is also the preferred host stage of *Bathyplectes curculionis* (Kingsley et al. 1993). The literature suggests that although mortality was present with the use of lambda cyhalothrin, that shouldn't change our estimated parasitism rates.

Our studies indicate that the use of diflubenzuron, kaolin or novaluron would benefit the parasitoid complex when compared to a synthetic pyrethroid treatment. This benefit may be reduced if the synthetic pyrethroid treatments were made earlier in the year. Studies by Davis (1970) have indicated no reductions in parasitism when applications of longer residual pesticides including carbofuran were made upon early alfalfa growth in the spring of the year.

Summary

Novaluron, a chitin synthesis inhibitor, was the most promising chemistry for managing AW larvae while minimizing impacts on natural enemies; however efficacy was low. The chemical offered some control of AW populations while causing little impact to parasitoids, lady bird beetles and/or damsel bugs. These benefits resulted in a greater predator-AW ratio when novaluron was used at four of five field sites with an average 0.15 in novaluron treated plots compared with 0.02 in lambda cyhalothrin treated plots. Higher predator-AW larvae ratios' may be of limited use due to a lack of efficacy towards pea aphids with the use of novaluron. Since pea aphids are the preferred prey of lady beetles and damsel bugs, we would expect little impact on AW larvae when they are present in high numbers (Kalaskar & Evans 2001; Giles et al. 1994).

Parasitism rates from *Bathyplectes curculionis and Oomyzus incertus* were unaffected by the use of diflubenzuron, novaluron and kaolin. This suggests that by using these experimental chemistries, parasitoids could be preserved when compared to the synthetic pyrethroid treatment. Diflubenzuron, novaluron and kaolin maintained

parasitism levels between 7.0 and 20.0% in screening trials compared to an average 2.0% with the use of lambda cyhalothrin across all field sites. This may assist in managing low AW larvae populations, not higher densities. Yeargan & Pass (1978) has confirmed that *Bathyplectes curculi*onis isn't effective at managing high AW populations.

Studies on the impacts of novaluron on second generation AW larvae and AW natural enemy complex in larger plots may be beneficial. The preservation of natural enemy populations may have residual impacts on second generation AW larvae when compared to the lambda cyhalothrin treatments. It is doubtful that these increased impacts would be economically viable due to the limited efficacy observed towards AW or pea aphids.

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CHAPTER 4

SUMMARY

Studies were conducted in Montana to evaluate the impacts of the insect growth regulators novaluron, diflubenzuron, azadirachtin and the particle barrier film, kaolin, on the primary pest, alfalfa weevil (AW, *Hypera postica* [Gyllenhal], natural enemies of AW and the secondary pest, pea aphid, *Acyrthosiphon pisum (Harris*). The primary use of these experimental products could potentially be used for the alfalfa seed industry and/or growers wanting organically-approved or integrated management options for sustainable AW control.

In field studies kaolin, diflubenzuron and azadirachtin treatments caused low (<53%) AW mortality and didn't protect alfalfa from AW feeding damage across field sites. Novaluron caused the highest mortality (peak 74%) while reducing feeding damage repeatedly across two of three field sites and two greenhouse trials. This was likely due to novaluron acting as a feeding deterrent with 30% of larvae noted not feeding while seeking alternative food sources in greenhouse trials. Feeding reductions and other behavioral changes have been noted with another similar compound that acts as a benzoyl phenyl urea chitin synthesis inhibitor, diflubenzuron (Braithwaite et al. 1976; Villavosa et al. 1995). Villavosa et al. (1995) noted applications of diflubenzuron resulted in decreased flight activity in boll weevils, *Anthonomus grandis* (Boheman), while Braithwaite et al. (1976) noted protection from AW feeding damage from applications of diflubenzuron. Our studies suggest that novaluron may be acting as a feeding deterrent

similar to diflubenzuron in previous studies. This resulted in more biomass at cutting in greenhouse trials however yield differences under field conditions were absent. Cuttings at the early bud stage (MSC 3.0) may take advantage of novalurons temporary feeding detterrant effect; thereby promoting a significant yield gain under field conditions. By cutting at the early bud stage growers could market premium quality alfalfa (crude protein > 20%) to the dairy industry at a much higher value.

Giles et al. (1994) and Kalaskar & Evans (2001) noted that reductions of pea aphids, *Acyrthosiphon pisum*, could promote further reductions in AW by limiting the primary food source of AW predators (Giles et al. 1994; Kalaskar & Evans 2001) while reducing secondary pest outbreaks of pea aphids. In our studies pea aphid populations were unaffected by applications of kaolin, azadirachtin, novaluron, and diflubenzuron across field sites. Nymphal mortality from these insect growth regulator compounds was likely negated by surviving adult aphids that have an extremely high reproductive rate (Blodgett 2006); while kaolin applications has difficulty penetrating the dense alfalfa canopy. Novaluron and diflubenzuron typically has high efficacy on immature larval stages of holo-metabolous insects not hemi-metabolous aphid nymphs (Villavaso et al. 1995; Cutler et al. 2005). Several studies have shown efficacy of azadirachtin on aphids (Shauer 1987; Stark and Rangus 1994; Lowery and Isman 1995); however studies demonstrating high efficacy towards pea aphids in alfalfa are lacking. Yardim et al. (2001) observed only a 41% reduction in aphids using the high rates of neem in alfalfa, while Stark and Rangus (1994) demonstrated reductions of pea aphids in beans, not forage alfalfa that has a much denser canopy.

All experimental chemistries provided some pre-harvest benefits to the predator-pea aphid complex at various field sites; however novaluron treatments provided significantly higher predator-AW ratios consistently across field sites when compared to the synthetic pyrethroid, lambda cyhalothrin ($P < 0.05$). Novaluron treated plots had an average predator-AW ratio of 0.15 compared to 0.02 in lambda cyhalothrin plots. Benefits to the predator-AW complex were primarily due to the conservation of beneficials, not from high rates of mortality. This was confirmed when predators were analyzed separately for each field trial. Novaluron applications conserved lady beetles and damsel bugs when compared to the synthetic pyrethroid treatment ($P < 0.05$).

Alfalfa weevil parasitism was primarily caused from *Bathyplectes curculionis* in our field trials, although we identified *Oomyzus incertus* at low levels. Total parasitism rates ranged from 7 – 23% across untreated, novaluron, kaolin, and diflubenzuron plots compared to an average 2.0% in the lambda cyhalothrin treated plots ($P < 0.05$). This was likely due to applications targeting second instar AW larvae which coincided with the preferred host stage of *Bathyplectes curculionis* (Kingsley et al. 1993). The added benefit of conserving predators and parasitoids in combination with direct pesticide efficacy never reduced densities of AW larvae to that of the synthetic pyrethroid treatment. Our results indicate that *Bathyplectes curculionis* parasitism rates are too low to effectively manage high AW populations. This agrees with previous studies by Yeargan & Pass (1978) and Ayedh et al. (1996).

A broad-spectrum insecticide treatment made earlier in the year may preserve the natural enemy complex compared to traditional timing of applications two to three weeks

prior to harvest. Studies by Davis (1970) indicated no reductions in parasitism rates when applications of longer residual pesticides including carbofuran were made upon alfalfa growth early in the spring. Early applications of synthetic pyrethoids may result in a loss of efficacy prior to vulnerable AW larval stages and/or unnecessary financial losses from pro-active applications when future AW densities are not at economic levels. Additional studies investigating longer residual pesticide formulations may be helpful, especially in areas with AW larval densities that predictably rise over the economic threshold. By preserving beneficial parasitoids and predators with a timely broad-spectrum application, AW larval densities may be reduced while minimizing impacts on natural enemies.

Theoretically, by conserving parasitoids and predators in the first harvest cycle, novaluron treatments should harbor higher predator and parasitoid densities in the second harvest cycle which could suppress AW and/or pea aphids; however we didn't see any benefit to using our top performing chemistry in the second harvest cycle. This is likely due to degradation of pre-harvest treatments of lambda cyhalothrin in the second harvest cycle thus resulting in an invasion of adult damsel bugs and lady beetles from untreated areas; in combination with a lack of AW in the second harvest cycle. Much larger scale field studies targeting second generation AW would be helpful in determining whether the use of novaluron could conserve predator or parasitoid populations into the second harvest cycle. By assessing multi-voltine AW larvae over a much larger field area a more accurate comparison of novaluron and lambda cyhalothrin in the second harvest cycle would be possible. Even if pest reductions in the second harvest cycle were possible, the inability of these chemistries to consistently reduce AW larval populations below the

economic threshold limits there practical use. An increase in the predator-prey ratio simply driven by conservation of natural enemies is of little value to managing AW larvae or pea aphids. This is due to the high reproductive potential of pea aphids (Blodgett 2006) and the pea aphid being the primary prey of AW predators. Since novaluron doesn't eliminate the primary prey of AW predators, predator impacts would be minimal towards AW larvae due to persistent pea aphid populations.

Studies on the impacts of novaluron on second generation AW larvae and AW natural enemy complex in larger plots may be beneficial; however it is doubtful that these increased impacts would be economically viable due to the limited efficacy observed towards AW larvae or pea aphids. Future studies taking advantage of novalurons mode of action as a feeding deterrent should be explored. Novaluron may potentially be used with early cutting to increase yields to that of conventional broad-spectrum insecticides. If that were the case novaluron could be a preferred management tool as it also preserves AW larvae and pea aphid natural enemies.

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APPENDICES

APPENDIX A

AW EFFICACY, AW GROWTH RATES, AW DAMAGE, ALFALFA STAGE, DEGREE DAYS & YIELD

Table 1. GLM analysis of alfalfa weevil larvae / sweep, leaf defoliation, and yield after forage alfalfa was treated with azadirachtin, novaluron, kaolin, lambda cyhalothrin and neem oil over multiple timing intervals in 2006.

Alfalfa Weevil Larvae	**DF**	**F-Statistic**	**Pr>F**
Timing	3	7.11	0.0003*
Trt	5	31.37	<0.0001*
Date	1	3.30	0.17
Date x Timing	3	0.69	0.56
Trt x Timing	5	3.57	0.006*
Trt x Date	5	1.84	0.11
Leaf Defoliation	**DF**	**F-Statistic**	**Pr>F**
Timing	3	3.08	0.03*
Trt	5	54.16	<0.0001*
Date	1	144.85	<0.0001*
Date x Timing	3	1.95	0.12
Trt x Timing	5	4.36	0.001*
Trt x Date	5	6.02	<0.0001*
Yield	**DF**	**F-Statistic**	Pr>F
Timing	3	1.82	0.15
Trt	5	0.68	0.64
Timing*Trt	5	1.98	0.10

* Represents values significant at $P<0.05$.

Table 2. GLM analysis of alfalfa weevil larvae / sweep by pesticide treatment after forage alfalfa was treated over multiple timing intervals in 2006.

Kaolin	**DF**	**F-Statistic**	**Pr>F**
Timing	3	16.86	<0.0001*
Date	1	7.04	0.02*
Timing x Date	3	1.12	0.36
Diflubenzuron	DF	F-Statistic	Pr>F
Timing	3	0.11	0.95
Date	1	5.72	0.02*
Timing x Date	3	0.39	0.76
Azadirachtin	**DF**	**F-Statistic**	**Pr>F**
Timing	1	0.68	0.42
Date	1	4.18	0.07
Timing x Date	1	0.35	0.57
Novaluron	**DF**	**F-Statistic**	**Pr>F**
Timing	1	0.02	0.89
Date	1	2.08	0.18
Timing x Date	1	23.66	0.0009*

* Represents values significant at $P<0.05$.

Table 3. GLM analysis of leaf defoliation by pesticide treatment after forage alfalfa was treated over multiple timing intervals in 2006.

Kaolin	**DF**	**F-Statistic**	**Pr>F**
Timing	3	0.95	0.43
Date	1	153.03	<0.0001*
Timing x Date	3	1.22	0.32
Diflubenzuron	**DF**	**F-Statistic**	**Pr>F**
Timing	3	0.87	0.42
Date	1	204.74	<0.0001*
Timing x Date	3	1.08	0.38
Azadirachtin	**DF**	**F-Statistic**	**Pr>F**
Timing	1	2.40	0.15
Date	1	86.40	<0.0001*
Timing x Date	1	5.40	0.07
Novaluron	**DF**	**F-Statistic**	**Pr>F**
Timing	1	21.43	0.001*
Date	1	45.05	0.0001*
Timing x Date	1	23.66	0.0008*

* Represents values significant at $P<0.05$.

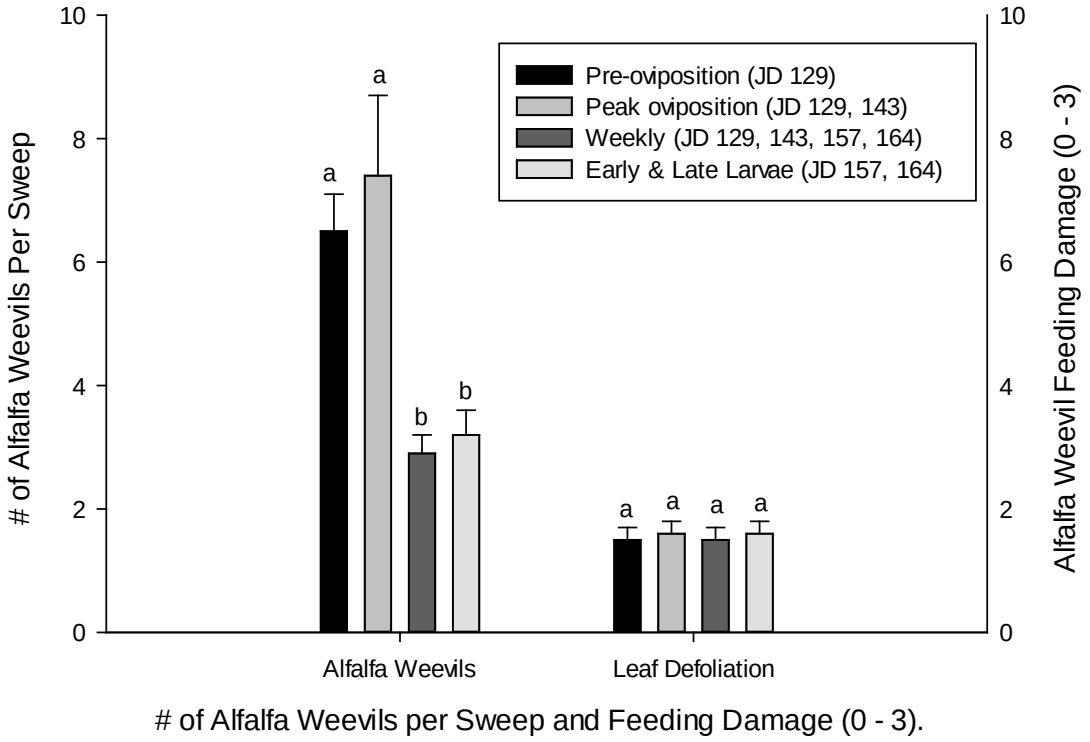


Figure 1. Average number of alfalfa weevils / sweep and feeding damage (0 = no leaf defoliation, 1 = 1 – 25%, 2 = 26 – 75%, 3 = > 75% leaf defoliation) at JD 170 and 177 in kaolin treated plots using various application timings in Bozeman, 2006. Means within bars followed by different letters are significantly different (LSD Test; $P = 0.05$).

Table 4. GLM analysis of application timings when evaluating leaf defoliation (0-3) and alfalfa weevils / sweep at various post application sample dates within novaluron treated alfalfa plots in 2006.

Alfalfa Weevils	**DF**	**F-Statistic**	**Pr>F**
Timing at Julian Date 157	1	9.72	0.05*
Timing at Julian Date 164	1	31.21	0.01*
Leaf Defoliation	**DF**	**F-Statistic**	**Pr>F**
Timing at Julian Date 157	1	10.07	0.06
Timing at Julian Date 164	1	11.00	0.04*

* Represents values significant at $P<0.05$.

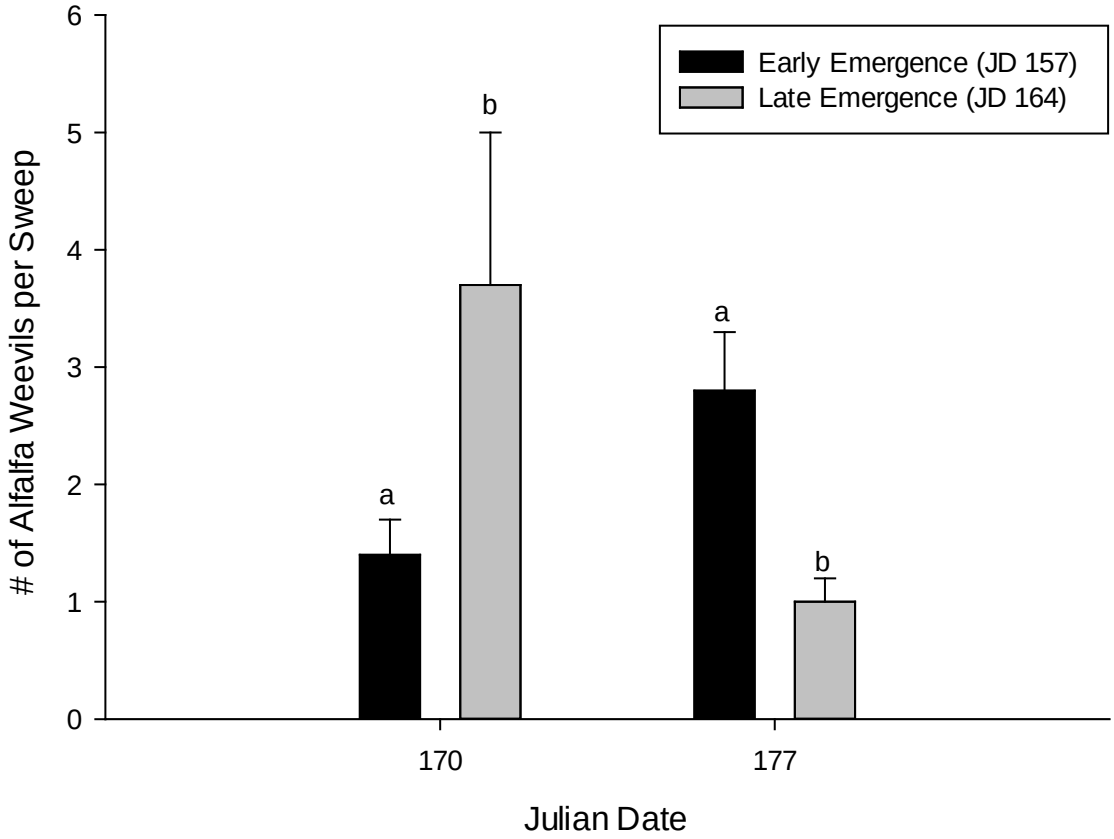


Figure 2. Comparison of application timings to suppress alfalfa weevils at various Julian dates (JD) in novaluron treated plots near Bozeman, 2006. Means within bars with different letters are significantly different (LSD Test; $P < 0.05$).

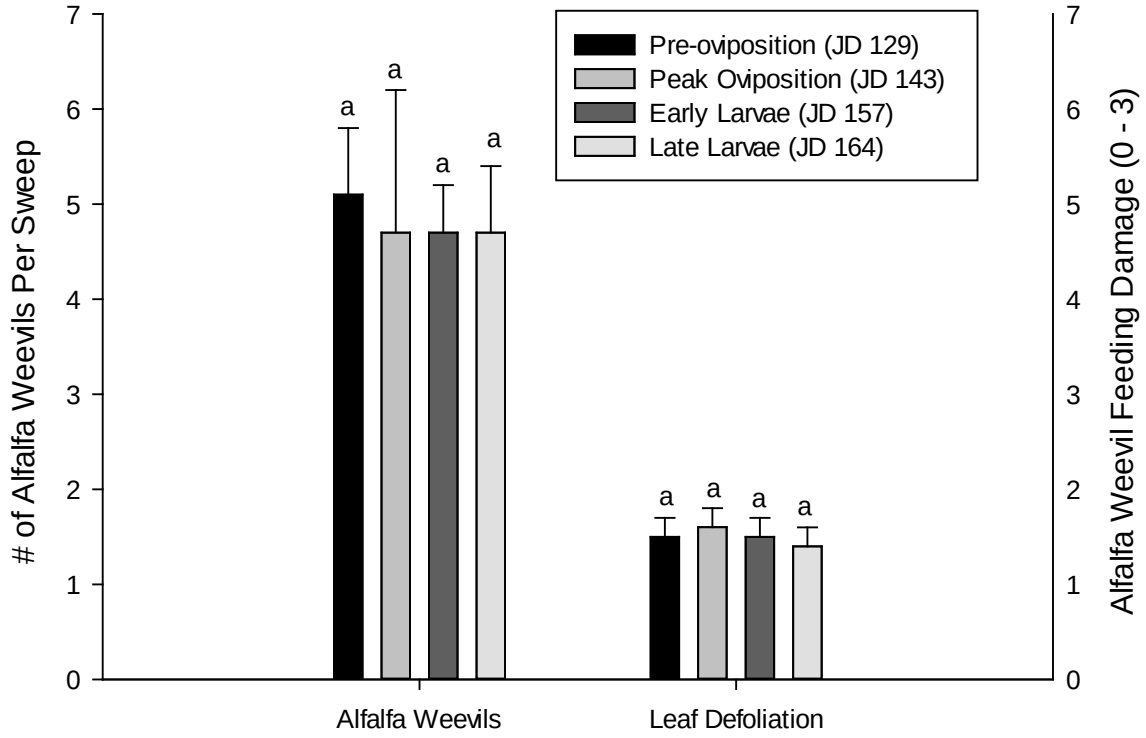


Figure. 3. Average number of alfalfa weevils / sweep and feeding damage (0 = no leaf defoliation, 1 = 1 – 25%, 2 = 26 – 75%, 3 = > 75% leaf defoliation) over Julian dates 170 and 177 in diflubenzuron treated plots using various application timings in 2006. Means within bars followed by different letters are significantly different (LSD Test; $P < 0.05$).

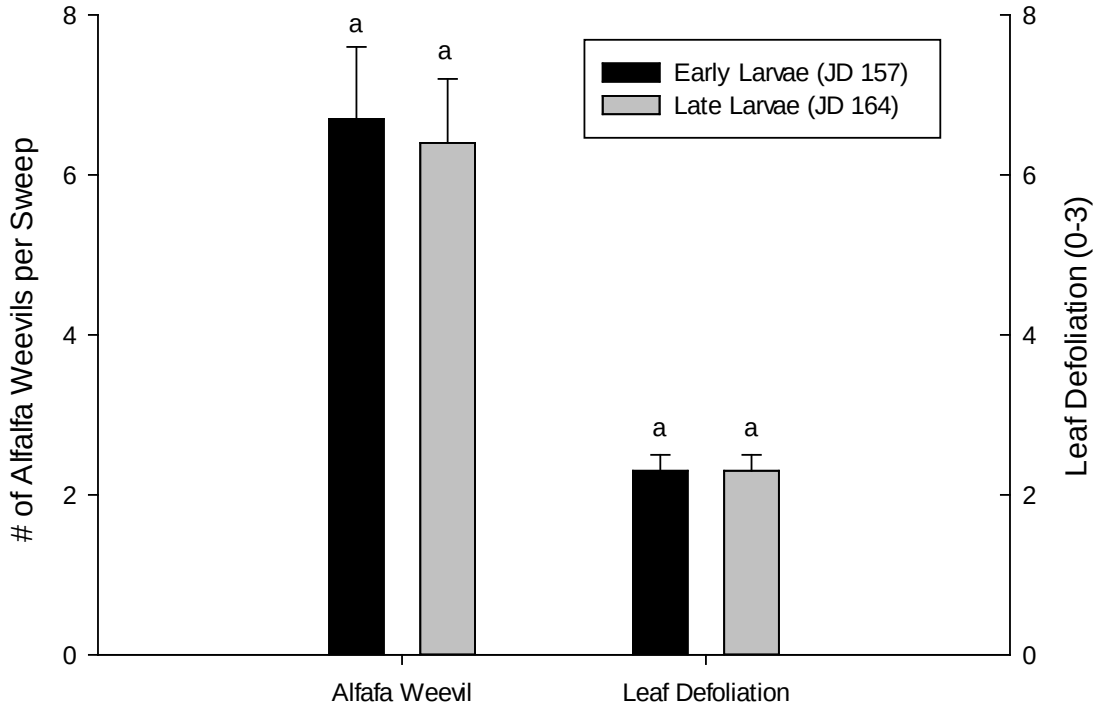


Figure 4. Average number of alfalfa weevils / sweep and feeding damage (0 = no leaf defoliation, 1 = 1 – 25%, 2 = 26 – 75%, 3 = > 75% leaf defoliation) over Julian dates 170 and 177 in azadirachtin treated plots using various application timings at Bozeman, 2006. Means within bars followed by different letters are significantly different (LSD Test; $P < 0.05$).

Table 5. Stem height, alfalfa mean stage by count (MSC), alfalfa weevil larval (AWL) growth stage, AWL degree day development, AWL / sweep and adult alfalfa weevil / sweep ± SE in untreated plots at various sample dates.

Field	Untreated Parameters	Julian Dates			
2006 Bozeman		157[a]	164	170	177
	Stem Height (cm)	54 ± 2.9	70.3 ± 2.6	86.0 ± 6.7	93.0 ± 9.0
	MSC	3.0 ± 0.0	3.8 ± 0.3	4.0 ± 0.0	5.8 ± 0.3
	AWL Growth Stage	2.0 ± 0.1	2.1 ± 0.1	2.2 ± 0.3	2.8 ± 0.1
	Degree Days	400	460	500	620
	AWL / Sweep	3.9 ± 1.0	7.8 ± 2.1	5.5 ± 1.1	7.6 ± 1.0
	Adults / Sweep	0.3 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
2009 Huntley		142[a]	147	155	162
	Stem Height (cm)	24.3 ± 0.3	38.2 ± 2.4	55.5 ± 7.0	70.0 ± 3.3
	MSC	1.0 ± 0.0	2.0 ± 0.0	2.5 ± 0.0	3.3 ± 0.3
	AWLl Growth Stage	1.8 ± 0.1	2.0 ± 0.2	2.6 ± 0.1	3.8 ± 0.1
	Degree Days	233	305	421	540
	AWL / Sweep	4.0 ± 0.6	18.2 ± 6.1	23.5 ± 1.4	28.3 ± 4.8
	Adults / Sweep	0.4 ± 0.2	0.9 ± 0.4	0.4 ± 0.2	0.6 ± 0.3
2009 Bozeman		162[a]	169	176	182
	Stem Height (cm)	46.0 ± 2.5	69.6 ± 0.4	78.8 ± 8.6	95.6 ± 3.7
	MSC	2.0 ± 0.0	3.0 ± 0.0	5.0 ± 0.0	5.8 ± 0.0
	AWL Growth Stage	2.1 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.8 ± 0.1
	Degree Days	325	433	500	606
	Weevils / Sweep	3.5 ± 0.3	3.8 ± 0.3	8.1 ± 0.7	13.9 ± 1.4
	Adults / Sweep	0.8 ± 0.2	1.0 ± 0.1	1.5 ± 0.2	1.7 ± 0.5

Data presented is untransformed.

[a] Applications of novaluron, kaolin and diflubenzuron were made on this date, while applications of lambda cyhalothrin and azadirachtin were made on the next sample date.

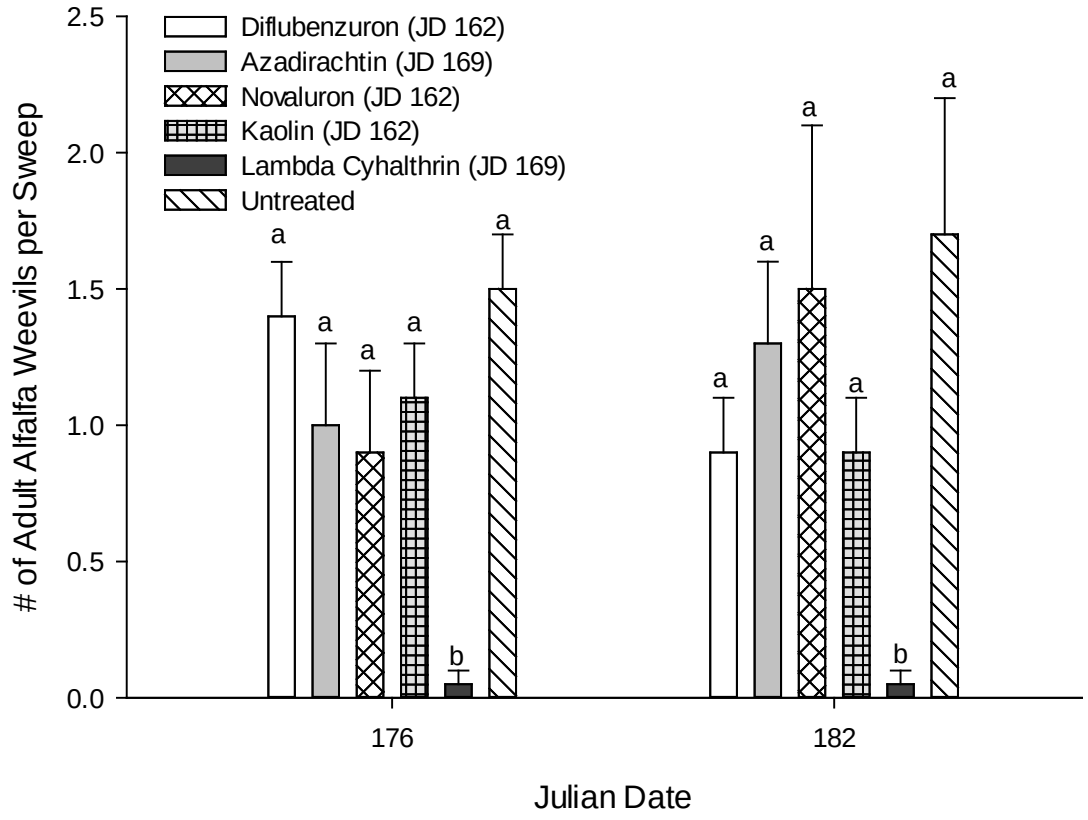


Figure 5. Number of adult alfalfa weevils / sweep ± SE after forage alfalfa was treated with various pesticide formulations at various treatment timings near Bozeman, Montana in 2009. Data transformed using square root + 0.5 transformation prior to analysis (LSD Test; Data presented is untransformed; $P < 0.05$).

Table 6. GLM analysis of yield (kg/ha) ± SE after forage alfalfa was treated with azadirachtin, novaluron, kaolin, and azadirachtin over multiple timing intervals at Bozeman, 2006.

		Yield (kg/ha)			
Application Timing Window	Julian Date of Application	Kaolin	Diflubenzuron	Azadirachtin	Novaluron
Pre-Oviposition	129	10,309 ± 448	10,085 ± 1,344	-	-
Pre & Peak Oviposition	129 and 143	7,620 ± 672	-	-	-
Peak Ovipostion	143	-	7,172 ± 1,120	-	-
Early Larvae	157	-	10,085 ± 672	8,965 ± 1,120	9,861 ± 1,120
Early Larvae and Peak Larvae	157 and 164	8,965 ± 896	-	-	-
Peak Larvae	164	-	9,189 ± 224	9,861 ± 672	9,413 ± 448
Weekly	All Dates	7,844 ± 672	-	-	-
F-Statistic		2.87	1.9	0.25	0.25
DF (model, error)		6, 9	6, 9	4, 3	4, 3
P-value		NS	NS	NS	NS

*Means within columns followed by similar letters are not significantly different (LSD Test; $P<0.05$).

Table 7. GLM analysis of percent reduction in alfalfa weevil larvae / sweep after treatment with azadirachtin, novaluron, kaolin, lambda cyhalothrin and azadirachtin at three field sites over four sample dates in 2006 and 2009.

Day 0[a b]	**DF**	**F-Statistic**	**Pr>F**
Field - Year	2	3.14	0.58
Trt	3	5.06	0.006*
Field x Trt	6	1.61	0.20
Day 7[c]	**DF**	**F-Statistic**	**Pr>F**
Field - Year	2	0.86	0.42
Trt	5	2.63	0.03
Field x Trt	10	1.97	0.06
Day 14	**DF**	**F-Statistic**	**Pr>F**
Field - Year	2	8.09	0.001*
Trt	5	36.00	<0.0001*
Field x Trt	10	3.04	0.005*
Day 21	**DF**	**F-Statistic**	**Pr>F**
Field - Year	2	1.76	0.18
Trt	5	48.98	<0.0001*
Field x Trt	10	1.97	0.06

* Represents values significant at $P<0.05$.

a Represents approximate sample date intervals relatative to applications.

b Novaluron, kaolin and diflubenzuron were applied on day 0.

c Azadirachtin and lambda cyhalothrin were applied on day 7.

Table 8. Percent reduction in alfalfa weevil larvae (AWL) / sweep ± SE following treatment with azadirachtin, novaluron, kaolin, lambda cyhalothrin and azadirachtin.

Field	Treatment	Rate (gai/ha)	Julian Date		
2006 Bozeman			164[a]	170	177
	Diflubenzuron	22.7	26 ± 16	29 ± 14*	21 ± 8*
	Azadirachtin	7.8	0 ± 0	16 ± 16	22 ± 16
	Novaluron	31.0	51 ± 14	74 ± 3*	62 ± *8**
	Kaolin	6,544.6	24 ± 10	48 ± 12*	52 ± 4*
	Lambda Cyhalothrin	5.5	0 ± 0	92 ± 2*	95 ± 4*
	F- Statistic		NS	18.72	16.59
	df(model, error)		6, 9	8, 15	8, 15
	P - value		NS	0.0001	0.0001
2009 Huntley			147[a]	155	162
	Diflubenzuron	22.7	32 ± 16	10 ± 8	21 ± 12*
	Azadirachtin	7.8	0 ± 0	8 ± 8	11 ± 5
	Novaluron	31.0	5 ± 4	22 ± 20	27 ± 16*
	Kaolin	6,544.6	0 ± 0	0 ± 0	18 ± 12
	Lambda Cyhalothrin	5.5	0 ± 0	87 ± 3*	99 ± 2*
	F - Statistic		3.09	9.34	19.3
	df(model, error)		7, 10	7, 10	7, 10
	P - value		0.06	0.001	<0.0001
2009 Bozeman			169[a]	176	182
	Diflubenzuron	22.7	0 ± 0	21 ± 8*	12 ± 7
	Azadirachtin	7.8	0 ± 0	39 ± 9*	42 ± 4*
	Novaluron	31.0	14 ± 7	21 ± 8*	31 ± 10*
	Kaolin	6,544.6	11 ± 11	14 ± 10*	21 ± 9*
	Lambda Cyhalothrin	5.5	0 ± 0	99 ± 2*	98 ± 3*
	F - Statistic		1.45	19.87	31.52
	df(model, error)		8, 15	8, 15	8, 15
	P - value		0.26	<0.0001	<0.0001

*Means within columns followed by * are significantly different than the untreated (LSD Test after square root arc-sine transformation; $P < 0.05$).

[a] Applications of lambda cyhalothrin and azadirachtin were made on this date, while applications of novaluron, kaolin and diflubenzuron were made approximately 7 d prior at each field site.

Table 9. GLM analysis of leaf defoliation after forage alfalfa was treated with azadirachtin, novaluron, kaolin, lambda cyhalothrin and azadirachtin at three field sites at four sample dates in 2006 and 2009.

Day 0[a b]	**DF**	**F-Statistic**	**Pr>F**
Field	2	0.01	0.99
Trt	3	0.01	0.99
Field x Trt	6	0.01	0.99
Day 7[c]	**DF**	**F-Statistic**	**Pr>F**
Field	2	23.76	<0.0001*
Trt	5	1.51	0.20
Field x Trt	10	1.88	0.07
Day 14	**DF**	**F-Statistic**	**Pr>F**
Field	2	33.07	<0.0001*
Trt	5	18.74	<0.0001*
Field x Trt	10	2.58	0.01*
Day 21	**DF**	**F-Statistic**	**Pr>F**
Field	2	63.12	<0.0001*
Trt	5	19.51	<0.0001*
Field x Trt	10	10.94	<0.0001*

* Represents values significant at $P<0.05$.

[a] Represents approximate sample date intervals relative to timing of applications.

[b] Novaluron, kaolin and diflubenzuron were applied on day 0.

[c] Azadirachtin and lambda cyhalothrin were applied on day 7.

Table 10. Alfalfa weevil leaf defoliation index (0 – 3) ± SE after forage alfalfa was treated with diflubenzuron, azadirachtin, novaluron, kaolin, and lambda cyhalothrin.

Field[b]	Treatment	Rate gai/ha	Julian Dates			
2006-B			157[a]	164[a]	170	177
	Diflubenzuron	22.7	0.0 ± 0.0	0.0 ± 0.0	1.1 ± 0.2ab	2.0 ± 0.2a
	Azadirachtin	7.8	-	0.0 ± 0.0	0.9 ± 0.1b	2.0 ± 0.1a
	Novaluron	31.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.1c	1.1 ± 0.2b
	Kaolin	6,544.6	0.0 ± 0.0	0.0 ± 0.0	1.2 ± 0.1a	1.9 ± 0.1a
	λ Cyhalothrin	5.5	-	0.0 ± 0.0	0.1 ± 0.1d	0.3 ± 0.1c
	Untreated		0.0 ± 0.0	0.0 ± 0.0	1.3 ± 0.1a	2.2 ± 0.2a
	F - Statistic		.	.	34.46	41.66
	df(model, error)		6, 9	8, 15	8, 15	8, 15
	P – value		NS	NS	<0.0001	<0.0001
2009-H			142[a]	147	155	162
	Diflubenzuron	22.7	0.0 ± 0.0	0.6 ± 0.2	1.8 ± 0.2a	1.7 ± 0.3c
	Azadirachtin	7.8	-	0.2 ± 0.2	2.1 ± 0.2a	3.0 ± 0.1a
	Novaluron	31.0	0.0 ± 0.0	0.2 ± 0.2	0.3 ± 0.1b	1.0 ± 0.1d
	Kaolin	6,544.6	0.0 ± 0.0	0.4 ± 0.1	1.5 ± 0.2a	2.3 ± 0.3b
	λ Cyhalothrin	5.5	-	0.2 ± 0.2	0.6 ± 0.2b	0.7 ± 0.3d
	Untreated		0.0 ± 0.0	0.5 ± 0.2	1.9 ± 0.2a	2.7 ± 0.3ab
	F – Statistic		.	1.51	14.35	22.42
	df(model, error)		6, 9	7, 10	7, 10	7, 10
	P – value		NS	0.27	0.0003	<0.0001
2009-B			162[a]	169	176	182
	Diflubenzuron	22.7	0.0 ± 0.0	0.1 ± 0.1	0.8 ± 0.3	1.0 ± 0.0
	Azadirachtin	7.8	-	0.1 ± 0.1	0.5 ± 0.3	1.0 ± 0.3
	Novaluron	31.0	0.0 ± 0.0	0.1 ± 0.1	0.3 ± 0.3	1.0 ± 0.1
	Kaolin	6,544.6	0.0 ± 0.0	0.1 ± 0.1	0.3 ± 0.3	0.3 ± 0.3
	λ Cyhalothrin	5.5	-	0.2 ± 0.1	0.0 ± 0.0	1.0 ± 0.3
	Untreated		0.0 ± 0.0	0.1 ± 0.1	0.8 ± 0.3	0.3 ± 0.3
	F – Statistic		.	0.81	1.57	2.33
	df(model, error)		6, 9	8, 15	8, 15	8, 15
	P - value		NS	0.56	0.22	0.09

*Means within columns followed by similar letters are not significantly different (LSD Test; $P < 0.05$).

[a] Shaded areas represent date of application.

[b] B = Bozeman sites; H = Huntley site.

Table 11. GLM analysis of alfalfa weevil adults after forage alfalfa was treated with azadirachtin, novaluron, kaolin, lambda cyhalothrin and azadirachtin at three field sites at four sample dates in 2006 and 2009.

Day 0[a b]	**DF**	**F-Statistic**	**Pr>F**
Field – Year	2	16.57	<0.0001*
Trt	3	0.70	0.56
Field x Trt	6	0.35	0.90
Day 7[c]	**DF**	**F-Statistic**	**Pr>F**
Field – Year	2	34.00	<0.0001*
Trt	5	0.83	0.53
Field x Trt	10	0.63	0.78
Day 14	**DF**	**F-Statistic**	**Pr>F**
Field – Year	2	40.16	<0.0001*
Trt	5	5.40	0.0006*
Field x Trt	10	4.82	0.0001*
Day 21	**DF**	**F-Statistic**	**Pr>F**
Field - Year	2	20.78	0.0001*
Trt	5	4.38	0.002*
Field x Trt	10	2.14	0.04*

* Represents values significant at $P < 0.05$.

[a] Represents approximate sample date intervals relative to date of initial applications.

[b] Novaluron, kaolin and diflubenzuron were applied on day 0.

[c] Azadirachtin and lambda cyhalothrin were applied on day 7.

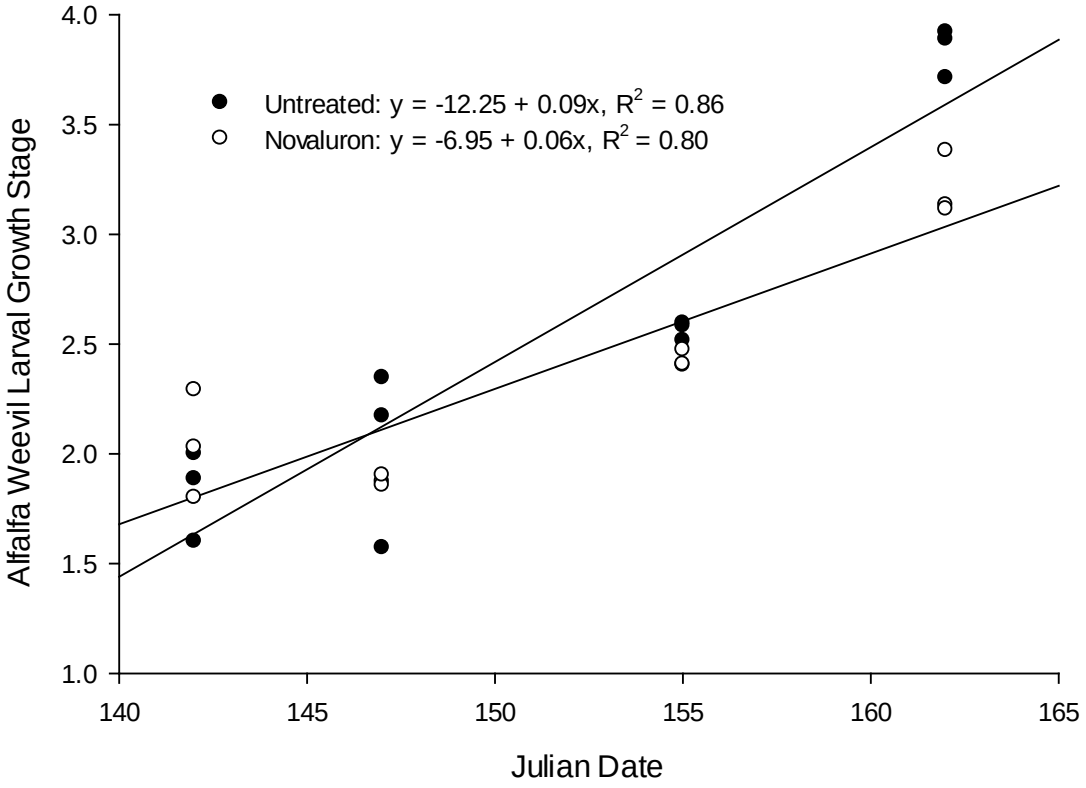


Figure 6. Regression of alfalfa weevil growth stage (1^{st} – 4^{th} instar) over time in untreated and novaluron treated plots near Huntley, Montana in 2009. Slopes were significantly different (PROC REG, 95% Confidence Interval).

Table 12 GLM analysis of alfalfa height ± SE after forage alfalfa was treated with azadirachtin, novaluron, kaolin, lambda cyhalothrin and azadirachtin at three field sites at four sample dates in 2006 and 2009.

Day 0[a b]	**DF**	**F-Statistic**	**Pr>F**
Field	2	101.55	<0.0001*
Trt	3	1.63	0.20
Field x Trt	6	1.0	0.44
Day 7[c]	**DF**	**F-Statistic**	**Pr>F**
Field	2	297.29	<0.0001*
Trt	5	1.60	0.17
Field x Trt	10	1.54	0.15
Day 14	**DF**	**F-Statistic**	**Pr>F**
Field	2	7.25	0.002*
Trt	5	0.23	0.94
Field x Trt	10	0.73	0.69
Day 21	**DF**	**F-Statistic**	**Pr>F**
Field	2	6.04	0.005*
Trt	5	0.41	0.84
Field x Trt	10	2.10	0.04*

* Represents values significant at $P < 0.05$.

[a] Represents approximate sample date intervals after initial application.

[b] Novaluron, kaolin and diflubenzuron were applied on day 0.

[c] Azadirachtin and lambda cyhalothrin were applied on day 7.

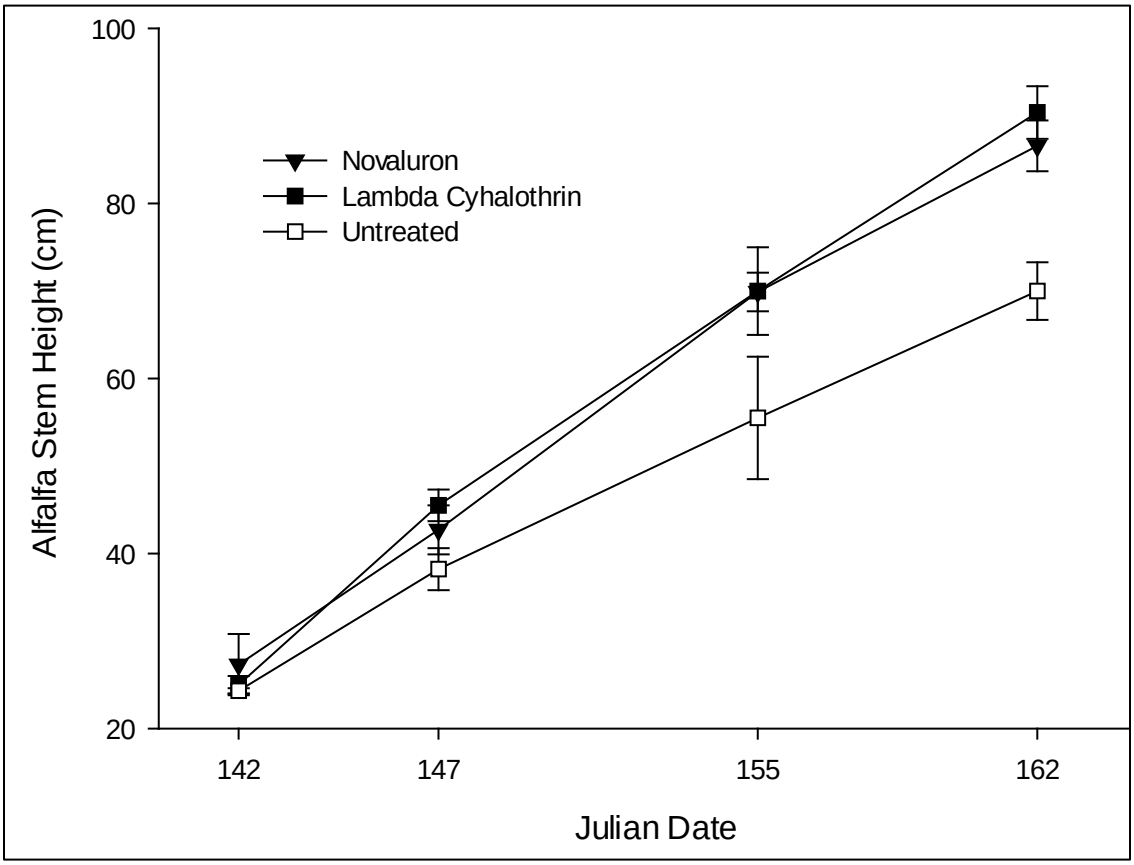


Figure 7. Forage alfalfa stem height (cm) ± SE over time after forage alfalfa was treated with novaluron at SARC, Huntley, Montana in 2009.

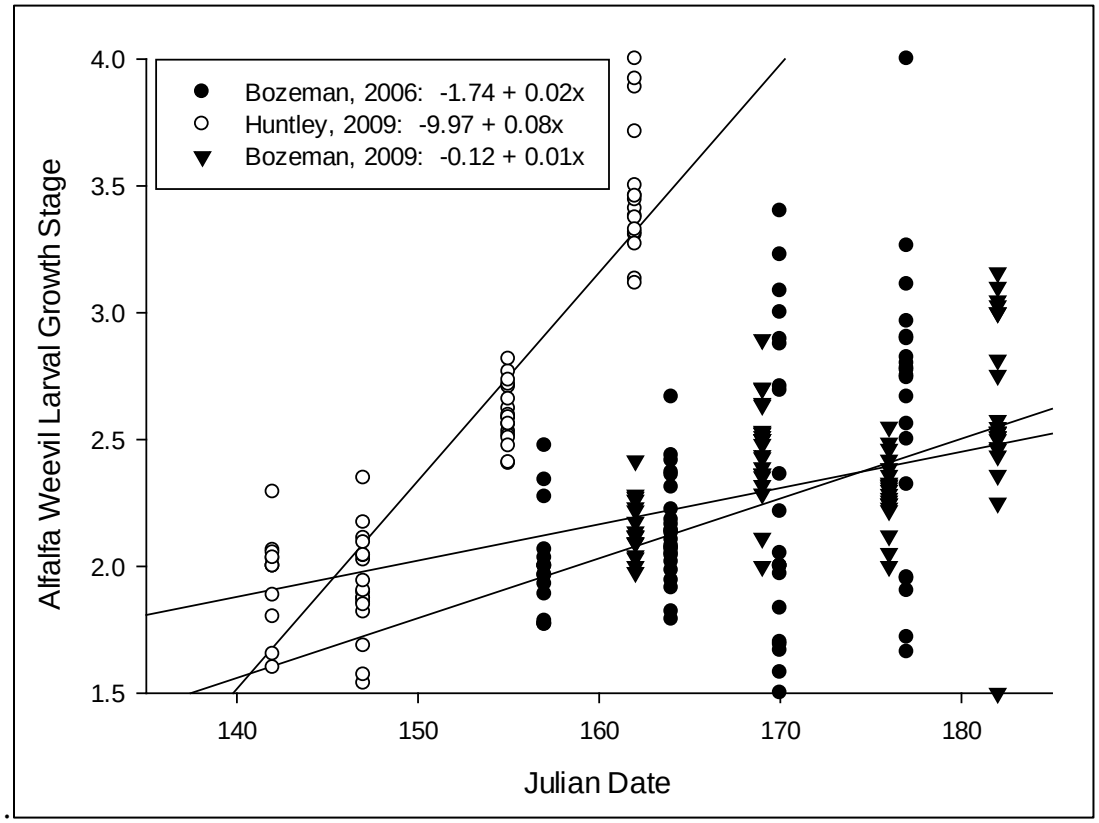


Figure 8. Linear regressions of alfalfa weevil larval growth stage (1^{st} – 4^{th} instar) by field. Alfalfa weevils at the Huntley 2009 site had a significantly different slope (growth rate) over time than the other field sites in this study (Proc Reg, 95% Confidence Interval).

Table 13. Regression of alfalfa weevil larval growth stage over time after forage alfalfa was treated with azadirachtin, novaluron, kaolin, lambda cyhalothrin and diflubenzuron.

Field [a]	Treatment	Rate (gai/ha)	n	Intercept ± SE*	Slope ± SE*	95% CI (slope)*	r^{2*}	*P(slope)*
2006-B	Diflubenzuron	22.7	16	-4.33 ± 1.75	0.04 ± 0.01	0.01 – 0.06	0.50	0.002
	Azadirachtin	7.8	16	0.95 ± 5.33	0.01 ± 0.03	-0.01 – 0.08	0.01	0.82
	Novaluron	31.0	16	-1.11 ± 2.09	0.02 ± 0.01	0.01 – 0.04	0.15	0.13
	Kaolin	6,544.6	16	-1.07 ± 2.46	0.02 ± 0.01	0.01 – 0.05	0.12	0.19
	Lambda Cyhalothrin	5.5	16	2.96 ± 10.68	0.00 ± 0.06	-0.14 – 0.13	0.01	0.94
	Untreated		16	-4.18 ± 2.10	0.03 ± 0.01	0.01 – 0.07	0.40	0.008
	Overall		96	-1.74 ± 1.32	0.02 ± 0.01	0.01 – 0.04	0.09	0.003
2009-H	Diflubenzuron	22.7	12	-8.31 ± 1.24	0.07 ± 0.01	-0.05 – 0.08	0.89	<0.0001
	Azadirachtin	7.8	12	-12.01 ± 1.07	0.09 ± 0.01	0.07 – 0.11	0.96	<0.0001
	Novaluron	31.0	12	-6.95 ± 1.50	0.06 ± 0.01	0.05 – 0.07	0.80	<0.0001
	Kaolin	6,544.6	12	-9.15 ± 1.33	0.07 ± 0.01	0.05 – 0.09	0.88	<0.0001
	Lambda Cyhalothrin	5.5	12	-16.31 ± 2.15	0.12 ± 0.01	0.08 – 0.15	0.93	<0.0001
	Untreated		12	-12.26 ± 1.92	0.10 ± 0.01	0.07 – 0.13	0.86	<0.0001
	Overall		72	-9.97 ± 0.70	0.08 ± 0.01	0.07 – 0.09	0.84	<0.0001
2009-B	Diflubenzuron	22.7	16	-1.06 ± 1.35	0.2 ± 0.01	0.00 – 0.03	0.31	0.02
	Azadirachtin	7.8	16	-6.54 ± 0.05	0.05 ± 0.02	0.00 – 0.10	0.31	0.06
	Novaluron	31.0	16	1.52 ± 0.92	0.01 ± 0.01	-0.01 – 0.01	0.05	0.36
	Kaolin	6,544.6	16	-1.31 ± 1.18	0.02 ± 0.01	0.01 – 0.03	0.41	0.007
	Lambda Cyhalothrin	5.5	16	11.73 ± 7.04	0.06 - 0.04	-0.15 – 0.03	0.21	0.21
	Untreated		16	-2.85 ± 1.46	0.03 ± 0.01	0.01 – 0.05	0.47	0.003
	Overall		96	-0.12 ± 0.96	0.01 ± 0.01	0.00 – 0.02	0.07	0.01

*Values were obtained using proc reg on SAS.

[a] B = Bozeman sites; H = Huntley site.

Table 14. Total alfalfa weevil eggs / stem ± SE in untreated plots in forage alfalfa at multiple field sites in 2006 and 2009.

	AW development correlated with Julian Dates			
Field Sites	Peak Adult Activity	Early Instar Larvae	Peak Larvae	Pre-pupation
	JD 143	JD 157	JD 164	JD 177
Bozeman 2006	0.8 ± 0.3	0.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
	JD 130	JD 142	JD 147	JD 164
Huntley 2009	0.0 ± 0.0	0.1 ± 0.1	0.4 ± 0.1	0.2 ± 0.1
	JD 150	JD 162	JD 169	JD 182
Bozeman 2009	0.1 ± 0.1	0.4 ± 0.3	0.3 ± 0.1	0.2 ± 0.1

Table 15. Yield (kg/ha) ± SE and final plant height ± SE at harvest after forage alfalfa was treated with azadirachtin, novaluron, kaolin, and azadirachtin at three field locations, Montana.

Treatment	Rate (gai/ha)	Biomass (kg/ha)		
		2006 Bozeman	2009 Huntley	2009 Bozeman
Diflubenzuron	22.7	9992 ± 642	7090 ± 727	5855 ± 559
Azadirachtin	7.8	9832 ± 563	8394 ± 347	4174 ± 602
Novaluron	31.0	9745 ± 893	8891 ± 534	7101 ± 2693
Kaolin	6,544.6	8928 ± 887	7681 ± 374	6887 ± 629
Lambda Cyhalothrin	5.5	8630 ± 545	7464 ± 608	4952 ± 1402
Untreated		8252 ± 642	7709 ± 971	6381 ± 357
Mean Stage by Count		5.8	5.3	5.7
F – Statistic		1.33	0.94	0.77
DF (model, error)		8, 15	7, 10	8, 15
P-value		0.30	0.94	0.58

*Application windows with similar letters within columns are not significantly different (LSD Test; $P < 0.05$).

Table 16. Summary of repeated measures analysis of alfalfa weevil mortality and leaf defoliation in two greenhouse trials at Montana State University, Bozeman, Montana.

Mortality	**DF**	**F-Statistic**	**Pr>F**
Days	5	1.99	0.08*
Trial	1	0.47	0.49
Trial x Days	5	1.72	0.13
Days x Trt	10	3.19	0.0008*
Trial x Trt	2	7.01	0.001*
Trt	2	362.05	<0.0001*
Leaf Defoliation	**DF**	**F-Statistic**	**Pr>F**
Days	5	71.27	<0.0001*
Trial	1	2.68	0.10
Trial x Days	5	0.96	0.44
Days x Trt	10	15.33	<0.0001*
Trial x Trt	2	1.47	0.23
Trt	2	389.24	<0.0001*

* Represents values significant at $P<0.05$.

Table 17. Summary of repeated measures analysis of stem height and percent displaced alfalfa weevil larvae in greenhouse trials at Montana State University, Bozeman, Montana.

Displaced Larvae	**DF**	**F-Statistic**	**Pr>F**
Days	5	16.36	<0.0001*
Trial	1	0.04	0.83
Trial x Days	5	1.04	0.39
Days x Trt	10	17.56	<0.0001*
Trial x Trt	2	3.53	0.03*
Trt	2	197.70	<0.0001*
Stem Height	**DF**	**F-Statistic**	**Pr>F**
Days	5	762.76	<0.0001*
Trial	1	27.97	<0.0001*
Trial x Days	5	15.07	<0.0001*
Days x Trt	10	11.26	<0.0001*
Trial x Trt	2	2.29	0.10
Trt	2	17.47	<0.0001*

* Represents values significant at $P<0.05$.

Table 18. Percent corrected mortality of alfalfa weevil larvae ± SE at various days after treatment (DAT) after infested forage alfalfa was treated with novaluron and lambda cyhalothrin under laboratory conditions at MSU, Bozeman, MT in 2010.

Trial	Treatment	Rate (gai/ha)	Percent Corrected Mortality				
			1 DAT	2 DAT	3 DAT	7 DAT	14 DAT
Trial #1	Novaluron	31.0	3 ± 2	10 ± 4	12 ± 5	23 ± 8*	22 ± 8*
	Lambda Cyhalothrin	5.5	90 ± 4*	93 ± 4*	93 ± 4*	93 ± 4*	93 ± 4*
	F- Statistic		145.30	76.74	76.16	48.04	44.12
	df(model, error)		7, 10	7, 10	7, 10	7, 10	7, 10
	P - value		<0.0001	< 0.0001	<0.0001	<0.0001	<0.0001
Trial #2	Novaluron	31.0	4 ± 3	10 ± 4	13 ± 5	14 ± 7*	31 ± 16
	Lambda Cyhalothrin	5.5	89 ± 5*	92 ± 3*	91 ± 4*	93 ± 4*	75 ± 16*
	F - Statistic		165.23	135.69	129.03	121.71	8.76
	df(model, error)		7, 10	7, 10	7, 10	7, 10	7, 10
	P – value		<0.0001	<0.0001	<0.0001	<0.0001	NS

*Means within columns followed by * are significantly different than the untreated (LSD Test after arc-sine transformation; *P=0.05*).

Table 19. GLM analysis of stem height and yield after plants were cut from pots in greenhouse trials at Montana State University, Bozeman, Montana.

Stem Height	**DF**	**F-Statistic**	**Pr>F**
Trial	1	12.55	0.002*
Trial x Trt	2	0.24	0.78
Trt	2	9.24	0.001*
Biomass	**DF**	**F-Statistic**	**Pr>F**
Trial	1	8.49	0.007*
Trial x Trt	2	0.83	0.44
Trt	2	9.87	0.0007*

* Represents values significant at $P < 0.05$.

Table 20. Biomass (grams) ± SE and final plant height ± SE 14 d post application after forage alfalfa was treated with novaluron and lambda cyhalothrin in two laboratory trials, MSU, Bozeman, MT.

Treatment	Rate (gai/ha)	Greenhouse Trial #1		Greenhouse Trial #2		Overall	
		Plant Ht (cm)	Biomass (g)	Plant Ht (cm)	Biomass (g)	Plant Ht (cm)	Biomass (g)
Novaluron	31.0	34.4 ± 1.5a	3.4 ± 0.3b	28.9 ± 2.0ab	2.7 ± 0.2ab	31.7 ± 1.5b	3.1 ± 0.2b
Lambda Cyhalothrin	5.5	36.0 ± 2.0a	3.5 ± 0.2b	31.9 ± 2.0b	2.9 ± 0.2b	33.9 ± 1.5b	3.2 ± 0.2b
Untreated		29.4 ± 1.7a	2.4 ± 0.3a	22.7 ± 1.8a	2.2 ± 0.1a	26.0 ± 1.6a	2.3 ± 0.2a
F – Statistic		3.44	5.52	5.35	12.88	6.81	7.87
DF (model, error)		7, 10	7, 10	7, 10	7, 10	7, 28	7, 28
P-value		NS	0.02	0.02	0.001	0.004	0.001

*Application windows with similar letters within columns are not significantly different (LSD Test; $P < 0.05$).

APPENDIX B

PRE & POST HARVEST NATURAL ENEMIES & SECONDARY PEST, PEA APHID

Table 1. Lady beetles (*Coccinellid spp*.), total predators (nabids + lady beetles), & predator- AW ratio after treatment with pesticides at a field site near Bozeman, 2006.

Total Lady Beetles	**DF**	**F-Statistic**	**Pr>F**
Date	3	9.49	<0.0001*
Trt	5	4.28	0.002*
Date x Trt	13	4.17	<0.0001*
Rep	3	0.22	0.88
H. convergens	**DF**	**F-Statistic**	**Pr>F**
Date	3	2.19	0.09
Trt	5	4.20	0.002*
Date x Trt	13	3.81	0.0002*
Rep	3	0.18	0.91
C. septumpunctata	**DF**	**F-Statistic**	**Pr>F**
Date	3	8.91	<0.0001*
Trt	5	**2.95**	0.02*
Date x Trt	13	2.90	0.002*
Rep	3	0.10	0.95
C. transversoguttata	**DF**	**F-Statistic**	**Pr>F**
Date	3	2.58	0.06
Trt	5	1.44	0.22
Date x Trt	13	1.76	0.06
Rep	3	1.00	0.39
C. trifasciata	**DF**	**F-Statistic**	**Pr>F**
Date	3	0.97	0.41
Trt	5	0.95	0.45
Date x Trt	13	1.05	0.42
Rep	3	1.00	0.39
H. parenthesis	**DF**	**F-Statistic**	**Pr>F**
Date	3	0.46	0.70
Trt	5	1.39	0.24
Date x Trt	13	0.89	0.56
Predators	**DF**	**F-Statistic**	**Pr>F**
Date	3	30.85	<0.0001*
Trt	5	6.27	<0.0001*
Date x Trt	13	3.64	0.003*
Predator/Prey AW	**DF**	**F-Statistic**	**Pr>F**
Date	3	9.64	<0.0001*
Trt	5	5.98	0.0001*
Date x Trt	13	1.60	0.10
Rep	3	1.68	0.18

* Represents values significant at $P<0.05$ (GLM after square root + 0.5 transformation for all factors except predator/prey ratio; predator prey ratio data was analyzed after square root arc sine transformation*).*

Table 2. GLM analysis of alfalfa weevils, pea aphids, damsel bugs (Nabidae), parasitoid wasps, predator-pea aphid ratio and spiders after alfalfa was treated with novaluron, diflubenzuron, kaolin, lambda cyhalothrin and azadirachtin near Bozeman in 2006.

Total Alfalfa Weevils	**DF**	**F-Statistic**	**Pr>F**
Date	3	19.25	<0.0001*
Trt	5	16.79	<0.0001*
Date x Trt	13	4.08	<0.0001*
Rep	3	8.02	0.0001*
Alfalfa Weevil Adults	**DF**	**F-Statistic**	**Pr>F**
Date	3	1.30	0.28
Trt	5	0.14	0.98
Date x Trt	13	1.01	0.45
Rep	3	0.87	0.46
Alfalfa Weevil Larvae	**DF**	**F-Statistic**	**Pr>F**
Date	3	19.35	<0.0001*
Trt	5	28.33	<0.0001*
Date x Trt	13	6.94	<0.0001*
Rep	3	7.74	0.0002*
Parasitic Wasps	**DF**	**F-Statistic**	**Pr>F**
Date	3	7.02	0.0004*
Trt	5	1.70	0.17
Date x Trt	13	1.52	0.07
Rep	3	0.24	0.86
Pea Aphids	**DF**	**F-Statistic**	**Pr>F**
Date	3	19.05	<0.0001*
Trt	5	20.00	<0.0001*
Date x Trt	13	4.10	<0.0001*
Rep	3	1.29	0.28
Damsel Bugs	**DF**	**F-Statistic**	**Pr>F**
Date	3	20.42	<0.0001*
Trt	5	1.68	0.15
Date x Trt	13	1.92	0.04*
Spiders	**DF**	**F-Statistic**	**Pr<F**
Date	3	23.29	<0.0001*
Trt	5	2.18	0.06
Date x Trt	13	2.36	0.01*
Predator/Prey Aphid	**DF**	**F-Statistic**	**Pr<F**
Date	3	20.88	<0.0001*
Trt	5	5.37	0.004*
Date x Trt	13	2.23	0.02*
Rep	3	0.56	0.64

* Represents values significant at $P < 0.05$ (GLM after square root + 0.5 transformation for every factor except aphids and alfalfa weevils which were log + 1 transformed).

Table 3. GLM analysis of total lady beetles (Coccinellidae), each lady beetle species, total predators (damsel bugs + lady beetles) predator / pea aphid ratio's, predator / alfalfa weevil (AW) ratio's after forage alfalfa was treated with diflubenzuron, novaluron, kaolin, lambda cyhalothrin and azadirachtin at a field site near Huntley in 2009.

Total Lady Beetles	**DF**	**F-Statistic**	**Pr>F**
Date	3	6.84	0.0007*
Trt	5	2.12	0.08
Date x Trt	13	0.90	0.55
Rep	2	0.06	0.94
C. septumpunctata	**DF**	**F-Statistic**	**Pr>F**
Date	3	5.93	0.0018*
Trt	5	2.36	0.05*
Date x Trt	13	1.04	0.43
Rep	2	0.17	0.84
H. parenthesis	**DF**	**F-Statistic**	**Pr>F**
Date	3	1.79	0.16
Trt	5	0.88	0.50
Date x Trt	13	0.85	0.61
Rep	2	2.10	0.13
Total Predators	**DF**	**F-Statistic**	**Pr>F**
Date	3	7.36	0.005*
Trt	5	2.65	0.03*
Date x Trt	13	0.55	0.87
Rep	2	0.76	0.47
Predator/Aphid Ratio	**DF**	**F-Statistic**	**Pr<F**
Date	3	4.82	0.006*
Trt	5	1.50	0.21
Date x Trt	13	0.74	0.71
Rep	2	0.82	0.44
Predator/AW Ratio	**DF**	**F-Statistic**	**Pr>F**
Date	3	11.79	<0.0001*
Trt	5	1.22	0.31
Date x Trt	13	0.49	0.91
Rep	2	0.25	0.77

* Represents values significant at $P<0.05$ (GLM after square root + 0.5 transformation for all factors except predator/prey ratio; predator prey ratio data was analyzed after square root arc sine transformation*)*.

Table 4. GLM analysis of alfalfa weevils, pea aphids, parasitoid wasps, damsel bugs and spiders (Areneae) after alfalfa was treated with diflubenzuron, novaluron, kaolin, lambda cyhalothrin and azadirachtin at a field site near Huntley in 2009.

Total Alfalfa Weevils	**DF**	**F-Statistic**	**Pr>F**
Date	3	71.28	<0.0001*
Trt	5	36.07	<0.0001*
Date x Trt	13	8.99	<0.0001*
Rep	2	4.67	0.01*
Alfalfa Weevil Adults	**DF**	**F-Statistic**	**Pr>F**
Date	3	4.91	0.005*
Trt	5	2.14	0.07
Date x Trt	13	0.66	0.78
Rep	2	2.87	0.06
Alfalfa Weevil Larvae	**DF**	**F-Statistic**	**Pr>F**
Date	3	86.90	<0.0001*
Trt	5	79.79	<0.0001*
Date x Trt	13	24.66	<0.0001*
Rep	2	6.17	0.004*
Parasitic Wasps	**DF**	**F-Statistic**	**Pr>F**
Date	3	6.60	0.0009*
Trt	5	2.00	0.08
Date x Trt	13	1.69	0.09
Rep	2	0.04	0.96
Pea Aphids	**DF**	**F-Statistic**	**Pr>F**
Date	3	4.61	0.007*
Trt	5	2.38	0.05*
Date x Trt	13	0.91	0.54
Rep	2	0.96	0.39
Damsel Bugs	**DF**	**F-Statistic**	**Pr>F**
Date	3	2.49	0.07
Trt	5	0.96	0.45
Date x Trt	13	0.47	0.92
Rep	2	1.51	0.23
Spiders	**DF**	**F-Statistic**	**Pr>F**
Date	3	3.91	0.02*
Trt	5	0.40	0.84
Date x Trt	13	0.22	0.99
Rep	2	2.32	0.11

* Represents values significant at $P<0.05$ (GLM after square root + 0.5 transformation for every factor except aphids and alfalfa weevils which were log + 1 transformed).

Table 5. GLM analysis of total lady beetles (Coccinellidae), each lady beetle species, total predators (damsel bugs and lady beetles) and predator-AW ratio after treatment with various pesticides at a field site near Bozeman in 2009.

Total Coccinellids	**DF**	**F-Statistic**	**Pr>F**
Date	3	0.62	0.60
Trt	5	2.62	0.03*
Date x Trt	13	0.97	0.48
Rep	3	0.56	0.64
H. convergens	**DF**	**F-Statistic**	**Pr>F**
Date	3	1.46	0.23
Trt	5	0.37	0.86
Date x Trt	13	0.89	0.57
Rep	3	0.67	0.57
C. septumpunctata	**DF**	**F-Statistic**	**Pr>F**
Date	3	1.20	0.31
Trt	5	*0.70*	0.43
Date x Trt	13	4.07	0.003*
Rep	3	0.38	0.76
C. transversoguttata	**DF**	**F-Statistic**	**Pr>F**
Date	3	0.77	0.51
Trt	5	0.51	0.76
Date x Trt	13	1.81	0.06
C. trifasciata	**DF**	**F-Statistic**	**Pr>F**
Date	3	1.03	0.38
Trt	5	0.62	0.68
Date x Trt	13	1.00	0.46
Rep	3	1.24	0.30
H. parenthesis	**DF**	**F-Statistic**	**Pr>F**
Date	3	0.97	0.41
Trt	5	0.95	0.45
Date x Trt	13	1.05	0.42
Rep	3	1.00	0.39
Total Predators	**DF**	**F-Statistic**	**Pr>F**
Date	3	0.87	0.48
Trt	5	3.05	0.01*
Date x Trt	13	1.04	0.43
Rep	3	0.77	0.51
Predator/Prey AW	**DF**	**F-Statistic**	**Pr<F**
Date	3	10.79	<0.0001*
Trt	5	1.60	0.17
Date x Trt	13	0.80	0.66
Rep	3	0.21	0.88

* Represents values significant at P < 0.05 (GLM after square root + 0.5 transformation).

Table 6. Alfalfa weevils, pea aphids, parasitoid wasps, nabids, predator-pea aphid ratio and spiders (Areneae) after alfalfa was treated with various pesticides, Bozeman, 2009.

Total Alfalfa Weevils	**DF**	**F-Statistic**	**Pr>F**
Date	3	57.32	<0.0001*
Trt	5	68.87	<0.0001*
Date x Trt	13	9.73	<0.0001*
Rep	3	0.55	0.65
Alfalfa Weevil Adults	**DF**	**F-Statistic**	**Pr>F**
Date	3	0.36	0.78
Trt	5	5.49	0.0003*
Date x Trt	13	3.15	0.0011*
Rep	3	0.55	0.64
Alfalfa Weevil Larvae	**DF**	**F-Statistic**	**Pr>F**
Date	3	30.19	<0.0001*
Trt	5	106.80	<0.0001*
Date x Trt	13	12.56	<0.0001*
Rep	3	1.27	0.29
Parasitic Wasps	**DF**	**F-Statistic**	**Pr>F**
Date	3	2.82	0.04*
Trt	5	1.43	0.22
Date x Trt	13	1.11	0.36
Rep	3	1.49	0.22
Pea Aphids	**DF**	**F-Statistic**	**Pr>F**
Date	3	20.35	<0.0001*
Trt	5	20.27	<0.0001*
Date x Trt	13	8.95	<0.0001*
Rep	3	1.27	0.25
Nabids	**DF**	**F-Statistic**	**Pr>F**
Date	3	6.23	0.0009*
Trt	5	0.91	0.48
Date x Trt	13	1.65	0.09
Spiders	**DF**	**F-Statistic**	**Pr>F**
Date	3	18.46	<0.0001*
Trt	5	0.25	0.93
Date x Trt	13	0.49	0.91
Predator/Prey Aphid	**DF**	**F-Statistic**	**Pr>F**
Date	3	6.59	0.006*
Trt	5	1.43	0.22
Date x Trt	13	0.42	0.95
Rep	3	0.32	0.81

* Represents values significant at $P<0.05$ (GLM after square root + 0.5 transformation for every factor except aphids and alfalfa weevils which were log + 1 transformed; and predator/prey ratio which was analyzed after square root arc sine transformation).

Table 7. Total lady beetles (Coccinellidae) ± SE / 10 sweeps at various Julian dates after forage alfalfa was treated with diflubenzuron, azadirachtin, novaluron, kaolin, and lambda cyhalothrin.

Field	Treatment	Total Lady Beetles			
2006 Bozeman		JD 157[a]	JD 164[a]	JD 170	JD 177
	Diflubenzuron	1.3 ± 0.6	0.5 ± 0.5	0.8 ± 0.3ab	2.5 ± 0.9a
	Azadirachtin	-	1.5 ± 0.5	0.5 ± 0.3ab	0.8 ± 0.3b
	Novaluron	0.3 ± 0.3	1.5 ± 0.3	1.5 ± 0.9a	6.0 ± 0.7a
	Kaolin	0.5 ± 0.5	2.3 ± 0.8	0.0 ± 0.0b	1.0 ± 0.4b
	Lambda Cyhalothrin	-	1.5 ± 0.6	0.0 ± 0.0b	0.8 ± 0.4b
	Untreated	1.3 ± 0.6	0.5 ± 0.5	1.8 ± 0.5a	2.5 ± 0.5a
	F - Statistic	1.89	1.39	3.21	8.73
	df(model, error)	6, 9	8, 15	8, 15	8, 15
	P – value	NS	NS	0.03	0.0005
2009 Huntley		JD 142[a]	JD 147	JD 155	JD 162
	Diflubenzuron	2.7 ± 0.7	6.7 ± 2.7	1.3 ± 1.3	2.0 ± 0.0
	Azadirachtin	-	5.3 ± 1.3	4.7 ± 2.4	4.7 ± 1.8
	Novaluron	3.3 ± 1.3	9.3 ± 4.0	2.0 ± 1.1	1.3 ± 0.7
	Kaolin	2.0 ± 1.2	6.0 ± 2.0	0.7 ± 0.7	3.3 ± 2.4
	Lambda Cyhalothrin	-	3.3 ± 1.3	0.0 ± 0.0	0.0 ± 0.0
	Untreated	1.3 ± 0.7	2.7 ± 0.7	1.3 ± 0.7	4.7 ± 2.7
	F – Statistic	0.67	0.91	1.25	1.67
	df(model, error)	5, 6	7, 10	7, 10	7, 10
	P – value	NS	NS	NS	NS
2009 Bozeman		JD 162[a]	JD 169	JD 176	JD 182
	Diflubenzuron	3.0 ± 0.6	2.3 ± 0.9	2.0 ± 0.4ab	2.3 ± 1.4
	Azadirachtin	-	1.5 ± 0.5	3.8 ± 0.8a	2.5 ± 0.9
	Novaluron	2.5 ± 0.6	2.5 ± 1.0	2.3 ± 0.8ab	3.5 ± 1.7
	Kaolin	3.5 ± 0.6	3.0 ± 1.3	3.0 ± 0.4ab	3.0 ± 0.6
	Lambda Cyhalothrin	-	2.5 ± 1.0	0.0 ± 0.0c	0.0 ± 0.0
	Untreated	3.3 ± 0.4	2.5 ± 1.3	1.3 ± 0.4ab	3.0 ± 1.3
	F – Statistic	0.45	0.17	6.46	1.22
	df(model, error)	6, 9	8, 15	8, 15	8, 15
	P - value	NS	NS	0.002	NS

*Means within columns followed by similar letters are not significantly different (LSD Test after square root + 0.5 transformation; $P < 0.05$; Data presented is untransformed).

[a] Shaded areas represent date of application.

Table 8. Composition of lady beetle (Coccinellidae) species ± SE / 10 sweeps averaged across treatments and blocks at various Julian dates (JD) after forage alfalfa was treated with various pesticides at multiple field sites.

Field*	Species Composition	%	Total	Percent Composition of Lady Beetles			
2006-B				JD 157[a]	JD 164	JD 170	JD 177
	H. convergens	10	2.8 ± 1.7	0.0 ± 0.0 (0%)	1.0 ± 0.5 (13%)	0.4 ± 0.2 (8%)	1.4 ± 0.9 (10%)
	C. septempunctata	74	21.9 ± 9.1	2.9 ± 1.0 (89%)	5.9 ± 2.5 (75%)	2.9 ± 1.6 (62%)	10.2 ± 4.0 (74%)
	C. transversoguttata	11	3.4 ± 2.6	0.4 ± 0.3 (11%)	0.5 ± 0.5 (6%)	0.7 ± 0.5 (15%)	1.8 ± 1.3 (13%)
	C. trifasciata	1	0.4 ± 0.2	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)	0.4 ± 0.2 (8%)	0.0 ± 0.0 (0%)
	H. parenthesis	4	1.3 ± 1.1	0.0 ± 0.0 (0%)	0.5 ± 0.5 (6%)	0.4 ± 0.2 (8%)	0.4 ± 0.4 (3%)
	S. punctum	0	0.0 ± 0.0	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)
	Total		29.8	3.3	7.9	4.8	13.8
		100					
2009-H				JD 142[a]	JD 147	JD 155	JD 162
	H. convergens	0	0.0 ± 0.0	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)
	C. septempunctata	97	66.6 ± 28.4	9.3 ± 3.4(100%)	31.3 ± 11.5 (94%)	10.0 ± 6.0 (100%)	16.0 ± 7.5 (100%)
	C. transversoguttata	0	0.0 ± 0.0	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)
	C. trifasciata	0	0.0 ± 0.0	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%
	H. parenthesis	3	2.0 ± 1.0	0.0 ± 0.0 (0%)	2.0 ± 1.0 (6%)	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)
	S. punctum	0	0.1 ± 0.1	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)	0.1 ± 0.1 (1%)
	Total	100	68.7	9.3	33.3	10.0	16.1
2009-B				JD 162[a]	JD 169	JD 176	JD 182
	H. convergens	6	3.0 ± 3.0	1.0 ± 1.0 (8%)	1.5 ± 1.5 (10%)	0.5 ± 0.5 (4%)	0.0 ± 0.0 (0%)
	C. septempunctata	82	44.4 ± 13.0	10.6 ± 2.0 (87%)	10.0 ± 5.0 (67%)	11.0 ± 3.0 (89%)	12.8 ± 3.0 (89%)
	C. transversoguttata	8	4.5 ± 4.0	0.6 ± 0.6 (5%)	2.0 ± 1.5 (13%)	0.9 ± 0.9 (7%)	1.0 ± 1.0 (7%)
	C. trifasciata	2	1.1 ± 1.1	0.0 ± 0.0 (0%)	0.5 ± 0.5 (3%)	0.0 ± 0.0 (0%)	0.6 ± 0.6 (4%)
	H. parenthesis	2	1.0 ± 1.0	0.0 ± 0.0 (0%)	1.0 ± 1.0 (7%)	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)
	S. punctum	0	0.0 ± 0.0	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%	0.0 ± 0.0 (0%)
	Total	100	54.0	12.2	15.0	12.4	14.4

[a] Applications of novaluron, kaolin and diflubenzuron were made on this date. All other application were made on the next date.
*B = Bozeman site, H = Huntley site.

Table 9. Total lady beetles (Coccinellidae) ± SE / 10 sweeps at various Julian dates (JD) after treatment with various pesticides at multiple field sites.

Field	Treatment	Total Lady Beetles / 10 Sweeps			
2006 Bozeman		JD 157[a]	JD 164[a]	JD 170	JD 177
	Diflubenzuron	1.3 ± 0.6	0.5 ± 0.5	0.8 ± 0.3ab	1.8 ± 0.3bc
	Azadirachtin	-	1.5 ± 0.5	0.3 ± 0.3bc	0.8 ± 0.3bc
	Novaluron	0.3 ± 0.3	1.3 ± 0.3	1.0 ± 0.4a	3.8 ± 0.8a
	Kaolin	0.3 ± 0.3	1.3 ± 0.5	0.0 ± 0.0c	1.0 ± 0.4bc
	Lambda Cyhalothrin	-	1.5 ± 0.6	0.0 ± 0.0c	0.8 ± 0.8c
	Untreated	1.0 ± 0.4	0.5 ± 0.5	1.3 ± 0.3a	2.3 ± 0.3ab
	F - Statistic	2.40	0.90	5.26	4.78
	df(model, error)	6, 9	8, 15	8, 15	8, 15
	P – value	NS	NS	0.005	0.008
2009 Huntley		JD 142[a]	JD 147	JD 155	JD 162
	Diflubenzuron	3.3 ± 1.3	6.7 ± 2.7	1.3 ± 1.3	2.0 ± 0.0
	Azadirachtin	-	5.3 ± 1.3	4.7 ± 2.4	4.7 ± 1.8
	Novaluron	2.0 ± 1.0	9.3 ± 4.1	2.0 ± 1.1	1.3 ± 0.7
	Kaolin	1.3 ± 0.7	6.0 ± 2.0	0.7 ± 0.7	3.3 ± 2.4
	Lambda Cyhalothrin	-	2.7 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
	Untreated	2.7 ± 0.7	2.0 ± 1.0	1.3 ± 0.7	4.7 ± 2.7
	F – Statistic	0.67	1.26	1.25	1.67
	df(model, error)	5, 6	7, 10	7, 10	7, 10
	P – value	NS	NS	NS	NS
2009 Bozeman		JD 162[a]	JD 169	JD 176	JD 182
	Diflubenzuron	2.8 ± 0.5	1.3 ± 0.8	1.8 ± 0.3ab	2.3 ± 1.4
	Azadirachtin	-	1.0 ± 0.6	3.0 ± 0.4a	2.0 ± 0.8
	Novaluron	2.3 ± 0.5	2.0 ± 0.8	2.3 ± 0.8ab	2.5 ± 0.9
	Kaolin	3.0 ± 0.4	2.0 ± 0.8	3.0 ± 0.4a	2.5 ± 0.5
	Lambda Cyhalothrin	-	1.0 ± 0.6	0.0 ± 0.0c	0.0 ± 0.0
	Untreated	2.8 ± 0.3	2.3 ± 1.0	1.3 ± 0.5b	2.0 ± 0.8
	F – Statistic	0.49	0.44	7.16	1.27
	df(model, error)	6, 9	8, 15	8, 15	8, 15
	P - value	NS	NS	0.001	NS

*Means within columns followed by similar letters are not significantly different (LSD Test after square root + 0.5 transformation; *P*=0.05*;* Data presented is untransformed).

[a] Shaded areas represent date of application.

Table 10. Total *H. convergens* and damsel bug species (*Nabid spp.)* ± SE / 10 sweeps at various Julian dates (JD) after forage alfalfa was treated with diflubenzuron, azadirachtin, novaluron, kaolin, and lambda cyhalothrin in Bozeman, 2006.

Field	Treatment	Rate (gai/ha)	Total *H. Convergens* / 10 Sweeps			
H. convergens			JD 157[a]	JD 164[a]	JD 170	JD 177
	Diflubenzuron	22.7	0.0 ± 0.0	0.0 ± 0.0b	0.0 ± 0.0	0.3 ± 0.3b
	Azadirachtin	7.8	-	0.0 ± 0.0b	0.0 ± 0.0	0.0 ± 0.0b
	Novaluron	31.0	0.0 ± 0.0	0.0 ± 0.0b	0.3 ± 0.3	1.0 ± 0.4a
	Kaolin	6,544.6	0.3 ± 0.3	0.8 ± 0.3a	0.0 ± 0.0	0.0 ± 0.0b
	Lambda Cyhalothrin	5.5	-	0.0 ± 0.0b	0.0 ± 0.0	0.0 ± 0.0b
	Untreated		0.0 ± 0.0	0.0 ± 0.0b	0.0 ± 0.0	0.0 ± 0.0b
	F - Statistic		1.00	9.00	1.00	3.75
	df(model, error)		6, 9	8, 15	8, 15	8, 15
	P – value		NS	0.0004	NS	0.02
Damsel Bugs			JD 142[a]	JD 147	JD 155	JD 162
	Diflubenzuron	22.7	0.3 ± 0.3	1.5 ± 0.6	0.0 ± 0.0	3.5 ± 0.9a
	Azadirachtin	7.8	-	1.8 ± 0.5	0.3 ± 0.3	2.5 ± 0.3a
	Novaluron	31.0	1.3 ± 0.3	1.3 ± 0.8	0.5 ± 0.3	1.8 ± 0.3a
	Kaolin	6,544.6	0.8 ± 0.5	1.5 ± 0.3	0.0 ± 0.0	2.3 ± 0.3a
	Lambda Cyhalothrin	5.5	-	1.8 ± 0.3	0.0 ± 0.0	0.3 ± 0.3b
	Untreated		1.3 ± 0.6	2.0 ± 0.7	0.5 ± 0.3	2.3 ± 1.0a
	F – Statistic		1.16	0.26	1.58	4.06
	df(model, error)		6, 9	8, 15	8, 15	8, 15
	P – value		NS	NS	NS	0.01

*Means within columns followed by similar letters are not significantly different (LSD Test after square root ± 0.5 transformation; $P < 0.05$; Data presented is untransformed).

[a] Shaded areas represent date of application.

Table 11. Composition of parasitoid wasps ± SE / 10 sweeps averaged over all treated plots at various Julian dates (JD) after forage alfalfa was treated with diflubenzuron, azadirachtin, novaluron, kaolin, and lambda cyhalothrin at multiple field sites.

Field	Hymenopteran Families/Superfamilies	Overall Proportion	Percent Composition of Parasitoid Wasps			
2006 Bozeman			JD 157[a]	JD 164	JD 170	JD 177
	Chalcidoidea	18	0.0 ± 0.0	1.5 ± 1.0	0.5 ± 0.5	2.5 ± 0.5
	Braconidae	31	1.0 ± 1.0	2.5 ± 2.5	1.5 ± 0.5	2.5 ± 0.5
	Ichneumonidae	50	0.5 ± 0.5	7.5 ± 4.0	0.5 ± 0.5	3.5 ± 1.5
	N	-	16	24	24	24
	Average Wasps In Sample	-	1.5 ± 1.5	11.5 ± 7.5	2.5 ± 1.5	8.5 ± 2.5
2009 Huntley			JD 142[a]	JD 147	JD 155	JD 162
	Chalcidoidea	4	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.5	0.0 ± 0.0
	Braconidae	0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Ichneumonidae	96	4.0 ± 1.5	5.0 ± 1.5	0.0 ± 0.0	2.5 ± 1.5
	N	-	12	18	18	18
	Average Wasps In Sample	-	4.0 ± 1.5	5.0 ± 1.5	0.5 ± 0.5	2.5 ± 1.5
2009 Bozeman			JD 162[a]	JD 169	JD 176	JD 182
	Chalcidoidea	20	0.0 ± 0.0	0.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
	Braconidae	0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Ichneumonidae	80	0.0 ± 0.0	1.5 ± 1.0	0.0 ± 0.0	0.5 ± 0.5
	N		16	24	24	24
	Average Wasps In Sample		0.0 ± 0.0	2.0 ± 1.0	0.0 ± 0.0	0.5 ± 0.5

[a] Applications of novaluron, kaolin and diflubenzuron were made on this date. Applications of lambda cyhalothrin and azadirachtin were made on the next sample date.

Table 12. Total alfalfa weevil larvae ± SE / 10 sweeps at various Julian dates (JD) after forage alfalfa was treated with various pesticide treatments at multiple field sites.

Field[b]	Treatment	Alfalfa Weevil Larvae / 10 Sweeps			
2006-B		JD 157[a]	JD 164[a]	JD 170	JD 177
	Diflubenzuron	28.3 ± 8.8	53.0 ± 10.4a	34.5 ± 1.8ab	59.5 ± 4.5ab
	Azadirachtin	-	76.0 ± 9.0a	49.8 ± 11.5a	67.0 ± 17.5a
	Novaluron	30.0 ± 15.0	31.5 ± 5.5b	14.0 ± 3.5c	27.5 ± 4.5c
	Kaolin	37.5 ± 11.5	57.5 ± 7.4a	25.8 ± 2.4b	37.5 ± 6.0c
	λ Cyhalothrin	-	81.3 ± 23.8a	4.3 ± 0.8d	4.0 ± 3.0d
	Untreated	38.5 ± 9.9	77.8 ± 21.6a	55.0 ± 10.7a	75.5 ± 9.4a
	F - Statistic	1.00	5.34	30.30	18.79
	df	6, 9	8, 15	8, 15	8, 15
	P – value	NS	0.005	<0.0001	<0.0001
2009-H		JD 142[a]	JD 147	JD 155	JD 162
	Diflubenzuron	45.3 ± 9.3	111.3 ± 11.1	214.0 ± 21.0ab	238.0 ± 31.4ab
	Azadirachtin	-	206.7 ± 15.7	262.0 ± 50.7ab	286.0 ± 9.9ab
	Novaluron	52.7 ± 9.0	198.7 ± 26.0	178.0 ± 40.4b	202.7 ± 23.3b
	Kaolin	38.7 ± 4.0	234.6 ± 41.5	287.5 ± 23.7a	259.3 ± 41.3ab
	λ Cyhalothrin	-	192.0 ± 3.5	30.0 ± 6.0c	2.0 ± 1.2c
	Untreated	40.0 ± 6.4	182.0 ± 61.0	234.7 ± 14.9ab	283.5 ± 48.4a
	F – Statistic	0.87	3.10	23.32	74.23
	df	5, 6	7, 10	7, 10	7, 10
	P – value	NS	NS	<0.0001	<0.0001
2009-B		JD 162[a]	JD 169	JD 176	JD 182
	Diflubenzuron	25.0 ± 0.9	48.3 ± 15.9	65.3 ± 9.6ab	125.0 ± 15.8ab
	Azadirachtin	-	39.5 ± 5.9	48.0 ± 5.3b	79.0 ± 4.0d
	Novaluron	25.0 ± 2.4	45.5 ± 14.4	64.3 ± 6.4ab	92.5 ± 5.0cd
	Kaolin	30.3 ± 2.4	37.0 ± 6.6	69.5 ± 12.5ab	107.0 ± 11.5bc
	λ Cyhalothrin	-	22.5 ± 5.7	0.8 ± 0.5c	2.0 ± 0.8e
	Untreated	34.5 ± 2.6	38.0 ± 2.5	81.0 ± 7.0a	138.8 ± 14.4a
	F – Statistic	3.46	0.97	105.42	91.48
	df	6, 9	8, 15	8, 15	8, 15
	P - value	NS	NS	<0.0001	<0.0001

*Means within columns followed by similar letters are not significantly different (LSD Test after log + 1.0 transformation; $P < 0.05$; Data presented is untransformed).

[a] Shaded areas represent date of application.

[b] B = Bozeman site, H = Huntley site.

Table 13. Total pea aphids ± SE / 10 sweeps at various Julian dates (JD) after forage alfalfa was treated with diflubenzuron, azadirachtin, novaluron, kaolin, and lambda cyhalothrin.

Field[b]	Treatment	Pea Aphids / 10 Sweeps			
2006 B		JD 157[a]	JD 164[a]	JD 170	JD 177
	Diflubenzuron	199.0 ± 42.2	462.5 ± 42.5	504.3 ± 55.7a	1174.3 ± 272.7a
	Azadirachtin	-	448.3 ± 27.2	551.3 ± 98.0a	1214.0 ± 238.0a
	Novaluron	211.3 ± 42.5	443.8 ± 39.8	505.3 ± 102.1a	1312.0 ± 87.8a
	Kaolin	265.8 ± 70.3	465.8 ± 44.5	570.0 ± 49.9a	1310.8 ± 335.8a
	λ Cyhalothrin	-	480.5 ± 62.2	35.0 ± 15.4b	361.0 ± 329.8b
	Untreated	265.0 ± 16.2	484.8 ± 85.1	563.8 ± 54.9a	1037.5 ± 177.8a
	F - Statistic	0.77	0.08	15.44	6.09
	df	6, 9	8, 15	8, 15	8, 15
	P – value	NS	NS	<0.0001	0.002
2009 H		JD 142[a]	JD 147	JD 155	JD 162
	Diflubenzuron	56.0 ± 6.1	54.0 ± 20.3	106.0 ± 12.9a	80.0 ± 14.8b
	Azadirachtin	-	55.5 ± 6.8	110.0 ± 4.0a	52.7 ± 26.5b
	Novaluron	48.7 ± 24.0	74.6 ± 30.8	98.0 ± 13.0a	39.3 ± 8.4b
	Kaolin	54.0 ± 34.1	54.0 ± 8.3	96.0 ± 10.0a	50.0 ± 5.3b
	λ Cyhalothrin	-	53.3 ± 15.7	21.3 ± 2.4b	19.3 ± 1.8a
	Untreated	40.0 ± 4.2	44.0 ± 7.0	98.0 ± 1.2a	47.3 ± 4.4b
	F – Statistic	0.37	0.16	33.77	3.01
	df	5, 6	7, 10	7, 10	7, 10
	P – value	NS	NS	<0.0001	0.05
2009 B		JD 162[a]	JD 169	JD 176	JD 182
	Diflubenzuron	10.8 ± 3.0	16.8 ± 1.8	17.0 ± 1.2a	41.3 ± 5.0a
	Azadirachtin	-	9.5 ± 3.8	17.3 ± 4.3a	35.0 ± 5.0a
	Novaluron	8.3 ± 1.7	18.5 ± 1.7	19.3 ± 5.9a	51.8 ± 6.2a
	Kaolin	11.5 ± 2.1	8.0 ± 2.5	14.5 ± 1.8a	47.0 ± 10.3a
	λ Cyhalothrin	-	16.5 ± 3.4	4.0 ± 0.8b	1.3 ± 1.0b
	Untreated	12.8 ± 2.0	11.8 ± 1.8	17.3 ± 1.7a	39.0 ± 4.5a
	F – Statistic	0.54	1.97	6.49	62.32
	df	6, 9	8, 15	8, 15	8, 15
	P - value	NS	NS	0.002	<0.0001

*Means within columns followed by similar letters are not significantly different (LSD Test after log + 1 transformation; *P=0.05;* Data presented is untransformed).

[a] Shaded areas represent date of application.

[b] B = Bozeman site, H = Huntley site.

Table 14. Total prey (pea aphids and alfalfa weevil larvae) ± SE / 10 sweeps at various Julian dates after forage alfalfa was treated with diflubenzuron, azadirachtin, novaluron, kaolin, and lambda cyhalothrin at multiple field sites.

Field[b]	Treatment	Prey / 10 Sweeps			
2006-B		JD 157[a]	JD 164[a]	JD 170	JD 177
	Diflubenzuron	228 ± 51	516 ± 37	539 ± 55a	1234 ± 273a
	Azadirachtin	-	524 ± 25	601 ± 105a	1281 ± 248a
	Novaluron	244 ± 49	475 ± 36	519 ± 104a	1340 ± 89a
	Kaolin	303 ± 79	523 ± 42	596 ± 51a	1348 ± 329a
	λ Cyhalothrin	-	562 ± 57	39 ± 16b	365 ± 333b
	Untreated	304 ± 22	563 ± 72	619 ± 55a	1113 ± 180a
	F - Statistic	0.76	0.52	17.90	4.18
	df(model, error)	6, 9	8, 15	8, 15	8, 15
	P – value	NS	NS	<0.0001	0.01
2009-H		JD 142[a]	JD 147	JD 155	JD 162
	Diflubenzuron	101 ± 14	165 ± 26	320 ± 32a	318 ± 45a
	Azadirachtin	-	262 ± 17	372 ± 54a	338 ± 28a
	Novaluron	101 ± 22	273 ± 39	276 ± 54a	242 ± 15a
	Kaolin	93 ± 38	289 ± 38	383 ± 34a	309 ± 46a
	λ Cyhalothrin	-	245 ± 18	51 ± 8b	21 ± 2b
	Untreated	80 ± 8	226 ± 60	332 ± 16a	331 ± 52a
	F – Statistic	0.41	2.55	15.85	24.32
	df(model, error)	5, 6	7, 10	7, 10	7, 10
	P – value	NS	NS	0.0002	<0.0001
2009-B		JD 162[a]	JD 169	JD 176	JD 182
	Diflubenzuron	36 ± 3	65 ± 15	82 ± 9ab	166 ± 19ab
	Azadirachtin	-	55 ± 18	65 ± 7b	114 ± 8c
	Novaluron	33 ± 3	58 ± 8	84 ± 12ab	144 ± 5b
	Kaolin	41 ± 3	45 ± 6	84 ± 14ab	154 ± 20b
	λ Cyhalothrin	-	39 ± 5	5 ± 1c	3 ± 1d
	Untreated	47 ± 4	50 ± 4	99 ± 6a	178 ± 16a
	F – Statistic	2.23	0.66	30.85	103.73
	df(model, error)	6, 9	8, 15	8, 15	8, 15
	P - value	NS	NS	<0.0001	<0.0001

*Means within columns followed by similar letters are not significantly different (LSD Test after log + 1 transformation; *P=0.05;* Data presented is untransformed).

[a] Shaded areas represent date of application.

[b] B = Bozeman site, H = Huntley site.

Table 15. Total predators (damsel bugs + lady beetles) ± SE / 10 sweeps at various Julian dates (JD) after forage alfalfa was treated with diflubenzuron, azadirachtin, novaluron, kaolin, and lambda cyhalothrin at multiple field sites.

Field	Treatment	Predators / 10 Sweeps			
2006 Bozeman		JD 157[a]	JD 164[a]	JD 170	JD 177
	Diflubenzuron	1.5 ± 0.5	2.0 ± 0.4	0.8 ± 0.3bc	6.0 ± 1.7ab
	Azadirachtin	-	3.3 ± 0.5	0.8 ± 0.3bc	3.3 ± 1.5b
	Novaluron	1.5 ± 0.3	2.8 ± 0.8	2.0 ± 1.1ab	7.8 ± 0.9a
	Kaolin	1.3 ± 0.5	3.8 ± 0.6	0.0 ± 0.0c	3.3 ± 0.3b
	λ Cyhalothrin	-	3.3 ± 0.6	0.0 ± 0.0c	1.0 ± 1.0c
	Untreated	2.5 ± 0.6	2.5 ± 0.3	2.3 ± 0.5a	4.8 ± 1.5ab
	F - Statistic	1.26	1.33	4.74	5.74
	df(model, error)	6, 9	8, 15	8, 15	8, 15
	P – value	NS	NS	0.008	0.004
2009 Huntley		JD 142[a]	JD 147	JD 155	JD 162
	Diflubenzuron	4.7 ± 1.8	10.0 ± 3.0	4.0 ± 2.3	3.3 ± 1.3a
	Azadirachtin	-	10.0 ± 2.0	6.0 ± 3.1	6.7 ± 1.3a
	Novaluron	4.7 ± 3.7	12.0 ± 4.2	4.0 ± 1.2	5.3 ± 1.8a
	Kaolin	3.7 ± 1.7	11.3 ± 3.7	3.3 ± 2.4	6.7 ± 3.5a
	λ Cyhalothrin	-	7.3 ± 1.8	0.0 ± 0.0	0.0 ± 0.0b
	Untreated	6.0 ± 2.3	6.0 ± 2.0	3.3 ± 0.7	6.7 ± 1.8a
	F – Statistic	0.14	0.50	1.00	3.01
	df(model, error)	5, 6	7, 10	7, 10	7, 10
	P – value	0.93	NS	NS	0.05
2009 Bozeman		JD 162[a]	JD 169	JD 176	JD 182
	Diflubenzuron	3.0 ± 0.6	2.5 ± 1.0	2.0 ± 0.4a	3.0 ± 1.9
	Azadirachtin	-	1.5 ± 0.5	3.8 ± 0.8a	3.0 ± 1.0
	Novaluron	2.5 ± 0.6	4.0 ± 0.4	2.3 ± 0.8a	3.5 ± 1.7
	Kaolin	3.5 ± 0.6	3.5 ± 1.0	3.0 ± 0.4 a	3.0 ± 0.5
	λ Cyhalothrin	-	3.0 ± 1.3	0.0 ± 0.0b	0.0 ± 0.0
	Untreated	3.3 ± 0.5	3.3 ± 1.5	1.3 ± 0.5a	3.8 ± 1.4
	F – Statistic	0.47	0.43	7.63	1.39
	df(model, error)	6, 9	8, 15	8, 15	8, 15
	P - value	0.71	NS	0.001	NS

*Means within columns followed by similar letters are not significantly different (LSD Test after square root + 0.5 transformation; *P=0.05;* Data presented is untransformed).

[a] Shaded areas represent date of application.

Table 16. Predator-alfalfa weevil ratio ± SE after forage alfalfa was treated with diflubenzuron, azadirachtin, novaluron, kaolin, and lambda cyhalothrin at various field sites.

Field[b]	Treatment	Predator-Alfalfa Weevil Ratio			
2006 B		JD 157[a]	JD 164[a]	JD 170	JD 177
	Diflubenzuron	0.12 ± 0.07	0.04 ± 0.01	0.02 ± 0.01bc	0.10 ± 0.02b
	Azadirachtin	-	0.04 ± 0.01	0.01 ± 0.01bc	0.06 ± 0.02b
	Novaluron	0.09 ± 0.03	0.09 ± 0.02	0.15 ± 0.01a	0.31 ± 0.06a
	Kaolin	0.04 ± 0.02	0.07 ± 0.01	0.00 ± 0.00c	0.10 ± 0.04b
	λ Cyhalothrin	-	0.06 ± 0.02	0.00 ± 0.00c	0.10 ± 0.10b
	Untreated	0.08 ± 0.03	0.04 ± 0.01	0.05 ± 0.01ab	0.06 ± 0.02b
	F - Statistic	1.21	2.39	3.76	2.97
	df(model, error)	6, 9	8, 15	8, 15	8, 15
	P – value	0.35	0.09	0.02	0.05
2009 H		JD 142[a]	JD 147	JD 155	JD 162
	Diflubenzuron	0.13 ± 0.07	0.09 ± 0.02	0.02 ± 0.01	0.01 ± 0.01ab
	Azadirachtin	-	0.05 ± 0.01	0.02 ± 0.01	0.02 ± 0.01ab
	Novaluron	0.09 ± 0.07	0.07 ± 0.03	0.02 ± 0.01	0.03 ± 0.01a
	Kaolin	0.12 ± 0.05	0.05 ± 0.02	0.01 ± 0.01	0.02 ± 0.01ab
	λ Cyhalothrin	-	0.04 ± 0.01	0.00 ± 0.00	0.00 ± 0.00b
	Untreated	0.16 ± 0.09	0.05 ± 0.03	0.01 ± 0.01	0.02 ± 0.01ab
	F – Statistic	0.32	0.71	1.31	3.69
	df(model, error)	5, 6	7, 10	7, 10	7, 10
	P – value	0.80	0.63	0.33	0.04
2009 B		JD 162[a]	JD 169	JD 176	JD 182
	Diflubenzuron	0.12 ± 0.02	0.08 ± 0.05	0.04 ± 0.01ab	0.02 ± 0.01
	Azadirachtin	-	0.07 ± 0.04	0.09 ± 0.03a	0.04 ± 0.01
	Novaluron	0.10 ± 0.03	0.11 ± 0.04	0.03 ± 0.01ab	0.04 ± 0.01
	Kaolin	0.12 ± 0.03	0.09 ± 0.01	0.05 ± 0.01ab	0.03 ± 0.01
	λ Cyhalothrin	-	0.11 ± 0.04	0.00 ± 0.00c	0.00 ± 0.00
	Untreated	0.09 ± 0.01	0.09 ± 0.04	0.02 ± 0.01bc	0.03 ± 0.01
	F – Statistic	0.23	0.15	5.08	1.51
	df(model, error)	6, 9	8, 15	8, 13	8, 14
	P - value	0.87	0.97	0.008	0.24

*Means within columns followed by similar letters are not significantly different (LSD Test after square root + 0.5 arc sine transformation; *P=0.05;* Data presented is untransformed).

[a] Shaded areas represent date of application.

[b] B = Bozeman site, H = Huntley site.

Table 17. Predator-pea aphid ratio ± SE at various Julian dates (JD) after forage alfalfa was treated with diflubenzuron, azadirachtin, novaluron, kaolin, and lambda cyhalothrin.

Field[b]	Treatment	Predator-Pea Aphid Ratio			
2006 B		JD 157[a]	JD 164[a]	JD 170	JD 177
	Diflubenzuron	0.008 ± 0.003	0.004 ± 0.001	0.002 ± 0.001bc	0.006 ± 0.002a
	Azadirachtin	-	0.007 ± 0.001	0.001 ± 0.001bc	0.003 ± 0.001a
	Novaluron	0.008 ± 0.002	0.006 ± 0.001	0.005 ± 0.003b	0.006 ± 0.001a
	Kaolin	0.005 ± 0.002	0.008 ± 0.002	0.000 ± 0.000c	0.003 ± 0.001a
	λ Cyhalothrin	-	0.007 ± 0.002	0.000 ± 0.000c	0.001 ± 0.001b
	Untreated	0.009 ± 0.001	0.006 ± 0.001	0.047 ± 0.007a	0.005 ± 0.001a
	F - Statistic	1.72	0.95	5.08	5.10
	df	6, 9	8, 15	8, 15	8, 15
	P – value	NS	NS	0.006	0.006
2009 H		JD 142[a]	JD 147	JD 155	JD 162
	Diflubenzuron	0.09 ± 0.04	0.25 ± 0.10	0.04 ± 0.02	0.03 ± 0.01
	Azadirachtin	-	0.18 ± 0.02	0.05 ± 0.03	0.39 ± 0.31
	Novaluron	0.10 ± 0.04	0.39 ± 0.31	0.04 ± 0.01	0.14 ± 0.04
	Kaolin	0.16 ± 0.01	0.21 ± 0.06	0.04 ± 0.03	0.12 ± 0.07
	λ Cyhalothrin	-	0.15 ± 0.04	0.00 ± 0.00	0.00 ± 0.00
	Untreated	0.14 ± 0.04	0.15 ± 0.07	0.03 ± 0.01	0.14 ± 0.04
	F – Statistic	0.47	0.45	1.08	1.64
	df	5, 6	7, 10	7, 10	7, 10
	P – value	NS	NS	NS	NS
2009 B		JD 162[a]	JD 169	JD 176	JD 182
	Diflubenzuron	0.40 ± 0.20	0.16 ± 0.06	0.12 ± 0.02ab	0.08 ± 0.05
	Azadirachtin	-	0.35 ± 0.22	0.28 ± 0.12a	0.10 ± 0.04
	Novaluron	0.40 ± 0.20	0.23 ± 0.09	0.12 ± 0.03ab	0.09 ± 0.05
	Kaolin	0.37 ± 0.13	0.83 ± 0.43	0.21 ± 0.02a	0.07 ± 0.02
	λ Cyhalothrin	-	0.23± 0.13	0.00 ± 0.00c	0.00 ± 0.00
	Untreated	0.29 ± 0.08	0.31 ± 0.13	0.07 ± 0.02b	0.11 ± 0.04
	F – Statistic	0.15	0.36	7.64	0.47
	df	6, 9	8, 14	8, 15	8, 13
	P - value	NS	NS	0.001	NS

*Means within columns followed by similar letters are not significantly different (LSD Test after square root arc sine transformation; *P*=0.05*;* Data presented is untransformed).

[a] Shaded areas represent date of application.

[b] B = Bozeman site, H = Huntley site.

Table 18. Total spiders (Araneae) / sweep ± SE at various Julian dates (JD) after treatment with diflubenzuron, azadirachtin, novaluron, kaolin, and lambda cyhalothrin in Bozeman, 2006.

Field	Treatment	Rate (gai/ha)	Julian Dates			
Spiders			JD 142[a]	JD 147	JD 155	JD 162
	Diflubenzuron	22.7	0.3 ± 0.2	0.4 ± 0.1a	0.0 ± 0.0	0.2 ± 0.1
	Azadirachtin	7.8	-	0.5 ± 0.1a	0.1 ± 0.1	0.4 ± 0.1
	Novaluron	31.0	0.5 ± 0.1	0.2 ± 0.1b	0.0 ± 0.0	0.3 ± 0.1
	Kaolin	6,544.6	0.9 ± 0.1	0.1 ± 0.1b	0.1 = 0.1	0.3 ± 0.1
	Lambda Cyhalothrin	5.5	-	0.5 ± 0.1a	0.1 ± 0.1	0.1 ± 0.1
	Untreated		0.7 ± 0.2	0.4 ± 0.1a	0.1 ± 0.1	0.2 ± 0.1
	F – Statistic		3.43	4.91	1.00	0.91
	df(model, error)		6, 9	8, 15	8, 15	8, 15
	P – value		NS	0.007	NS	NS

*Means within columns followed by similar letters are not significantly different (LSD Test after square root + 0.5 transformation; *P=0.05;* Data presented is untransformed).

[a] Shaded areas represent date of application.

Table 19. Parasitism rates ± SE after rearing alfalfa weevil larvae after application of various pesticides at multiple field sites.

			Larval Mortality, Parasitism Rate and Adult Emergence Rate				
Field[a]	Treatment	Rate (gai/ha)	Mortality	Adult Emergence	*% O. incertus*	*% B. curculionis*	Parasitism
2006-B	Diflubenzuron	22.7	3 ± 2a	83 ± 4ab	2 ± 2	12 ± 6a	14 ± 6ab
	Azadirachtin	7.8	2 ± 2a	95 ± 2a	3 ± 2	0 ± 0b	3 ± 2b
	Novaluron	31.0	1 ± 2a	78 ± 5b	8 ± 3	12 ± 4a	20 ± 5a
	Kaolin	6,544.6	3 ± 2a	80 ± 5b	7 ± 3	10 ± 4a	17 ± 6a
	λ Cyhalothrin	5.5	12 ± 3b	84 ± 4a	0 ± 0	4 ± 2b	4 ± 2b
	Untreated		5 ± 2a	72 ± 3b	9 ± 4	14 ± 3a	23 ± 3a
	F - Statistic		3.00	3.22	1.90	2.82	3.12
	df(model, error)		8, 15	8, 15	8, 15	8, 15	8, 15
	P – value		0.04	0.03	NS	0.05	0.04
2009-H	Diflubenzuron	22.7	0 ± 0a	91 ± 2	1 ± 1	8 ± 1a	9 ± 2a
	Azadirachtin	7.8	5 ± 5ab	77 ± 2	3 ± 2	16 ± 2a	19 ± 4a
	Novaluron	31.0	4 ± 2ab	77 ± 4	6 ± 3	12 ± 2a	18 ± 4a
	Kaolin	6,544.6	8 ± 8ab	78 ± 6	3 ± 1	13 ± 2a	15 ± 1a
	λ Cyhalothrin	5.5	23 ± 5b	77 ± 6	0 ± 0	1 ± 1b	1 ± 1b
	Untreated		0 ± 0a	83 ± 1	3 ± 1	13 ± 1a	17 ± 1a
	F – Statistic		3.22	2.74	1.31	12.79	7.66
	df(model, error)		7, 10	7, 10	7, 10	7, 10	7, 10
	P – value		0.05	NS	NS	0.004	0.003
2009-B	Diflubenzuron	22.7	4 ± 4a	89 ± 4a	0 ± 0	7 ± 3a	7 ± 3a
	Azadirachtin	7.8	9 ± 4a	82 ± 2a	0 ± 0	10 ± 2a	10 ± 2a
	Novaluron	31.0	2 ± 2a	89 ± 6a	0 ± 0	9 ± 6a	9 ± 6a
	Kaolin	6,544.6	8 ± 4a	83 ± 7a	2 ± 2	7 ± 3a	10 ± 4a
	λ Cyhalothrin	5.5	45 ± 5b	55 ± 5b	0 ± 0	0 ± 0b	0 ± 0b
	Untreated		13 ± 4a	82 ± 5a	0 ± 0	12 ± 3a	12 ± 3a
	F – Statistic		10.57	6.41	1.23	3.25	3.12
	df(model, error)		8, 12	8, 12	8, 12	8, 12	8, 12
	P - value		0.009	0.002	NS	0.03	0.04

*Means within columns followed by similar letters are not significantly different (LSD Test; Data analyzed after square root arc sine transformation; *P=0.05*).

Table 20. GLM analysis of alfalfa weevils, predators (lady beetles + damsel bugs), pea aphids, predator-pea aphid ratio and predator-alfalfa weevil ratio in 1st harvest cycle forage alfalfa after treatment with novaluron and lambda cyhalothrin near Toston & Huntley, MT in 2010.

Predator / AW Ratio	**DF**	**F-Statistic**	**Pr>F**
Field	1	0.48	0.49
Field x Treatment	2	7.93	0.004*
Treatment	2	0.09	0.91
Rep	3	0.69	0.57
Predators	**DF**	**F-Statistic**	**Pr>F**
Field	1	3.81	0.06
Field x Treatment	2	8.36	0.0006*
Treatment	2	15.67	<0.0001*
Rep	3	0.67	0.67
Predator / Aphid Ratio	**DF**	**F-Statistic**	**Pr>F**
Field	1	16.69	0.001*
Field x Treatment	2	19.59	<0.0001*
Treatment	2	10.14	0.001*
Rep	3	0.19	0.89
Alfalfa Weevil Totals	**DF**	**F-Statistic**	**Pr>F**
Field	1	18.74	<0.0001*
Field x Treatment	2	0.44	0.63
Treatment	2	87.98	<0.0001*
Rep	3	0.77	0.51
Alfalfa Weevil Larvae	**DF**	**F-Statistic**	**Pr>F**
Field	1	18.67	<0.0001*
Field x Treatment	2	0.04	0.95
Treatment	2	81.90	<0.0001*
Rep	3	**0.88**	**0.45**
Alfalfa Weevil Adults	**DF**	**F-Statistic**	**Pr>F**
Field	1	4.10	0.04*
Field x Treatment	2	6.67	0.002*
Treatment	2	4.22	0.01*
Rep	3	2.54	0.06
Pea Aphids	**DF**	**F-Statistic**	**Pr>F**
Field	1	73.15	<0.0001*
Field x Treatment	2	1.01	0.36
Treatment	2	6.55	0.002*
Rep	3	2.19	0.09

* Represents values significant at $P < 0.05$ (GLM after square root + 0.5 transformation for all factors except predator/prey ratio, alfalfa weevil and pea aphid data; predator prey ratio data was analyzed after square root arc sine transformation while alfalfa weevil and pea aphid data were log + 1 transformed).

Table 21. GLM analysis of alfalfa weevils (AW), predators (ladybeetles + damsel bugs), pea aphids, predator-pea aphid ratio, and predator-AW ratio in 2nd harvest cycle forage alfalfa after treatment of novaluron and lambda cyhalothrin near Toston & Huntley, 2010.

Predator / AW Ratio	**DF**	**F-Statistic**	**Pr>F**
Field	1	7.64	0.01*
Field x Treatment	2	0.69	0.51
Treatment	2	0.10	0.90
Rep	3	1.29	0.31
Predators	**DF**	**F-Statistic**	**Pr>F**
Field	1	1.11	0.29
Field x Treatment	2	0.69	0.50
Treatment	2	0.15	0.86
Rep	3	0.17	0.91
Predator / Aphid Ratio	**DF**	**F-Statistic**	**Pr>F**
Field	1	6.94	0.02*
Field x Treatment	2	0.61	0.55
Treatment	2	0.90	0.42
Rep	3	0.97	0.43
Alfalfa Weevil Totals	**DF**	**F-Statistic**	**Pr>F**
Field	1	26.03	<0.0001*
Field x Treatment	2	1.17	0.31
Treatment	2	0.80	0.45
Rep	3	0.73	0.53
Alfalfa Weevil Larvae	**DF**	**F-Statistic**	**Pr>F**
Field	1	9.27	0.003*
Field x Treatment	2	0.78	0.46
Treatment	2	0.39	0.67
Rep	3	0.75	0.52
Alfalfa Weevil Adults	**DF**	**F-Statistic**	**Pr>F**
Field	1	25.50	<0.0001*
Field x Treatment	2	0.38	0.68
Treatment	2	0.96	0.38
Rep	3	0.95	0.42
Pea Aphids	**DF**	**F-Statistic**	**Pr>F**
Field	1	18.79	<0.0001*
Field x Treatment	2	0.01	0.99
Treatment	2	0.79	0.45
Rep	3	0.49	0.68

* Represents values significant at $P<0.05$ (GLM after square root ± 0.5 transformation for all factors except predator/prey ratio, alfalfa weevil and pea aphid data; predator prey ratio data was analyzed after square root arc sine transformation while alfalfa weevil and pea aphid data were log + 1 transformed).

Table 22. First and second harvest cycle predators (lady beetles + damsel bugs), predator-pea aphid ratio and predator-alfalfa weevil (AW) ratio / 10 sweeps ± SE after treatment with novaluron and lambda cyhalothrin near Toston and Huntley, MT in 2010.

Field	Treatment	Rate gai/ha	Toston		Huntley	
Predators / AW			1^{s} Harvest Cycle	2nd Harvest Cycle	1st Harvest Cycle	2nd Harvest Cycle
	Novaluron	31.0	0.23 ± 0.06a	0.74 ± 0.24	0.08 ± 0.02	1.97 ± 0.77
	λ Cyhalothrin	5.5	0.00 ± 0.00b	1.13 ± 0.29	0.26 ± 0.04	1.73 ± 0.36
	Untreated		0.21 ± 0.04a	0.64 ± 0.16	0.04 ± 0.01	2.54 ± 1.00
	F - Statistic		34.35	1.02	2.06	0.39
	df		5, 6	5, 6	5, 6	5, 6
	P – value		0.0005	NS	NS	NS
Predators / Aphid			Toston		Huntley	
	Novaluron	31.0	0.12 ± 0.06a	0.05 ± 0.01	0.01 ± 0.01	0.18 ± 0.06
	λ Cyhalothrin	5.5	0.00 ± 0.00b	0.08 ± 0.02	0.04 ± 0.03	0.12 ± 0.01
	Untreated		0.14 ± 0.03a	0.11 ± 0.04	0.01 ± 0.01	0.20 ± 0.07
	F - Statistic		46.38	1.42	0.88	0.87
	df		5, 6	5, 6	5, 6	5, 6
	P – value		0.0002	NS	NS	NS
Predators			1st Harvest Cycle	2nd Harvest Cycle	1st Harvest Cycle	2nd Harvest Cycle
	Novaluron	31.0	4.3 ± 0.9a	3.8 ± 0.7	2.7 ± 0.7	5.4 ± 0.8
	λ Cyhalothrin	5.5	0.0 ± 0.0b	5.8 ± 1.8	1.6 ± 0.9	4.8 ± 0.9
	Untreated		5.4 ± 0.9a	5.3 ± 1.8	1.6 ± 0.4	5.1 ± 0.9
	F – Statistic		28.19	0.51	1.33	0.18
	df		5, 30	5, 30	5, 30	5, 30
	P – value		<0.0001	NS	NS	NS

*Means within columns followed by similar letters are not significantly different (LSD Test after predator data was square root + 0.5 transformed; while predator-prey ratios were square root arc sine transformed in the 1st harvest cycle; *P*=0.05*;* All data presented is untransformed).

Table 23. Alfalfa weevils (AW) and pea aphids ± SE / 10 sweeps after treatment with novaluron and lambda cyhalothrin at sites near Toston and Huntley, MT in 2010.

Field	Treatment	Rate gai/ha	Toston		Huntley	
Total AW			1^{s} Harvest Cycle	2nd Harvest Cycle	1st Harvest Cycle	2nd Harvest Cycle
	Novaluron	31.0	22.8 ± 2.3b	7.8 ± 1.7	33.3 ± 4.7a	3.9 ± 0.7
	λ Cyhalothrin	5.5	1.1 ± 0.1c	6.3 ± 0.7	9.6 ± 3.3b	3.2 ± 0.6
	Untreated		29.3 ± 1.6a	10.7 ± 2.2	42.7 ± 2.8a	3.2 ± 0.6
	F - Statistic		161.44	1.14	22.75	0.39
	df		5, 30	5, 30	5, 30	5, 30
	P – value		<0.0001	NS	<0.0001	NS
AW Larvae			1st Harvest Cycle	2nd Harvest Cycle	1st Harvest Cycle	2nd Harvest Cycle
	Novaluron	31.0	20.5 ± 2.5b	5.9 ± 1.7	32.6 ± 4.5b	3.5 ± 0.7
	λ Cyhalothrin	5.5	0.8 ± 0.1c	5.2 ± 0.9	8.3 ± 3.3c	2.9 ± 0.6
	Untreated		26.6 ± 1.8a	9.1 ± 2.4	41.4 ± 3.1a	2.9 ± 0.6
	F – Statistic		235.10	0.28	21.46	0.19
	df		5, 30	5, 30	5, 30	5, 30
	P – value		<0.0001	NS	<0.0001	NS
AW Adults			1st Harvest Cycle	2nd Harvest Cycle	1st Harvest Cycle	2nd Harvest Cycle
	Novaluron	31.0	2.3 ± 0.3a	1.8 ± 0.4	0.7 ± 0.4	0.4 ± 0.2
	λ Cyhalothrin	5.5	0.3 ± 0.2b	1.2 ± 0.3	1.3 ± 0.4	0.3 ± 0.1
	Untreated		2.7 ± 0.7a	1.6 ± 0.4	1.3 ± 0.4	0.3 ± 0.2
	F – Statistic		12.14	0.68	1.09	0.40
	df		5, 30	5, 30	5, 30	5, 30
	P - value		0.0001	NS	NS	NS
Pea Aphids			1st Harvest Cycle	2nd Harvest Cycle	1st Harvest Cycle	2nd Harvest Cycle
	Novaluron	31.0	36.4 ± 3.9a	65.4 ± 13.5	260.5 ± 56a	32.5 ± 2.9
	λ Cyhalothrin	5.5	17.3 ± 3.2b	66.8 ± 7.9	123.0 ± 33b	38.6 ± 7.1
	Untreated		39.6 ± 3.2a	57.5 ± 9.8	239.4 ± 42a	32.4 ± 6.4
	F – Statistic		10.91	0.50	4.01	1.24
	df		5, 30	5, 30	5, 30	5, 30
	P - value		0.0003	NS	0.03	NS

*Means within columns followed by similar letters are not significantly different (LSD Test after log + 1 transformation; *P=0.05;* Data presented is untransformed).

Table 24. GLM analysis of lady beetles (Coccinellidae) and each lady beetle species ± SE in the 1st harvest cycle after treatment with novaluron and lambda cyhalothrin near Toston & Huntley, MT in 2010.

Total Lady Beetles	**DF**	**F-Statistic**	**Pr>F**
Field	1	7.54	0.007*
Field x Treatment	2	10.07	0.0002*
Treatment	2	12.45	<0.0001*
Rep	3	0.61	0.61
C. septempunctata	**DF**	**F-Statistic**	**Pr>F**
Field	1	5.05	0.02*
Field x Treatment	2	8.12	0.0007*
Treatment	2	9.58	0.0002*
Rep	3	0.27	0.84
H. parenthesis	**DF**	**F-Statistic**	**Pr>F**
Field	1	3.10	0.08
Field x Treatment	2	1.03	0.36
Treatment	2	1.03	0.36
Rep	3	0.34	0.79
H. convergens	**DF**	**F-Statistic**	**Pr>F**
Field	1	4.10	0.04*
Field x Treatment	2	0.08	0.92
Treatment	2	0.22	0.80
Rep	3	0.98	0.40
C. transversoguttata	**DF**	**F-Statistic**	**Pr>F**
Field	1	8.54	0.004*
Field x Treatment	2	2.96	0.06
Treatment	2	2.96	0.06
Rep	3	0.64	0.59
H. caseyi	**DF**	**F-Statistic**	**Pr>F**
Field	1	5.99	0.01*
Field x Treatment	2	1.68	0.19
Treatment	2	1.68	0.19
Rep	3	1.52	0.21
H. tredecimpunctata	**DF**	**F-Statistic**	**Pr>F**
Field	1	7.46	0.008*
Field x Treatment	2	1.87	0.16
Treatment	2	1.87	0.16
Rep	3	1.38	0.25

* Represents values significant at $P<0.05$ (GLM after square root + 0.5 transformation).

Table 25. GLM analysis of total lady beetles (Coccinellidae) and each lady beetle species ± SE in the 2nd harvest cycle after forage alfalfa was treated with novaluron and lambda cyhalothrin near Toston & Huntley, MT in 2010.

Total Lady Beetles	**DF**	**F-Statistic**	**Pr>F**
Field	1	0.77	0.38
Field x Treatment	2	1.57	0.21
Treatment	2	0.29	0.75
Rep	3	0.14	0.93
C. septempunctata	**DF**	**F-Statistic**	**Pr>F**
Field	1	0.39	0.53
Field x Treatment	2	1.28	0.28
Treatment	2	0.26	0.77
Rep	3	0.14	0.93
H. parenthesis	**DF**	**F-Statistic**	**Pr>F**
Field	1	1.00	0.32
Field x Treatment	2	1.00	0.37
Treatment	2	1.00	0.37
Rep	3	1.00	0.39
H. convergens	**DF**	**F-Statistic**	**Pr>F**
Field	1	21.29	<0.0001*
Field x Treatment	2	0.43	0.64
Treatment	2	0.43	0.64
Rep	3	0.72	0.54
C. transversoguttata	**DF**	**F-Statistic**	**Pr>F**
Field	1	2.17	0.14
Field x Treatment	2	2.17	0.12
Treatment	2	2.17	0.12
Rep	3	0.72	0.54
H. caseyi	**DF**	**F-Statistic**	**Pr>F**
Field	1	2.17	0.14
Field x Treatment	2	2.17	0.12
Treatment	2	2.17	0.12
Rep	3	0.72	0.54

* Represents values significant at $P<0.05$ (GLM after square root + 0.5 transformation).

Table 26. Total lady beetles and composition of each lady beetle species ± SE / 10 sweeps after treatment with novaluron and lambda cyhalothrin at field sites near Toston and Huntley, MT in 2010.

Field	Treatment	Rate (gai/ha)	Toston		Huntley	
Total Lady Beetles			1^{s} Harvest Cycle	2nd Harvest Cycle	1st Harvest Cycle	2nd Harvest Cycle
	Novaluron	31.0	3.2 ± 0.6a	1.8 ± 0.6	1.7 ± 0.5	4.0 ± 0.6
	λ Cyhalothrin	5.5	0.0 ± 0.0b	4.5 ± 1.5	1.2 ± 0.7	3.2 ± 0.9
	Untreated		4.0 ± 0.5a	4.1 ± 1.5	0.8 ± 0.3	3.6 ± 0.8
	F - Statistic		31.24	1.15	0.82	0.40
	df(model, error)		5, 30	5, 30	5, 30	5, 30
	P – value		<0.0001	NS	NS	NS
Composition of Lady Beetles	Species	Proportion	1st Harvest Cycle	2nd Harvest Cycle	1st Harvest Cycle	2nd Harvest Cycle
	C. septempunctata	27.6 (86%)	5.1 ± 0.8 (69%)	9.9 ± 3.5 (95%)	3.0 ± 1.5 (79%)	9.6 ± 2.3 (90%)
	H. parenthesis	0.4 (1%)	0.3 ± 0.2 (4%)	0.1 ± 0.1 (1%)	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)
	H. convergens	2.0 (6%)	0.1 ± 0.1 (1%)	0.0 ± 0.0 (0%)	0.8 ± 0.5 (21%)	1.1 ± 0.4 (10%)
	C. transversoguttata	1.0 (3%)	0.8 ± 0.4 (11%)	0.2 ± 0.1 (2%)	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)
	H. caseyi	0.7 (2%)	0.5 ± 0.2 (7%)	0.2 ± 0.1 (2%)	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)
	H. tredecimpunctata	0.6 (2%)	0.6 ± 0.2 (8%)	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)

*Means within columns followed by similar letters are not significantly different (LSD Test after square root + 0.5 transformation; *P=0.05;* Data presented is untransformed

Table 27. GLM analysis of spiders (Araneae) and damsel bugs (Nabidae) in the 1st harvest cycle after treatment with novaluron and lambda cyhalothrin near Toston & Huntley, MT in 2010.

Araneae	**DF**	**F-Statistic**	**Pr>F**
Field	1	14.90	0.0003*
Field x Treatment	2	0.32	0.72
Treatment	2	9.72	0.0002*
Rep	3	1.03	0.38
Nabidae	**DF**	**F-Statistic**	**Pr>F**
Field	1	0.06	0.81
Field x Treatment	2	1.43	0.24
Treatment	2	6.30	0.003*
Rep	3	0.14	0.93

* Represents values significant at P<0.05 (GLM after square root + 0.5 transformation).

Table 28. GLM analysis of spiders (Araneae), damsel bugs (Nabidae), parasitoid wasps, alfalfa weevil (AW) growth stage (instar index: 1 – 4), stem height (cm) and yield in the 2nd harvest cycle after treatment with novaluron and lambda cyhalothrin near Toston & Huntley, MT in 2010.

Spiders	**DF**	**F-Statistic**	**Pr>F**
Field	1	24.74	<0.0001*
Field x Treatment	2	0.41	0.66
Treatment	2	0.67	0.51
Rep	3	0.78	0.51
Damsel Bugs	**DF**	**F-Statistic**	**Pr>F**
Field	1	0.89	0.34
Field x Treatment	2	0.26	0.77
Treatment	2	0.17	0.84
Rep	3	1.10	0.35
Parasitoid Wasps	**DF**	**F-Statistic**	**Pr>F**
Field	1	1.81	0.18
Field x Treatment	2	0.83	0.43
Treatment	2	1.94	0.15
Rep	3	0.94	0.42
Leaf Defoliation	**DF**	**F-Statistic**	**Pr>F**
Field	1	0.62	0.44
Field x Treatment	2	1.00	0.32
Treatment	2	1.44	0.26
Rep	3	0.41	0.73
AW Growth Stage	**DF**	**F-Statistic**	**Pr>F**
Field	1	4.08	0.04*
Field x Treatment	2	0.65	0.55
Treatment	2	0.79	0.47
Rep	3	1.00	0.40
Stem Height	**DF**	**F-Statistic**	**Pr>F**
Field	1	0.61	0.45
Field x Treatment	2	1.22	0.36
Treatment	2	0.93	0.40
Rep	3	0.39	0.74
Yield	**DF**	**F-Statistic**	**Pr>F**
Field	1	0.24	0.62
Field x Treatment	2	0.85	0.45
Treatment	2	1.44	0.23
Rep	3	0.76	0.52

* Represents values significant at $P<0.05$ (GLM after square root + 0.5 transformation).

Table 29. Spiders (Araneae), damsel bugs (Nabidae), and *C. septempunctata* ± SE / 10 sweeps after forage alfalfa was treated with novaluron and lambda cyhalothrin at field sites near Toston and Huntley, MT in 2010.

Field	Treatment	Rate gai/ha	Toston		Huntley	
Spiders			1^{s} Harvest Cycle	2nd Harvest Cycle	1st Harvest Cycle	2nd Harvest Cycle
	Novaluron	31.0	0.8 ± 0.2a	0.5 ± 0.2	1.7 ± 0.3a	1.3 ± 0.2
	λ Cyhalothrin	5.5	0.0 ± 0.0b	0.6 ± 0.3	0.8 ± 0.2b	1.9 ± 0.2
	Untreated		1.2 ± 0.4a	0.5 ± 0.2	1.8 ± 0.3a	1.6 ± 0.5
	F - Statistic		7.07	0.02	3.11	0.93
	df(model, error)		5, 30	5, 30	5, 30	5, 30
	P – value		0.003	NS	0.05	NS
C. septempunctata			1stHarvest Cycle	2nd Harvest Cycle	1st Harvest Cycle	2nd Harvest Cycle
	Novaluron	31.0	2.3 ± 0.5a	1.9 ± 0.6	1.4 ± 0.5	3.5 ± 0.6
	λ Cyhalothrin	5.5	0.0 ± 0.0b	4.3 ± 1.5	1.0 ± 0.7	2.8 ± 0.9
	Untreated		2.8 ± 0.3a	3.8 ± 1.4	0.6 ± 0.3	3.3 ± 8.8
	F – Statistic		28.59	0.95	0.89	0.33
	df(model, error)		5, 30	5, 30	5, 30	5, 30
	P – value		<0.0001	NS	NS	NS
Damsel Bugs			1stHarvest Cycle	2nd Harvest Cycle	1st Harvest Cycle	2nd Harvest Cycle
	Novaluron	31.0	1.1 ± 0.3a	1.4 ± 0.3	0.8 ± 0.2	1.4 ± 0.4
	λ Cyhalothrin	5.5	0.0 ± 0.0b	1.3 ± 0.3	0.4 ± 0.2	1.6 ± 0.2
	Untreated		1.2 ± 0.3a	1.3 ± 0.4	0.8 ± 0.3	1.5 ± 0.4
	F – Statistic		7.05	0.26	0.90	0.16
	df(model, error)		5, 30	5, 30	5, 30	5, 30
	P – value		0.003	NS	NS	NS

*Means within columns followed by similar letters are not significantly different (LSD Test after square root + 0.5 transformation; *P=0.05;* Data presented is untransformed).

Table 30. Composition of parasitoid wasps ± SE / 10 sweeps after forage alfalfa was treated with novaluron and lambda cyhalothrin at multiple field sites in 2010.

Field	Treatment	Rate (gai/ha)	Toston		Huntley	
Total Wasps			1^{s} Harvest Cycle	2nd Harvest Cycle	1st Harvest Cycle	2nd Harvest Cycle
	Novaluron	31.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	λ Cyhalothrin	5.5	0.1 ± 0.1	0.0 ± 0.0	0.2 ± 0.2	0.3 ± 0.3
	Untreated		0.6 ± 0.3	0.2 ± 0.1	0.0 ± 0.0	0.3 ± 0.1
	F - Statistic		2.87	2.14	1.00	1.30
	df(model, error)		5, 30	5, 30	5, 30	5, 30
	P – value		NS	NS	NS	NS
Composition	Hymenopteran Families/Superfamily	Proportion	1st Harvest Cycle	2nd Harvest Cycle	1st Harvest Cycle	2nd Harvest Cycle
	Chalcidoidea	0.3 (17%)	0.2 ± 0.1 (25%)	0.0 ± 0.0 (0%)	0.0 ± 0.0 (0%)	0.1 ± 0.1 (17%)
	Braconidae	0.7 (39%)	0.2 ± 0.2 (25%)	0.0 ± 0.0 (0%)	0.1 ± 0.1 (50%)	0.4 ± 0.2 (66%)
	Ichneaumonidae	0.8 (44%)	0.4 ± 0.2 (50%)	0.2 ± 0.1 (100%)	0.1 ± 0.1 (50%)	0.1 ± 0.1 (17%)
	N	144	36	36	36	36
	Total Wasps	1.8	0.8 ± 0.5	0.2 ± 0.1	0.2 ± 0.2	0.6 ± 0.4

*Means within columns followed by similar letters are not significantly different (LSD Test after square root + 0.5 transformation; *P=0.05;* Data presented is untransformed).

Table 31. GLM analysis of percent alfalfa weevil (AW) larvae parasitism, percent parasitism by *Oomyzus incertus*, percent parasitism by *Bathyplectes curculionis*, percent AW adult emergence from pupae and AW larval mortality from reared alfalfa weevil larvae after treatment with novaluron and lambda cyhalothrin near Toston & Huntley, MT in 2010.

Percent Parasitism	**DF**	**F-Statistic**	**Pr>F**
Field	1	0.39	0.54
Field x Treatment	2	1.44	0.26
Treatment	2	14.99	0.0003*
Rep	3	0.15	0.92
Percent Parasitism *Oomyzus incertus*	**DF**	**F-Statistic**	**Pr>F**
Field	1	0.24	0.62
Field x Treatment	2	1.05	0.37
Treatment	2	4.57	0.02*
Rep	3	0.68	0.58
Percent Parasitism of *Bathyplectes curculionis*	**DF**	**F-Statistic**	**Pr>F**
Field	1	1.37	0.25
Field x Treatment	2	3.90	0.04*
Treatment	2	20.45	<0.0001*
Rep	3	0.15	0.92
Percent Adult Emergence	**DF**	**F-Statistic**	**Pr>F**
Field	1	0.08	0.78
Field x Treatment	2	4.18	0.03*
Treatment	2	17.95	0.0001*
Rep	3	2.38	0.11
Larval Mortality through Pupation	**DF**	**F-Statistic**	**Pr>F**
Field	1	0.62	0.44
Field x Treatment	2	0.74	0.49
Treatment	2	48.79	<0.0001*
Rep	3	0.87	0.48

* Represents values significant at $P<0.05$ (GLM after square root arc sine transformation).

Table 32. Alfalfa weevil (AW) parasitism rates ± SE after rearing 50 larvae from each forage alfalfa plot that were treated with novaluron and lambda cyhalothrin at multiple field sites in 2010.

			Larval Mortality, Percent Parasitism and Adult Emergence*				
Field	Treatment	Rate gai/ha	AW Mortality	AW Adult Emergence from Pupae	*% Oomyzus incertus*	*% Bathyplectes curculionis*	% AW Parasitism
2010 Toston	Novaluron	31.0	4 ± 2b	82 ± 3a	6 ± 2a	9 ± 1a	15 ± 3a
	λ Cyhalothrin	5.5	36 ± 4a	64 ± 4b	0 ± 0b	1 ± 1b	1 ± 1b
	Untreated		6 ± 3b	74 ± 3ab	3 ± 1a	19 ± 2a	21 ± 2a
	F - Statistic		20.61	9.20	7.22	68.17	72.55
	df(model, error)		5, 6	5, 6	5, 6	5, 6	5, 6
	P – value		0.002	0.01	0.02	<0.0001	<0.0001
2010 Huntley	Novaluron	31.0	6 ± 1b	75 ± 3b	3 ± 1a	17 ± 3a	21 ± 4a
	λ Cyhalothrin	5.5	30 ± 6a	65 ± 4c	1 ± 1a	5 ± 4b	6 ± 5a
	Untreated		2 ± 1b	83 ± 3a	3 ± 2a	13 ± 1a	16 ± 2a
	F – Statistic		17.29	16.10	0.65	5.71	4.35
	df(model, error)		5, 6	5, 6	5, 6	5, 6	5, 6
	P – value		0.003	0.003	NS	0.04	NS

*Means within columns followed by similar letters are not significantly different (LSD Test after square root arc sine transformation; *P*=0.05*;* Data presented is untransformed).

Table 33. Alfalfa weevil (AW) growth stage (instar index: 1 - 4), AW degree days, & alfalfa growth stage (MSC) ± SE in untreated plots at sites in 2010.

Field	Treatment	Julian Dates						
2010 Toston		152[a]	158	165	174[b]	200	209	215[c]
	AW Growth Stage	1.7 ± 0.2	2.0 ± 0.2	2.8 ± 0.3	3.7 ± 0.2	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
	Degree Days	335	399	466	552	995	1165	1284
	MSC	2.0 ± 0.0	3.8 ± 0.1	3.8 ± 0.3	5.5 ± 0.3	3.0 ± 0.0	3.8 ± 0.3	5.0 ± 0.1
2010 Huntley		153[a]	159	166	173[b]	194	200	207[c]
	AW Growth Stage	2.5 ± 0.2	3.1 ± 0.3	3.7 ± 0.5	3.9 ± 0.3	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
	Degree Days	465	567	652	751	1180	1322	1489
	MSC	2.0 ± 0.0	3.8 ± 0.3	3.9 ± 0.2	5.3 ± 0.3	2.5 ± 0.3	4.0 ± 0.1	5.0 ± 0.2

[a] Applications of novaluron of lambda cyhalothrin were made on this sample date.
[b] First harvest cutting made on this sample date.
[c] Second harvest cutting made on this sample date.

Table 34. Alfalfa weevil (AW) growth stage (instar index: 1 – 4), AW degree days and alfalfa growth stage (MSC) ± SE in untreated plots at three field sites in 2006 and 2009.

Field	Untreated Parameters	Julian Dates			
2006 Bozeman		157[a]	164	170	177
	MSC	3.0 ± 0.0	3.8 ± 0.3	4.0 ± 0.0	5.8 ± 0.3
	Larval Growth Stage	2.0 ± 0.1	2.1 ± 0.1	2.2 ± 0.3	2.8 ± 0.1
	Degree Days	400	460	500	620
2009 Huntley		142[a]	147	155	162
	MSC	1.0 ± 0.0	2.0 ± 0.0	2.5 ± 0.0	3.3 ± 0.3
	Larval Growth Stage	1.8 ± 0.1	2.0 ± 0.2	2.6 ± 0.1	3.8 ± 0.1
	Degree Days	233	305	421	540
2009 Bozeman		162[a]	169	176	182
	MSC	2.0 ± 0.0	3.0 ± 0.0	5.0 ± 0.0	5.8 ± 0.0
	Larval Growth Stage	2.1 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.8 ± 0.1
	Degree Days	325	433	500	606

[a] Applications of novaluron, kaolin and diflubenzuron were made on this date. Applications of lambda cyhalothrin and azadirachtin were made on the next sample date.

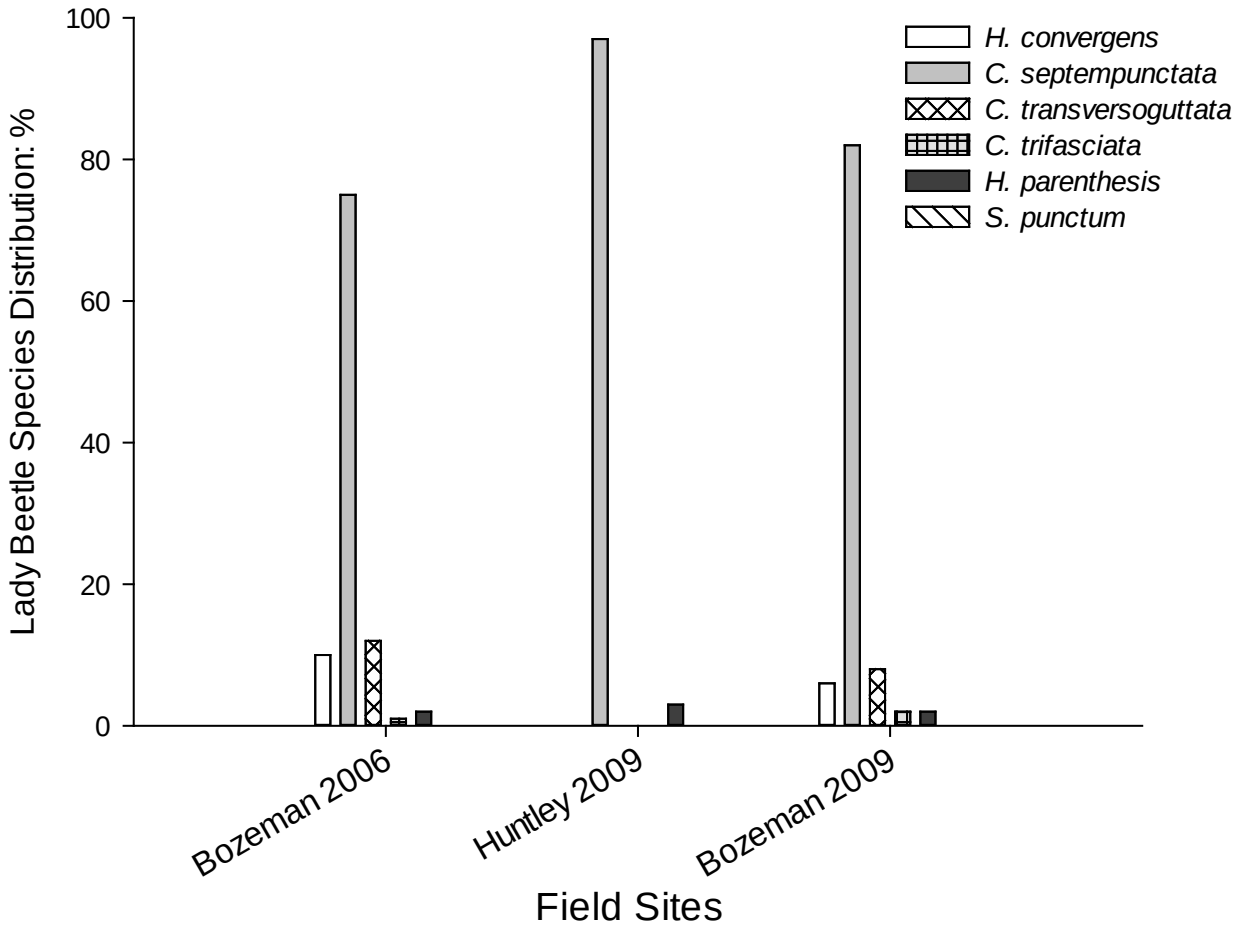


Figure 1. Lady Beetle species assemblages across all treatments and dates at the Bozeman 2006, Huntley 2009, and Bozeman 2009 field sites.