

2006

# Is Everything Connected? Following the Predators, Pests, and Plants Within a No-Till, Western Montana Agroecosystem

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IS EVERYTHING CONNECTED?  
FOLLOWING THE PREDATORS, PESTS, AND PLANTS  
WITHIN A NO-TILL, WESTERN MONTANA AGROECOSYSTEM

By

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Thesis  
presented in partial fulfillment of the requirements  
for the degree of:

Master of Science  
in Environmental Studies

The University of Montana  
Missoula, MT

Autumn 2006

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## ABSTRACT

Smith, Ethan A., M.S., Autumn 2006

Environmental Studies

An Evaluation of Interactions between the Imported Cabbage Worm (*Pieris rapae*), an Assemblage of Six Arthropod Predators, and Two Insecticides Within a Minimum-Till Brussels Sprouts Agroecosystem

Chairperson: Neva Hassanein

Agricultural systems are being re-engineered with hedgerows, living mulches, or minimum tillage activities in hopes of retaining populations of beneficial, predatory insects that may decrease the need for pesticide use. The purpose of this research was twofold. First, this on-farm research assessed the population and activity of six beneficial arthropod predators - the carabid beetle (*Carabidae*: Coleoptera), minute pirate bug (*Orius spp.*: Hemiptera), nabid bug (*Nabis spp.*: Hemiptera), lady beetle larvae (family *Coccinellidae*: Coleoptera), syrphid fly larvae (*Syrphidae*: Diptera) and spiders (Araneae) - and one crop pest - the imported cabbage worm (*Pieris rapae*: Lepidoptera) - within a no-till Brussels sprouts (*Brassica oleracea*, gemmifera group) and red clover (*Trifolium pratense*) living-mulch system. Second, using two common organic insecticides - *Bacillus thuringiensis var. kurstaki* (Bt) and a pyrethrin/rotenone blend - this research assessed the capacity of these aforementioned predatory or parasitic arthropods to control the *P. rapae* population and crop damage through biological (as compared to chemical) means.

Field investigations for predators/pests involved weekly sweep-net sampling, pitfall trap installation, and direct plant examination. Insecticides were applied as a bi-weekly "calendar" application (pyrethrin/rotenone) or as a pest-density "threshold" dependant application (Bt). Generally, pest control and damage prevention were more successful in Bt treatments than in pyrethrin/rotenone treatments. Bt pesticides had no significant effect on any arthropods sampled, while the pyrethrin/rotenone insecticide appeared to significantly reduce the activity or population levels of all arthropods sampled. *P. rapae* activity and crop damage was lowest in Bt treated plots, moderate in control plots (no pesticides were applied, yet natural levels of arthropods were present), and highest in plots treated with pyrethrin/rotenone sprays. Preliminary results indicate that Bt treatments worked as an additive control measure, which then augmented natural predator populations. The increased pest activity and damage in pyrethrin/rotenone treated plots - which coincided with reduced "beneficial" insect numbers as compared to the other treatments - may indicate a disruption of the multiple-arthropod predator assemblage that kept pest impacts lower in the "control" plots.

## ACKNOWLEDGEMENTS

First and foremost, I would like to express my gratitude to my thesis committee for their help in crafting, refining, and shaping this project: To Neva Hassanein, my adviser, who has always known when I needed a hug, when I needed a kick in the pants, and what to say in the times in-between. To Josh Slotnick, who has been a mentor, teacher, inspiration, and good friend - all performed seamlessly and with ease. To Helen Atthowe, truly a “mad-scientist” farmer, who has motivated an entire new generation of students to seek connections between our farms and the natural world around us.

I would also like to thank the Western Sustainable Agriculture Research and Education (WSARE) program for funding this research project, and for their part in spreading practical knowledge to people and places that need truly it.

Thanks also to: Joni Peterson and Lauren Preistman, two rays of sunshine in the field. Hans Zuuring, who patiently helped me re-learn statistics. Tom Deluca, one of the greatest teachers I have ever known. Sue Blodgett, for her excellent technical support.

To my graduate school classmates and professors, too numerous to fit on one page, thank you for being the peers that I’d hoped I’d meet in grad school – driven, idealistic, and tireless in the pursuit of the better world that we all know is possible.

And finally, last only so that I can keep thinking about them long after I’ve trailed off of this page, thank you to my family: My parents, Harlie and Marguerite Smith, and my brother Adam, who have supported me on every step of my journey. And, of course, to my beautiful wife Sarah, who has patiently waited for me on many late nights, who has ignored miscellaneous jars of larvae in the kitchen and bowls of Brussels sprouts in the bedroom, and who is probably as glad as I am that this project is complete. Thanks.

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## INTRODUCTION

Each year, farmers and gardeners across America battle pests in their fields and on their crops. Annually, nearly \$33 billion in agricultural products are lost (or fail to be realized) due to insect, weed, and disease infestations in the U.S.; \$15.9 billion of those losses are due to insect pests in agricultural crops, many of which are introduced or exotic species that have only recently come to this continent (Pimental, et al. 2000).

The imported cabbage worm (ICW), *Pieris rapae*: Lepidoptera, is a significant, introduced pest on farms and in gardens throughout the United States wherever *brassica* plants are cultivated. Current control measures vary by region and by the management style of the farms producing this plant family, which includes cabbage, Brussels sprouts, broccoli, and more. In many areas, producers are structuring their fields to encourage biological sources of pest control - such as predators and parasites – as a supplement for chemical pesticides. Prior research has been conducted on the parasites and individual predators of *P. rapae*, though usually in conventional, bare-soil agricultural systems. Little is known, however, about the efficacy of multiple-arthropod assemblages on controlling this pest, or how these effects may vary in “unconventional” agricultural systems that include minimum-tillage or living-mulch practices. This study probes connections between pest management techniques, the ICW, six predatory arthropods, and final crop damage within a minimum-till, living-mulch system.

The second section of this report is a review of current literature. It details the lifecycle of *P. rapae*, and highlights the positive and negative aspects of current biological and chemical approaches to its control. Relevant research on predator/parasite



interactions with crop pests – and specifically the ICW - are explored, and the gaps within published works identified. Finally, the production of Brussels sprouts (*Brassica oleracea*, Gemmifera Group) is addressed, followed by details and considerations of no-till and living-mulch systems of production.

The third section of this report details the research methods used for data collection. A brief site history and explanation of the farm's organization are included, followed by plot/treatment designs and detailed sampling methods.

Section four presents the experimental results in graphic and written form, followed by a detailed discussion of experimental results in section five. Finally, section six presents the conclusions of this research.

## REVIEW OF LITERATURE

### *Pieris rapae*, the Imported Cabbage Worm

#### History

The imported cabbage worm (*Pieris rapae*: Lepidoptera), or “ICW”, was originally introduced to Canada from Europe in the mid 1800’s. Aided by trade and efficient biological dispersal, the ICW is now nearly ubiquitous throughout North America, and can be found on every continent except for Antarctica (Antonelli 1987). The life-cycle and attributes of the ICW have been documented extensively since their introduction to North America, and publications concerning their biology and methods of control date back to the beginning of the 20<sup>th</sup> century. Moss (1933) and Richards (1940) published early articles detailing *P. rapae* biology, ecology, and controls that are still cited by authors and researchers today. Many other studies have since filled gaps in regional understanding, probed deeper ecological connections, and explored the impacts of new insecticides.

#### Biology and Lifecycle

Adults of this genus are familiar to almost anyone who has worked in a garden or walked through a field in the evening hours of summer. Often incorrectly identified as moths, the white or cream hued adults are actually butterflies. Smaller than 5 cm from wing-tip to wing-tip, this species can be identified by the simple black dots that adorn the tip of the forewing and the small black spot on the front edge of each hind wing. Females have two black spots on each forewing, while males have only one (Opler 1984, 1992).

*P. rapae* over-winter as pupae, often attached to host-plant debris. Adults may emerge as early as March or April, and lay eggs singly on cultivated host plants if available. The first generation often begins on wild host plants in the *Brassicaceae* family, however, because few farms or gardens have established brassica crop-plants so early in the spring. Larvae (see lifecycle, Fig. 1) will hatch from their eggs in 4-8 days, and subsequently feed on their host plants for 14-21+ days. During this period, larvae pass through

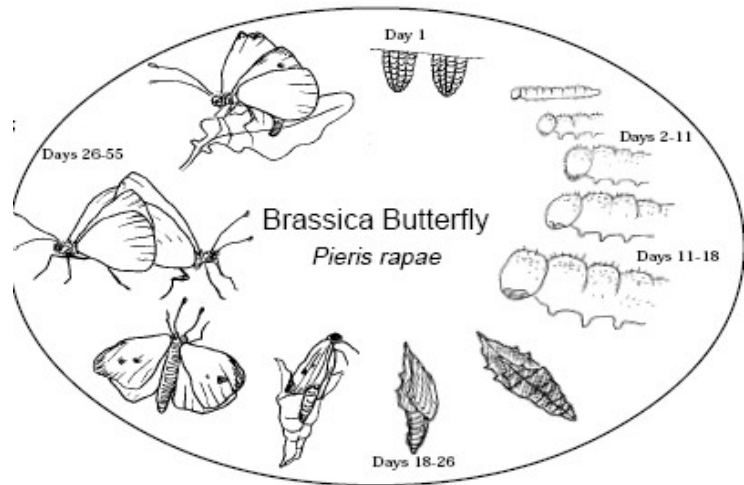


Fig 1. Life cycle of *Pieris rapae*. “Days” ranges indicate the variability of duration for each stage. Note that this variability leads to life cycles of four to eight weeks. (WFPP n.d.)

five instar phases, molting and enlarging each time. Mature larvae then form pupae that are secured by silk bands to the undersides of host-plants. Adults (butterflies) generally emerge from their pupae in another 7-12 days to begin the next generation. An exception to this occurs within the last generation of ICW in a season, when individuals pause at the pupae stage and remain there to over-winter and emerge the following spring (Antonelli 1987). Factors such as temperature, rainfall, food supply, cause this time span to be quite variable across different regions and climates. This temporal variability results in generations that span four to six weeks or more. It is estimated that there may be three to five generations of *P. rapae* per growing season in western Montana, but exact numbers are currently unknown.

### Existing Controls for *P. rapae*

Farmers and gardeners have employed many different techniques to combat cabbage worm infestation, and most fall into two broad categories: “chemical” control and “biological” control. The following section explores these two strategies, as well as the known benefits and drawbacks of each.

#### Chemical Control

Chemical control of insect pests involves the application of toxic compounds - either synthetic or natural in origin - to a crop system or surrounding area with the aim of killing a target species. These toxins have a mode of action that is either “broad spectrum” or pest-specific. An insecticide’s mode of toxicity – how it kills or otherwise affects an organism – has a large bearing on the scope and scale of its effects within an agroecosystem and the greater environment.

#### *Broad Spectrum Insecticides*

Broad-spectrum insecticides are compounds that have a capacity to kill a wide variety of insect species. Many of these are contact poisons that affect most arthropod species in a similar way, making them efficient and versatile while also increasing the risks to non-target species. Conventional (non-organically certified) broad-spectrum insecticides used to control pest larvae like *P. rapae* are often in the form of pyrethroids - synthetic neurotoxins that kill insects soon after contact. This class of compounds acts by disrupting the sodium channel within nerve membranes, cascading across various levels of physiological systems, quickly paralyzing and finally killing most arthropods (WHO

1989). Compounds such as permethrin and cypermethrin are commonly used pyrethroids, and appear in popular brands such as Pounce, Ambush, Demon, and Raid. Though listed within insect management literature for control of lepidopteran larvae, their use may negatively impact natural enemies of crop pests (Hollingsworth 2006). These compounds are specifically engineered to resist break down in the presence of water and sunlight, and can persist in the environment for weeks following application (Class 1992).

It is often difficult or impossible to prevent these insecticides from affecting non-target species. For example, Dempster (1967, 1968(a), 1968(b)) studied the ecological effects of using a persistent, broad-spectrum insecticide to control *P. rapae* within a Brussels sprouts system. These studies demonstrated a decrease in parasitoid populations, reductions of up to 50% in spider and other generalist predator populations, and 50-250% fewer pest deaths from predation in sprayed plots. Crop pests that survive these chemicals may then be free of beneficial insect controls in the field for several weeks, until predator/parasite populations can rebuild. Aside from the negative impacts upon non-target, predatory, and parasitic insects, pest resistance to entire classes of these pesticides can develop with continued applications (Beugnet 1995, Hemmingway 2002). Many synthetic pyrethroids are also extremely toxic to aquatic species and other vertebrates (Cox 2002), and may negatively impact soil biotic communities (Rangaswamy and Kenkateswarla 1993)

Pest control under National Organic Program (NOP) standards also allows the use of certain broad-spectrum insecticides that originate from natural sources. One such chemical class is the pyrethrins, natural derivatives of two chrysanthemum flowers:

*Chrysanthemum cinerariaefolium* and *C. cinereum*. Natural pyrethrins were the original template for synthesizing synthetic pyrethroids, and thus share similar chemical structures and modes of toxicity. Pyrethrins are often blended with another chemical, rotenone (derived from the tropical plant *Lonchocarpus spp.*) and sold as broad-spectrum insecticides for the organic control of dozens of insects (Casida 1973), including *P. rapae*. Pyrethrins have a much shorter persistence period (24-72 hours) than synthetic pyrethroids (2-4+ weeks), yet they may still reduce populations of beneficial insects such as pollinators, predators, or parasitoids. They must make dermal contact to be effective, thus lacking effectiveness on hidden larval pests due to their quick breakdown in sunlight and water (Casida and Quistad 1995, Extoxnet 1994). Like synthetic pyrethroids, pyrethrins may also have toxic effects on birds, fish, and humans, and negatively affect soil biota (Cox 2002).

### *Pest-Specific Insecticides*

While broad-spectrum insecticides are toxic to many different species, other chemicals are pest-specific, killing only certain types of insects. These compounds are designed to have a high toxicity to target pest-organisms while maintaining a low level of toxicity to most other organisms. The two most popular pest-specific chemicals used to control *P. rapae* larvae are spinosad and *bacillus thuringiensis* (Bt).

Spinosad is a compound derived from two secondary metabolites of the soil-dwelling actinomycete *Saccharopolyspora spinosa*, spynosin A and spynosin D. Spinosad is applied to plant surfaces in concentrated liquid form, and works as both an ingested or contact toxin that causes neurological disruption, involuntary spasm, and

paralysis. Unlike broad spectrum contact insecticides, spinosad has a low level of toxicity to many beneficial pollinators, predators, and parasitoids, while showing a high level of toxicity toward insects in the Lepidoptera, Diptera, and Thysanoptera orders. According to a review conducted at Cornell University, 33 of 47 ( $\approx 70\%$ ) recent studies indicate “good” levels of caterpillar control with spinosad (Caldwell et al. 2005). Despite these positive results, spinosad may still have unintended impacts upon non-target species in aquatic or terrestrial environments. Spinosad can decrease reproductive capacity and longevity in some parasitoids (Williams et al. 2003). It is also thought to be toxic to some beneficial predators such as Syrphid flies (Chaney 2003), and may therefore be undesirable for IPM use.

*Bacillus thuringiensis* is a gram-positive species of naturally occurring, soil-dwelling bacteria. The spores and proteins of Bt are manufactured into powdered or liquid insecticides, which are then applied to plant surfaces. Bt differs from most broad spectrum insecticides - which are dermal or contact poisons - in that it must be ingested to kill target pests. As part of their lifecycle, Bt bacteria produce crystalline proteins that accumulate on plant tissues and leaf surfaces. These protein endotoxins are then consumed during feeding, and bind to the gut of the larvae. This area of binding usually develops a hole through which contents of the digestive system enter the body cavity and the blood stream (Nester et al. 2002).

Unlike spinosad (which is not broad-spectrum, yet may impact several insect orders), Bt is highly specific to which species of insects it will effect. Strains commercially available include *B. thuringiensis* vars. *kurstaki*, *aizawi*, *tenebrionis/san diego*, and *isrealiensis*. *Bt* var. *tenebrionis* and var. *isrealiensis* are used on beetle larvae

and mosquitoes/black flies respectively. The most common strains used for ICW control are *Bt var. kurstaki* and *var. aizawi*. Bt has been shown as a supplemental control for many pests - including *P. rapae* - while having no negative affect upon beneficial arthropods (Lundgren et al. 2002).

Bt is hindered by rapid break down in sunlight and water, and works only on feeding larval stages of pests (Hines and Hutchison 2001). Excessive contact with Bt may lead to adaptive resistance in pests (Loseva et al. 2001), and as such an alternation of different Bt strains is recommended for instances where many repeated applications are made. Though Bt does not pose a threat to many of the predatory or parasitic insects within agroecosystems, it may nonetheless be toxic to non-target caterpillar species. The impact of Bt on the Monarch (*Danaus plexippus*) butterfly has been heavily debated (Pimentel and Raven, 2000), and there are still poorly understood ecological effects upon non-target species that may arise from cavalier dispersal of these bacteria (James et al. 1993, Naranjo 2005).

### Biological Control

Biological control is a portion of a larger pest management system known as Integrated Pest Management (IPM). While some IPM systems do use pesticides as a last resort, biological control methods are antonymous management tools that work independent of these chemical applications. Flint and van den Bosch (1981) nicely sum up the goal of biological control within IPM as: “An ecologically based pest control strategy that relies heavily on natural mortality factors...and seeks out control tactics that disrupt these factors as little as possible.” The “natural mortality factors” referred to here



may take the form of predation or parasitism (predatory/parasitic insects, birds, mammals, reptiles, amphibians, etc.), disease, inter- or intra-specific competition for resources, allelopathy, and more. Due to the extensive nature and complexity of the many types of biological control, this research focuses only on the portion of biological control using predatory or parasitoid (parasites that kill their hosts) insects.

### *Predators and Parasitoids*

Farmers may reduce crop damage and obtain non-additive crop gains by employing arthropods that are natural enemies of agricultural pests (Cardinale et al. 2003). For these natural enemies to be effective, their parasitic or predatory life-cycles must intersect with the appropriate lifecycles of their host or prey (the pest). An example of this is the egg-parasite *Trichogramma spp.*, whose adult (wasp) stage must be concurrent with their host's egg stage to achieve effective pest control (Knutson 1998). An agricultural producer must therefore understand the specific attributes of the predators and parasitoids that they wish to employ in order to align these traits with the lifecycles of their pest.

Arthropods that fall into the "predator" category tend to share several common attributes. Predators are often generalists, consuming many different species and varying developmental phases of prey, and will do so to fulfill part or all of their diet during one or more of their life-cycle stages. Males and females may be predatory, and are often - though not always - larger than their prey. Predators will also consume many prey through the course of their life-cycles (Hoffman and Frodsham 1993). A review of manipulative field-studies showed that in nearly 75% of studies, predatory arthropods

caused a significant reduction in crop pests (Symondson et al. 2002). Naturally occurring generalist predators such as ground beetles (Coleoptera: *Carabidae*), many species of spiders (*Araneae*), minute pirate bugs (*Orius spp.*: Hemiptera), nabid or damsel bugs (*Nabis spp.*: Hemiptera), and the larvae of lady beetles (Coleoptera: *Coccinellidae*) and syrphid flies (Diptera: *Syrphidae*) directly feed upon many agricultural pests, including the imported cabbage worm in either egg and/or larval stages (Dempster 1968, Ashby 1974, Schmaedick and Shelton 1999). Complete biology, life cycles and descriptions of these six predatory arthropods may be found in Appendix A.

Arthropod parasitoids differ from predators in several important ways. Parasitoids are specialists, usually attacking only within a certain genera or species of host, and then will attack only during certain host life-stages. Only female parasitoids search for hosts, depositing eggs on, within, or near their target. The small, immature parasitoids will feed upon (and thereby kill) their larger, single host before moving on to adulthood. Adult parasitoids can be - but are not always – predacious (Hoffman and Frodsham 1993). Arthropod parasitoids such as the wasp *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) have been purported to kill up to 90% of the mature ICW larvae during mid-summer generations (Boucher 1995). Findings by researchers such as Coleman et al. (1999), however, are raising questions as to whether or not high rates of parasitism are actually correlated to a reduction in plant damage. The egg parasitoid *Trichogramma spp.* is another wasp species used for IPM controls of *P.rapae*, and is currently in use in Oregon and Washington.

### *Augmentative Parasitoid/Predator Biological Control*

The implementation of biological control using predatory or parasitic insects is generally accomplished in one of two ways. The first method involves the release of a specific species of predator/parasitoid insect at the location of a current or anticipated pest outbreak. This method, known commonly as “augmentative” biological control, involves the release large numbers of predators/parasites to either augment natural populations or to overwhelm the pest directly. Often, this release is a reactionary attempt at control that *follows* a pest outbreak, but releases of these “beneficial” insects may also be used as a tool for pre-emptive establishment of predator/parasite populations. The release of predators such as the convergent lady beetle (Coleoptera: Coccinellidae) can be quite successful for short term control of pests such as aphids (Dreistadt and Flint 1996). A review of research on augmentative control, however, found that over 50% of establishment or augmentation attempts resulted in failure. Many of these failures were attributed to poor habitat and shelter, inaccessibility of pests, dispersal problems for the predators/parasites, and other environmental factors on-site which may have led to low beneficial arthropod populations in the first place (Collier and Van Steenwyk 2004).

### *Ecological Engineering for Biological Control*

The second method of applying biological control with insects is the practice of “ecologically engineering” an agricultural ecosystem, which in this case means the encouragement of *naturally* occurring predators and parasites in the crop area by altering, enhancing, or increasing the amount of available habitat. The term “ecological

engineering” was first used by the ecologist H.T. Odum (1962) to describe "...those cases where the energy supplied by man is small relative to the natural sources but sufficient to produce large effects in the resulting patterns and processes." Today, the term describes portions of many ecological fields, including research and writings on alternative agriculture and pest management (Gurr, Wratten, and Altieri 2004).

The idea of altering an agricultural system so that human inputs are minimized and ecological processes are maximized is far from new. Agriculture depended upon natural sources of pest control, weed abatement, and soil fertility for literally thousands of years. Only within the last half of the 19<sup>th</sup> century did these tools change over to synthetic fertilizers, powerful chemicals, and highly mechanized operations. A new field of researchers, is looking past these technologies - or perhaps looking *before* them - to learn more about the ecological inputs that might save money and time, reduce pollution and habitat destruction, and might actually increase crop yields.

Miguel Altieri, one of the leaders in the field of Agroecology, has found that weed or ground covers –as opposed to bare soil - can attract greater populations of beneficial insects (1979, 1982, 1986), these may then help control pest outbreaks (1984), and can help to bolster systemic stability with increased biodiversity. Leaving un-mowed borders or field margins will protect existing predators and can increase their populations (Thomas 1991, Denys and Tscharnke 2002). These un-disturbed areas also help control dispersal, diversify communities, and aid scavenging predators such as ground beetles (Clark et al. 1997, Kinnunen et al. 2001). Parasitoids can benefit as well from added habitat and cover, and may use flowering ground covers or perennials as nectar sources

(Rebek et al. 2004), though flower selections should match the desired parasitoids as some are ineffective and may even be repellent (Wäkers 2004).

The diverse communities of arthropod predators that develop in these engineered ecosystems can help control pests before they reach outbreak status (Flint and Dreistadt 1998). Multi-species assemblages of predators or parasites in these refuges can be as or more effective than an individual species is in augmentation releases, yet interactions between different predator and parasitoid species are still poorly understood and highly variable (Symondson 2002). Some assemblages of predators and parasites may have additive interactions, as generalist predators aid parasitoid searching by removing other low-preference species of prey (Cardinale et al. 2003). In the aforementioned study, Cardinale et al. also reported non-additive increases in the total crop system, resultant from concurrent suppression of multiple pests. Conversely, parasite regeneration rates may drop in the presence of generalist predators (as parasitized hosts are eaten), and these interactions may lead to delays in control of a burgeoning pest population (Snyder and Ives 2002). Disturbances in species or habitat composition (introduction of new arthropods, tillage that eliminates refuge, application of pesticides/herbicides, etc.) can potentially scatter many species, rendering the system ineffective for pest control (Jervis, Lee, and Heimpel 2004, Flint and Dreistadt 2004).

Though agricultural systems with diversified and enhanced arthropod habitats may well increase numbers of “beneficial” predators and parasitoids, little research has explored the actual IPM contributions made by these bolstered arthropod populations. Multiple-arthropod assemblages may prove to be an effective means for general pest control in such systems. More research is needed, however, to probe additive or

subtractive interspecies interactions, to understand connections between generalists and specialists, and to test the influence of chemical or physical disturbance upon these systems.

### *Brassica oleracea* (Gemmifera group) - Brussels Sprouts Production

The *Brassicaceae* family contains approximately 350 genera, and over 3000 species of herbaceous plants. Originating in Europe and Eastern Asia, this cool-season plant family includes the cultivated crops: Brussels sprouts, cabbage, broccoli, cauliflower, kale, turnips, mustards, radishes, and more. Additional species in this family are wild or “weed” species, and may serve as hosts or incubators for diseases or pests such as *P. rapae* (Antonelli 1987).

Although Brussels sprouts may be grown through direct seeding, cultivation in cool northern regions most often begins with seedlings grown under greenhouse conditions. Seedlings are grown indoors for 4-7 weeks, and then transplanted into the field by hand or mechanical means once the danger of frost has passed. To allow for plant growth and resource capture, spacing within rows is generally 40-60 cm, with 60-100 cm spacing between rows. Temperatures between 5° C and 25° C are desirable for growth, with the optimal range being 15-18° C. Nutrient requirements of all *Brassicaceae* species are fairly high, with particularly high demands in nitrogen and phosphorus, as well as moderate needs for potassium, sulfur, and other micronutrients. A soil pH of greater than 6.5 is desirable to prevent outbreaks of diseases such as clubroot (*Plasmodiophora brassicae*), and to maintain nutrient availability.

Harvest takes place when either the lower leaves of the plant turn yellow, or when the sprouts reach an optimal size. Sprouts that are allowed to get too large can have a bitter taste and tough texture. When harvested by hand, the lowest and largest sprouts are snapped from the stalk, allowing the upper, smaller sprouts to continue to mature. In this way, several harvests totaling two pounds or more may be achieved from each plant. Mechanized operations typically remove the entire plant; thus, large commercial yields are often lower in per-plant weight.

Brussels sprouts spend longer in the field than nearly any other brassica species, often taking 90-110+ days from germination to harvest. This extended residency may result in a longer period of time for nutrient acquisition, but may also expose the plants to a greater variety of crop pests. The greatest pest pressures for Brussels sprouts are from the cabbage worm complex (the imported cabbage worm, cabbage looper, and diamondback moth), cabbage maggots, and aphids. Different biological, cultural, and chemical controls for each of these pests exist, and range from the enlistment of beneficial insects to the use of highly persistent, synthetic pesticides.

#### *Considerations for No-Till Brussels Sprout Production*

The high resource needs of brassica species require the use of a well-designed agroecosystem. Heavy feeding requirements can deplete soil resources, and pest and disease pressures may build during successive years of planting in the same area. These needs may be satisfied by growing brassica plants - and specifically B. sprouts - in minimum-tillage systems with living-mulch ground covers.

Living mulches and reduced tillage B. sprouts systems may bolster populations of beneficial insects that could reduce pest pressures. Experiments growing brassica crops within a living-mulch have shown increased levels of beneficial predators such as ground beetles and members of the *Coccinellidae* family (Schellhorn 1997). Intercropped Brussels sprouts can have lower pest populations as compared to plants grown in bare soil (Theunissen 1980). Reduced tillage systems may help retain organic matter, which has been positively correlated to reductions in soil-borne pathogens (Hoitink et al. 1997, Stone et al. 2003). Nutrients may exhibit delayed or reduced availability in minimum-tillage systems as compared with conventional tillage (Stubbs 2004), yet long growing-season Brussels sprouts have more time to capture them.

Brassica plants may not always benefit from living mulches or intercropping, however. Ground covers may compete with crop plants, leading to low or no marketable crop yields (Dempster 1969, Bottenberg et al. 1997). Weaver (1984) concluded that this competition could be minimized by removing ground-cover or weeds four or five weeks prior to transplanting. Diseases may actually find refuge in minimum-till or living mulch systems if plant debris remains near crops from year to year, and if rotations are not implemented (Bockus and Shroyer 1998). Careful selection and management of living mulches and adherence to crop rotation are therefore important considerations in these systems.

This research will attempt to determine the presence of predatory and parasitic arthropods within a no-till Brussels sprouts system, and will assess the ability of these “beneficials” to control the population and resultant damage of *P. rapae*.



## METHODS AND MATERIALS

### Site History and Description

The site-location for this research was Biodesign Farm, owned and operated by Helen Atthowe, and located in Stevensville, Montana, at approximately 46°32'N-114°03'W. The experimental plots were established in a recently initiated six-acre field (see Figure 2) that was created with the dual goals of 1) increasing the habitats of wildlife and beneficial insects, and 2) providing a system for efficiently producing vegetables with a high economic yield.

For 13 years, Biodesign Farm has been following the lead of Miguel Altieri and other agroecologists in the experimentation with living mulches between and within vegetable rows. Beginning in 1995, on-farm research was done by owner/operator Helen Atthowe to study the management of these living mulches and the possible affects upon populations of beneficial insects. It was found that lightly-mowed but intact living mulches contained higher levels of beneficial predators than did bare-soil or tilled plots (Atthowe 1996). Atthowe also made specific identifications of predatory insects on site, including syrphid fly larvae (family *Syrphidae*), predaceous stinkbugs (family *Pentatomidae*), aphid parasitoids, spiders, carabid beetles (family *Carabidae*), lady bugs and their larvae, minute pirate bugs (*Orius spp.*), lacewings (family *Chrysopidae*), and nabid bugs (*Nabis spp.*). This experimental outcome led to the pursuit of minimum-tillage practices on the farm in 1997, followed by the cessation of all pesticide application for cabbage-worm pests in 2000.

## Management of Habitat for Beneficial Insects

The field containing the test plots was managed as a perpetual pasture for 50 years until the fall of 2004 when 600 sheep were pastured on the site and employed for what the farmer terms as “sheep tillage” (heavy, close grazing and manure deposits). In April of 2005 the field was undercut and turned to a depth of approximately 15 cm, disked twice, and then allowed to dry. One month later the site was disked again, and a cover-crop blend of triticale (a hybrid of wheat – *Triticum spp.*, and rye – *Secale spp.*) and red clover (*Trifolium pretense*) was seeded and allowed to establish. This cover-crop was designed to function as a “living mulch” to provide soil stabilization, suppress weeds, and to create possible beneficial insect habitat on what would otherwise be bare soil in many agricultural systems. The clover-triticale blend was specifically chosen for its ability to provide a quick, vigorous, and dense cover after sowing (triticale), and to provide nitrogen to the system while persisting through the winter (red clover).

The triticale germinated quickly after sowing, preventing the establishment of quack grass (*Elymus repens*) and other pioneer weed species. Within three weeks, however, the triticale was completely out-competed, and a virtually pure stand of red clover emerged. The clover was mowed close (8 cm) in September of 2005, and left undisturbed during the late fall and winter months. By April of 2006, the *T. pretense* still dominated the field. The depth of this clover averaged 10-15 cm throughout the field, and it comprised nearly 100 percent of the plant biomass.

The field possessed a central strip of undisturbed and untilled permanent pasture grass that measures approximately 200 m long (the full length of the field) by 10 m wide. As shown in Figure 2, this strip connects at both ends with the 5 m wide swath of un-

mowed, permanent pasture grass that surrounds the entire field margin. All rows within the field are separated by 1.5 - 2.5 m wide pathways of the red clover that has persisted since the 2005 planting. Living mulches and undisturbed borders such as these have been demonstrated to encourage predatory arthropod populations and to increase predator to prey ratios (Altieri and Letourneau 1982, Denys and Tscharntke 2002).

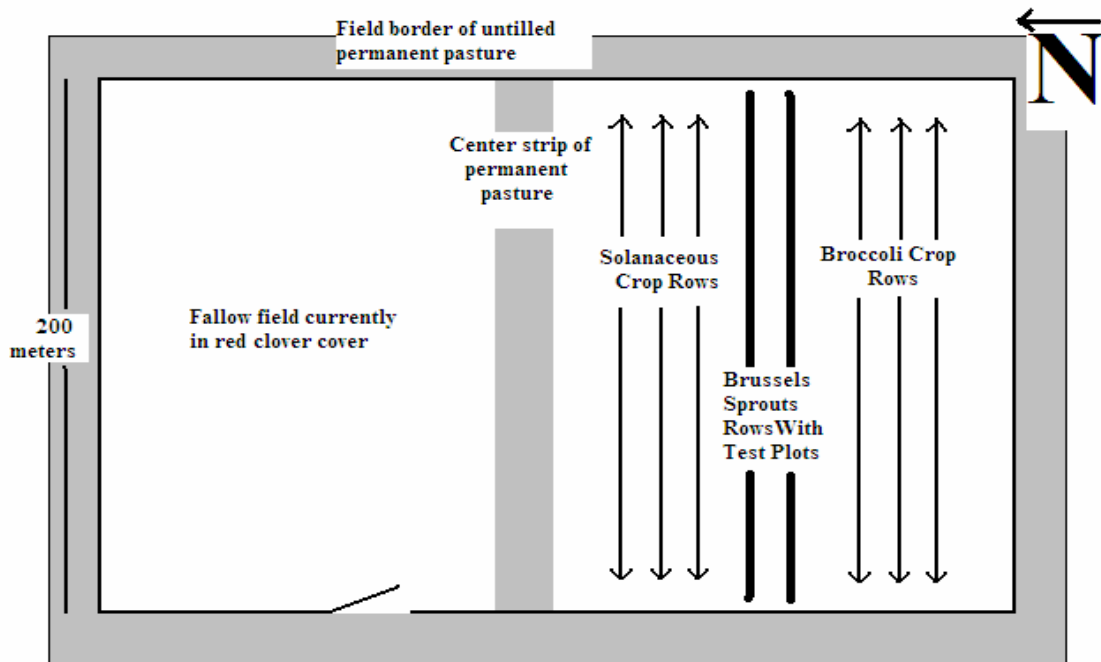


Figure 2. Overhead view of research site at Bidesign Farm. Unmowed center strip and borders of pasture grass have been no-till for 50 years. The white portion of the diagram indicates where the triticale-clover ground cover was established in 2005.

## Experimental Design

### Establishment of No-Till Brussels Sprouts

The red clover planted in 2005 over-wintered in the 6-acre field, and comprised the living mulch within and between the planting rows of the experimental plots. To prepare the No-till rows for the Brussels sprouts transplants, two parallel strips 0.5 m

wide and 2 m apart were cut into the clover with a single pass of a tractor mounted mower. This was followed by a series of passes with a Forevergreen™ “Agra” model infrared-weeding device, walking at approximately 50 meters per minute. By operating at 1000° C on the combustion of compressed propane within a ceramic element, this device wilted the mowed clover by boiling intracellular water and rupturing plant cells as it passed over. This wilted strip was designed to set the clover back – but not to kill it – so as to allow the Brussels sprouts seedlings to establish themselves prior to the recovery of the mulch. The plots were not tilled or disturbed in preparation for planting. Instead, a minimal application of composted manure (approximately 1000-2000 kg/ha) was spread on the planting beds as a top-dressing and spread with a tractor-mounted harrow two weeks prior to field transplantation of the crop seedlings.

Brussels sprouts (*Brassica oleracea (gemmifera group)*) seeds of two varieties, “Oliver” and “Diablo”, were started in early April. Seedlings were grown for six weeks under greenhouse conditions in 50-cell trays (cell size 1”L x 1”W x 1”D) and transplanted into the field in mid-May. The plants measured approximately 10-12 cm tall at transplanting. Seedlings were planted directly into the wilted strips of clover with a conventional, tractor-mounted waterwheel transplanter, spaced 0.5 m apart with 0.5 m spacing between rows. The transplanter operated by punching regularly spaced holes in the soil that were simultaneously filled with an aqueous solution of fish emulsion. Seedlings were then placed by hand into these holes with the soil firmly secured around their roots. Seedlings were watered in via overhead irrigation, which was continued throughout the growing season to supplement natural precipitation.

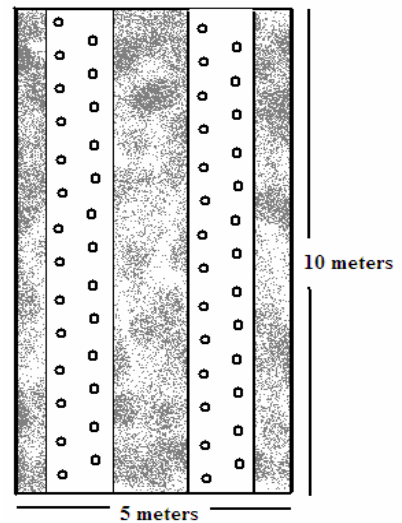
### Plot Design and Field Layout

Plots measured 10 m long by 5 m wide and encompassed the two double rows of Brussels sprouts, with the red clover living-mulch underneath (see Fig. 3). 55-57 Brussels sprouts were planted in each plot, with an equal number of “Oliver” (an early-harvest variety, planted in the north row) and “Diablo” (a late-harvest variety, planted in the south row) in each. Experimental plots were separated from neighboring plots by a buffer of approximately 8 meters of untreated Brussels sprouts and clover.

Three different treatments were used, with each assigned to a separate plot. These were then assembled into a randomized-block design over four replications, yielding a total of 12 plots.

### Treatments

The three treatments in this experiment were crafted to resemble three agricultural methods of controlling the ICW. Two treatments involved the application of pesticides, and were administered under specific conditions. The “calendar” treatment plots each received a biweekly application of Bonide® liquid pyrethrin/rotenone insecticide (0.8% pyrethrin and 1.1% rotenone) at a rate of 2.6 ml per liter (2 teaspoons per gallon) of H<sub>2</sub>O - as so labeled for control of *P. rapae* larvae. The pyrethrin/rotenone solution was



**Figure 3.** Example test plot layout. Patchy grey area indicates red clover cover, and white strips indicate area burned back by flaming. Circles indicate Brussels sprouts seedlings. Drawing is not to scale.

applied using a Solo model 473P3 backpack-pump sprayer, and was administered at approximately 1100-1115 hrs on each application date. Spraying in the “calendar” plots began with the first appearance of *P. rapae* adults on 31 May and continued on a bi-weekly basis until 4 October, constituting 10 total applications (see Fig 9). This treatment, though still recommended by some agencies for ICW control, was intended to disrupt populations of predatory or parasitic arthropods.

The “threshold” treatments received applications of Dipel Dust® Bt (*Bacillus thuringiensis var. kurstaki*) and Concern® insecticidal soap, but *only* when a pest-density based “action threshold” was met or surpassed on the weekly sampling date. Action threshold spray guidelines are an agricultural pest-management tool that bases pesticide application decisions upon pre-determined levels of pest density or crop damage. In this experiment, we based our action-threshold percentages on the presence of one or more larvae per plant. For example, if a sampling of plants showed that 3 of the 10 plants (i.e. 30% of plants sampled) contained one or more larvae, this constitutes a pest density of 30% regardless of how many total larvae were on those three plants. Because specific thresholds have not been established for *P. rapae* on Brussels sprouts in Montana, we adapted them from University of Minnesota Extension Service guidelines for ICW control in cabbage (Hines and Hutchison 2001). The action thresholds used in this experiment were: 30% larval infestation from transplantation until cupping (head formation) and 10% larval infestation from cupping/heading until harvest. The “threshold” treatment consisted of 60 g ( $\approx$  2 oz) of Bt powder mixed with 30 ml ( $\approx$  1 fl oz) of insecticidal soap (equivalent to approximately  $\frac{1}{2}$  the labeled rate and used as a surfactant) per 3.75 liters of H<sub>2</sub>O. This solution was thoroughly applied to all surfaces of

the Brussels sprouts plants at approximately 1100-1115 hrs on the same day that the threshold levels were met or exceeded. There were eight applications of this treatment, with dates indicated by the orange gridlines in Fig 9. This treatment was designed to mimic the standard, recommended control method for the ICW. It was designed to remove the pest species while having negligible impact on the other arthropods.

Finally, the “control” plots received no outside treatment for ICW control. This treatment was designed to retain the pest species as well as the arthropod predators and parasites, and to thus be a measure of the pest-control capacity of these “beneficials” within the system.

## Data Collection

### Weekly Measurements

Weekly measurements were taken on Wednesdays, between approximately 0900 and 1100 hrs. With the exception of ICW adult activity, all measurements were taken within each treatment plot. Whenever individual Brussels sprouts plants were sampled weekly, 10 individuals (based on equivalent sample sizes from Hines and Hutchison (2001) and Maltais et al. (1998)) were chosen every week from within each plot using a random number generator. These randomly chosen sample sets were used for all plant-sampling measurements for that given plot in that given week.

### *ICW Adult Presence and Activity*

Because detailed or exact population size surveys (which often involve catching and marking individual adult butterflies) can be time consuming, a simple method of

assessing *P. rapae* adult activity was used. Once per week, an east-to-west transect line was walked across the experimental field at approximately 1000 hrs. All *P. rapae* adults that were passed along this transect were counted. This method was repeated three times, and the results were then averaged together to give an overall adult ICW activity number for the week.

#### *ICW Egg Presence*

Once per week, the 10 randomly selected plants from each plot were examined for the presence of *P. rapae* eggs. All surfaces of each plant, including the leaf tops and bottoms, petioles, and the main plant stem were searched visually for eggs. Any eggs discovered on the primary search were examined using a 12x field lens to ensure proper identification. The presence of one or more of these eggs on a plant was recorded as a positive presence or “1”, while the complete lack of eggs on a plant was indicated as a negative presence with a “0”. The percentage of plants exhibiting egg presence within each treatment plot was then tabulated, and an average was obtained for each total treatment. Data was assembled from 31 May 2006 to 11 October 2006, and graphed for comparison between treatments.

#### *P. rapae Larval Presence*

Once per week, the 10 randomly selected plants from each plot were examined for the presence of *P. rapae* larvae. Early-instar (first or second instar) larvae were examined with the use of a 12x field lens to ensure proper species identification. The number of larvae present on each plant was counted and recorded. During the second



generation, sampling for larval presence was adapted to include size classes of “A” = less than or equal to 6 mm, “B” = 6 mm to 12 mm, and “C” = greater than 12 mm in length. Larval presence was averaged for each sampling day across each treatment, assembled from 31 May 2006 to 11 October 2006, and graphed for comparison between treatments.

#### *Larval Feeding - Foliar Damage*

Once per week, the 10 randomly selected plants from each plot were examined for evidence of ICW larval feeding. After counting the total number of leaves on each plant, a ratio was obtained between damaged and undamaged leaves. Data was recorded as both unreduced fractions (e.g., 4/16 was not reduced to 1/4) and also in decimal form. Unreduced fractions were used to track the progress of per-plant leaf production and growth, while the decimal forms were used to track the overall percentages of leaves damaged. Percentages of leaf damage were assembled and averaged for each treatment plot, and graphed for visual analysis of variation between treatments.

#### *Larval Frass*

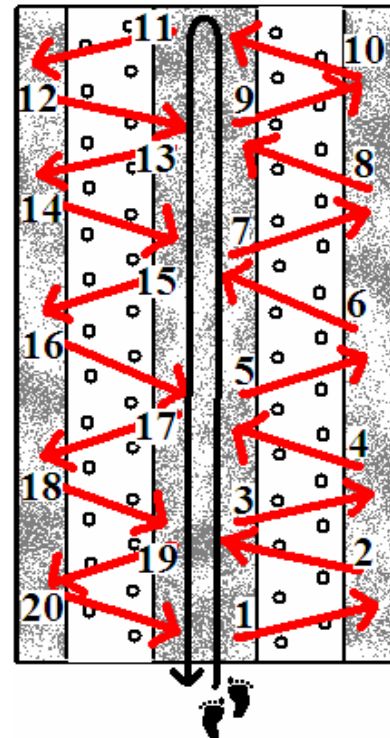
Initially, the ten randomly selected plants from each plot were examined each week for the presence of larval frass (excrement). This measurement was abandoned early in experimentation after uncontrollable variables such as irrigation and other plant disturbances caused variations in frass presence and retention on plants. Larval frass was instead measured for presence on the harvestable portions of Brussels sprouts at the time of harvest.

### *Sweep Net Sampling of Plots*

Sweep net sampling can be the most cost-effective, and time efficient way to collect insects, and can be as accurate as insect vacuums (Parajulee 2006). Once per week, each plot was swept with a Gemplers® R13101 15” sail-cloth sweep net. Sweeps were conducted the same time each week - approximately 1000 hrs - and were done using the following standardized method illustrated in Fig. 4: The net was grasped with a

forehand grip and swept with a quick stroke through the top 20 cm of red clover between the Brussels sprouts plants. Stroke number one began from left to right, and as a single step was taken forward stroke number two was taken immediately from right to left. Twenty strokes in all were completed for each plot, with ten strokes conducted through each of the double rows of Brussels sprouts. Following the 20 sweeps, the contents of the net were examined for quantities of minute pirate bugs (*Orius spp.*: Hemiptera), nabid bugs (*Nabis spp.*: Hemiptera), lady beetle larvae and adults (family *Coccinellidae*: Coleoptera), syrphid fly larvae and adults (*Syrphidae*: Diptera) and spiders (Araneae).

This accounting either occurred immediately on site,



**Figure 4.** Path and pattern of sweep-net sampling technique. Numbers indicate the beginning of each sweep, red arrows indicate sweep direction, and black arrow indicates the path of travel.

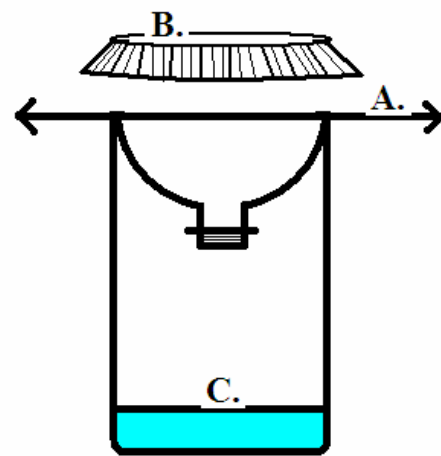
or the contents were emptied into 1-gallon zippered storage bags for later identification if pressed for time. To allow for quick and efficient identification, arthropods were identified only to the genus, family, or order listed here.

Arthropod counts were averaged for each treatment, and the per-treatment sweep results from 31 May 2006 to 11 October 2006 were 1) assembled graphically for analysis, and 2) tested with one-way ANOVA analysis.

### Bi-weekly and Irregularly Scheduled Measurements

#### *Pitfall Trap Sampling*

Pitfall-trap sampling was pursued to examine insect activity at the ground level where sweep net sampling is ineffective. Pitfall traps may be inaccurate measures of arthropod *population*, but they can offer comparison between treatments in terms of arthropod *activity* (Dempster 1968(b)). Pitfall traps (Fig. 5) were created by cutting the tops from 2-liter plastic bottles and inverting them inside the remaining bottle bases. Traps were buried with their tops flush with the soil surface, fitted with small aluminum tart tins for cover, and were filled with approximately 100 ml of 70% isopropyl alcohol. Traps were installed within one of the double rows of Brussels sprouts, located randomly in one of four corners of each plot. Pitfalls were installed on Wednesdays, and removed seven days later for sampling. Ground



**Figure 5.** Diagram of pitfall-trap construction. A.) Indicates ground level, B.) Aluminum tart-tin shelter, and C.) 100 ml 70% isopropyl alcohol solution.

beetles and spiders captured in the traps were counted and recorded. A three-week period was then allowed to pass before the traps were re-installed in different, randomly selected plot corners. This 28-day sequence was designed to prevent the over-harvest of ground-dwelling insects, and was created on the advice of Dr. Sue Blodgett, Associate Professor, Extension Specialist and Integrated Pest Management Coordinator at Montana State University.

Data was assembled from all traps in all plots for statistical analysis. The complete data set was initially tested for homogeneity of variance. After passing this initial test, data was run analyzed by One-way ANOVA that included Tukey HSD as a post-hoc, multiple comparison test.

#### *Parasitism Rates and Larval Rearing*

To measure for rates of larval parasitism by parasitoids, *P. rapae* larvae were collected three times during the growing season and reared in captivity. On 28 June, 16 August, and 06 Sept respectively, 25, 52, and 53 larvae were captured by hand from Brussels sprouts plants that lay within the buffer spaces between experimental plots. Larvae were selected based on their size class - the 4<sup>th</sup> or 5<sup>th</sup> instar, or approximately 2 cm long - in an attempt to ensure that they had been in the field long enough to have been exposed to any parasitic organisms. Larvae were collected from these buffer-areas to prevent sampling effects and the skewing of larval presence numbers within the test plots. Captured larvae were placed in quantities of two or three within small rearing chambers fashioned from 1-quart, glass mason jars for rearing. The bottoms of the jars were first lined with 4-5 cm of moist soil, and several small Brussels sprouts leaves were inserted

stem-down into the soil so as to retain turgor pressure and to stave off leaf desiccation. Subsequent to larval insertion, jar mouths were covered with a section of canvas cloth that was secured by metallic mason jar lid-rings. These coverings allowed for atmospheric exchange of oxygen and moisture, while preventing the inward or outward movement of either larvae or possible parasitoids. Rearing chambers were kept in a mixed-shade location outdoors to maintain climatic conditions similar to those within the field where they were collected. Larvae were inspected every 24 hours for signs of possible parasitism such as larval body discoloration, changes in activity or feeding, atrophy, or death. Once the captive larvae pupated, chambers were monitored every 48 hours for any signs of parasitoid emergence. The rearing chambers received no further monitoring once *P. rapae* butterflies had emerged from the pupae stage.

#### Harvest and Yield Measurements

For ease of obtaining comparable numbers in this experiment, plants were deemed to be of harvestable size when there were at least three perpendicular rows of sprouts on the plant in which all heads were greater than 3 cm in diameter. Plant specimens were clipped with pruning shears at ground level, and the entire plants were taken immediately indoors for measurement. Unless otherwise noted, all weights were measured using a digital produce scale with gradations of 0.005 pounds ( $\approx 2.26$  g). All plants that were deemed harvestable in each plot were harvested and measured, and a final 5-plant subset from each plot was selected for data analysis. Harvest took place once per week over a 4-week period.

### *Total Above Ground Plant Mass*

Lacking equipment and labor for oven-dry biomass measurement, entire plants were weighed immediately after harvest.

### *Total Foliar Damage At Harvest*

Following the measurement for whole-plant mass, the total number of leaves was counted and the percentage that was damaged by larval feeding was recorded. As with weekly damage ratings, both the unreduced fractions of damaged/undamaged and their decimal equivalents were recorded.

Harvest damage ratings were modified by an  $\arcsin\sqrt{p}$  transformation prior to statistical analysis. Once the damage data set passed homogeneity of variance tests, one-way ANOVA analysis with post-hoc, multiple comparison Tukey HSD was performed.

### *Frass Presence*

Following leaf damage assessment, the leaves were removed from the plant by passing a sharp knife through the petioles approximately 4-5 cm distal to the main plant stem. Once the leaves were removed, the harvestable Brussels sprouts were examined for signs of ICW larval frass. Any frass noted was recorded as a positive presence for the plant with a "1", and a lack of larval frass was recorded as a negative presence with a "0". This data collection was abandoned, however, when accurate frass presence was compromised by uncontrollable variables.

### *Brussels Sprouts Damage Assessment*

Following the examination for frass presence and leaf damage, individual sprouts were removed from their stem by grasping them between the thumb and fore-finger and snapping them loose in a direction perpendicular to the stalk. As sprouts were removed, they were assessed using a numerical damage-rating system adapted from the Greene et al. (1969) and Hutchison (2004) systems for *P. rapae* damage on cabbage. In this numerical damage-rating system (see Fig. 6), **1** = No larval feeding, **2** = minor feeding on wrapper or outer leaves totaling 1% of crop, no head damage, **3** = Moderate feeding on outer or wrapper leaves with no head damage and 2-5% of leaf area eaten, **4** = Moderate damage to outer or wrapper leaves with minor head damage and 1-10% of leaf area eaten, **5** = Moderate to heavy feeding on wrapper and head leaves and a moderate number of head-scars with 11-30% of leaf area eaten, **6** = Considerable feeding on head and wrapper leaves with numerous feeding scars and > 30% leaf area eaten. Vole damage was recorded by the number of sprouts damaged per plant.

### *Total Brussels Sprouts Mass*

Following removal from the stalk and the damage assessment, the total mass of Brussels sprouts from each plant was recorded. Any sprouts damaged by voles that were therefore unsalable irregardless of larval damage were omitted from all treatment totals.

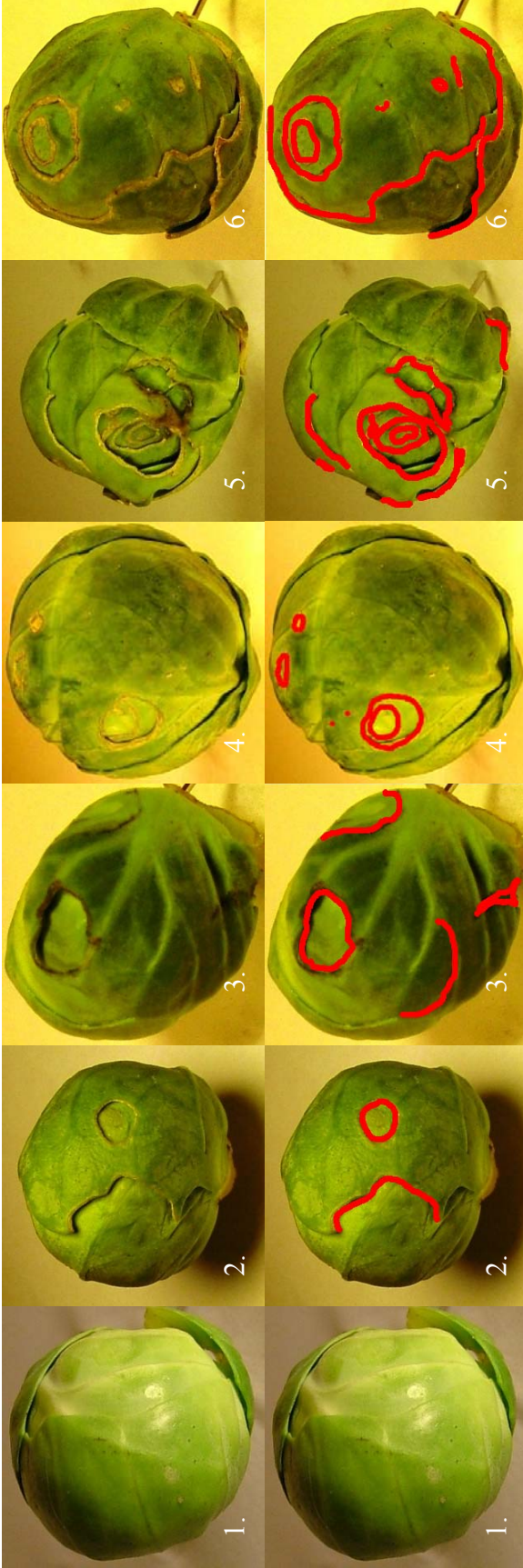
### *Unsalable Brussels Sprouts Mass*

Using a nearly identical 1-6 rating system on cabbage, Hines and Hutchison (2001) found that crops rating 3 or less yielded marketable crop, while those rated 4+

were unmarketable. Using these assertions as a guide, all sprouts that received a rating of 4-6 were separated and weighed separately with a digital scale to determine the mass of the unsalable portion of the crop for each plant. This unsalable Brussels sprouts mass was divided by the total Brussels sprouts mass for each plant, yielding a percentage of unsalable Brussels sprouts mass that could be compared across treatments.

Analysis of this data set was completed using a Cross-Tab Chi-Square test. For each treatment, all Brussels sprouts receiving a 1, 2, or 3 were summed together, as were all sprouts receiving a 4, 5, or a 6. These sums were tested by descriptive analysis using “1”(yes) and “0”(no) for marketability in the rows, and the three separate treatments in columns of the Chi Square.



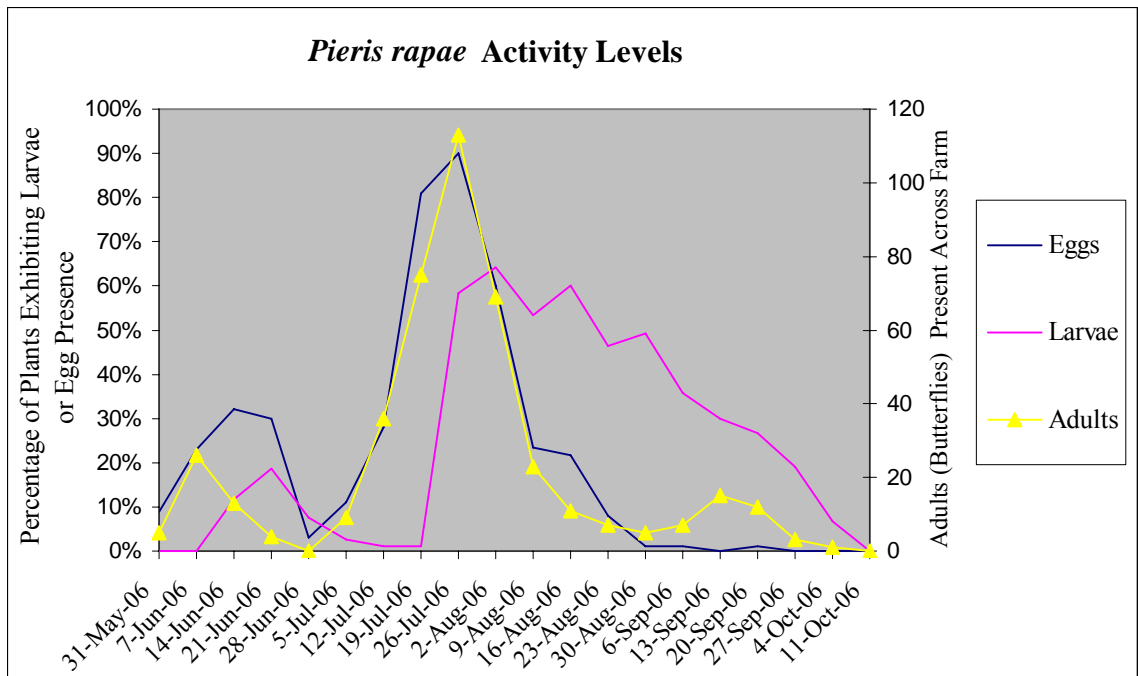


**Figure 6.** Visual representation of numerical damage-rating system in Brussels sprouts. Damage in bottom row pictures has been outlined in red for increased visibility. Single lines or circles, such as in pictures 2 and 3 indicate damage that affected one wrapper or outer leaf. Concentric circles such as those in pictures 4, 5, and 6 indicate damage to two or more layers, indicating head damage. **1** = No larval feeding, **2** = minor feeding on wrapper or outer leaves totaling 1% of crop, no head damage, **3** = Moderate feeding on outer or wrapper leaves with no head damage and 2-5% of leaf area eaten, **4** = Moderate damage to outer or wrapper leaves with minor head damage and 1-10% of leaf area eaten, **5** = Moderate to heavy feeding on wrapper and head leaves and a moderate number of head-scars with 11-30% of leaf area eaten, **6** = Considerable feeding on head and wrapper leaves with numerous feeding scars and > 30% leaf area eaten.

## RESULTS

### Farm-Wide ICW Lifecycles and Activity

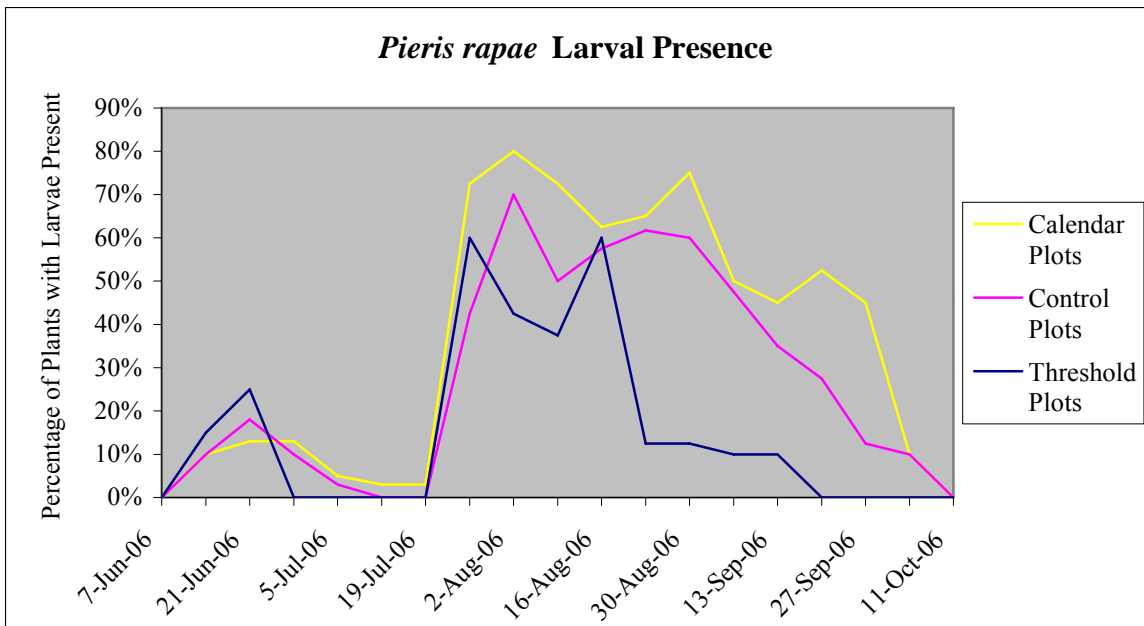
The *P. rapae* population at Biodesign Farm produced two generations during the growing season of 2006. Figure 1 shows the two population peaks within all three lifecycle phases. The first generation of *P. rapae*, which peaked in adult presence on June 6, was smaller than the second generation. The second generation of *P. rapae* adults peaked on August 7, and exceeded the first generation by over 400%. These peaks of adult activity were followed seven days later by a peak in egg presence during the first generation, and coincided perfectly with the peak in egg presence during the second generation. A third small peak of adults occurred on Sept 13, yet did not lead to any significant increases in egg or larvae presence.



**Figure 7.** *P. rapae* activity levels for three lifecycle phases: Egg, Larvae, and Adult. Egg and Larvae activity correspond with the left Y-axis, and are expressed by the percentage of plants sampled that had 1+ larvae or eggs. Adult activity corresponds with the right-hand Y-axis, and is measured by the number of *P. rapae* butterflies counted along the farm transect.

### ICW Larval Presence by Treatment

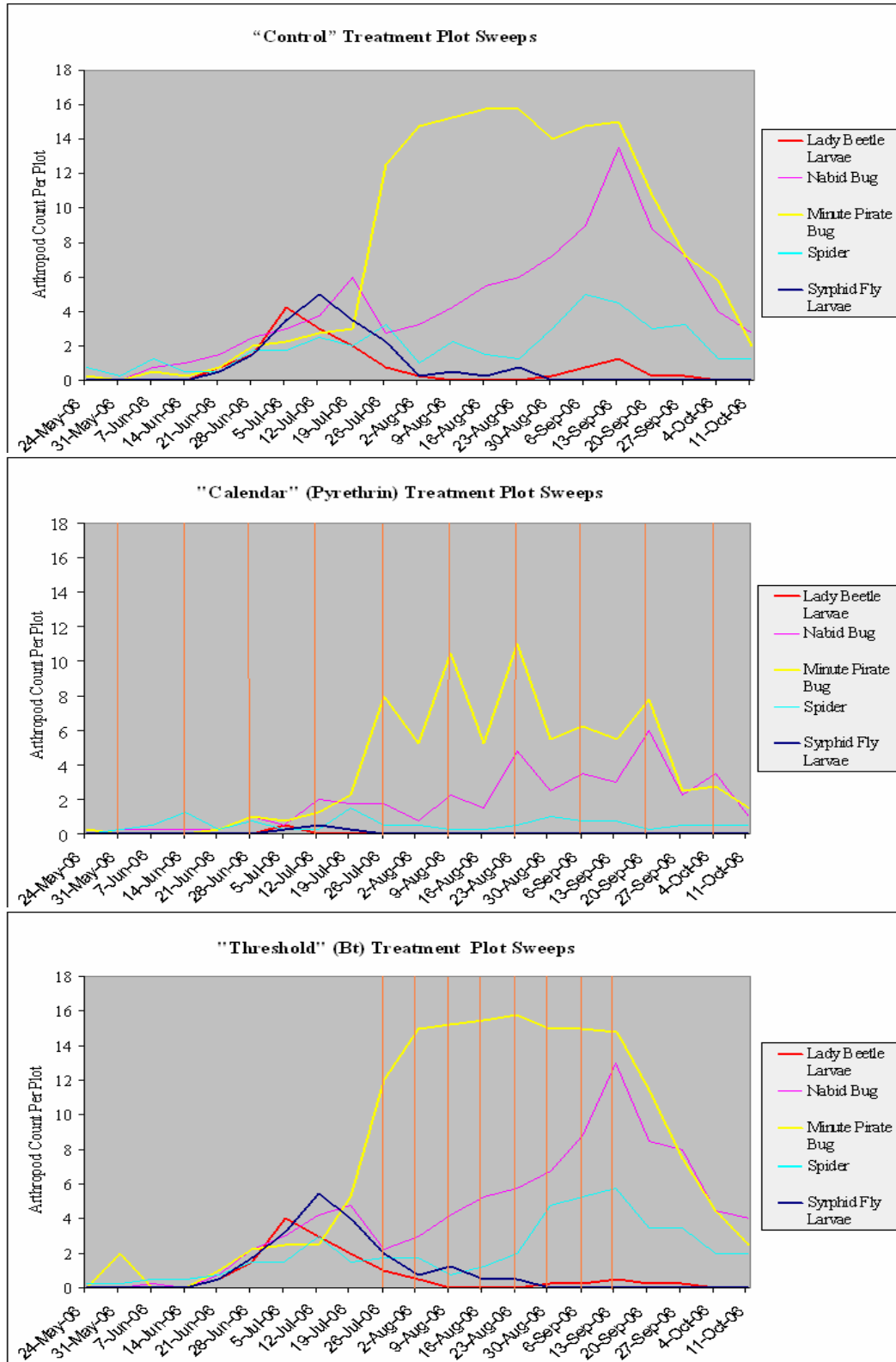
ICW larval activity differed between the two chemical treatment plots and the control (see Fig. 8). The “threshold” treatment lacked all larval presence during two separate three-week periods. The first occurred between 28 June and 19 July, while the second larvae free period began 20 September and lasted until the end of the study. The “control” plots also exhibited one period of zero larval presence, lasting one week between 12 July and 19 July. The “calendar” plot had no periods during the study when larval activity was at zero.



**Figure 8.** *Pieris rapae* larval presence – comparison of the three treatments. Percentages represent the average number of plants per plot in each treatment that had 1+ larvae present during sampling.

### Arthropod Presence Compared Between and Within Treatments

The sweep net sampling data collected from the three sets of treatment plots is expressed in Fig. 9. “Threshold” and “control” plot sweeps maintained similar numbers



**Figure 9.** Sweep-net sampling data – a comparison of treatments using three separate graphs. Y-axis indicates the number of arthropods captured in each plot, and X-axis values represent sampling dates. Orange gridlines indicate the dates of insecticide-treatment applications.

of individuals captured for all five arthropod groups measured. There was no statistically significant difference between these two treatment groups. The “calendar” treatment, however, was significantly different from both the “control” and the “threshold” treatments. Populations of all five arthropod groups were lower in the “calendar” treatment than in the “control”. The most significant differences occurred during July within populations of lady beetle larvae and syrphid fly larvae (each 500% lower in “calendar” plots than “control” plots), and during late summer in minute pirate bug and nabid bug populations (both approximately 50% lower in “calendar” plots than in “control” plots).

Within individual treatments, two arthropod groups exhibited clear multiple generation peaks – and are evident in both the “threshold” and “control” treatment plots. One was the nabid bug, which demonstrated peaks on 26 July and 20 September. The other arthropod with multiple peaks was the lady beetle, which had its largest larvae population peak on 12 July, and exhibited a second, smaller peak in mid-September. Populations of spiders also fluctuated, yet because no particular species was isolated, no separate generational peaks may be identified. The other arthropod groups sampled exhibited population fluctuations, yet none was significant enough to ascertain generational changes.

### Pitfall Traps

After passing statistical checks for homogeneity of variance, one-way ANOVA analysis indicated a significant difference ( $p < .001$ ) between treatments. As demonstrated in Table 1, the average numbers of carabid beetles and spiders captured in the “threshold”

and “control” plots wer consistently greater than the number captured in “calendar” treatments.

	Carabids			Spiders		
	Threshold	Control	Calendar	Threshold	Control	Calendar
<b>7/5/06</b>	2.75	3	0.25	7.75	8.25	2.5
<b>8/2/06</b>	2.5	2.5	0.25	7.5	9.5	2.5
<b>8/30/06</b>	2.5	2.25	0.25	8.25	9.75	2.5
<b>9/27/06</b>	3	2.75	0.25	9	9.5	1.25

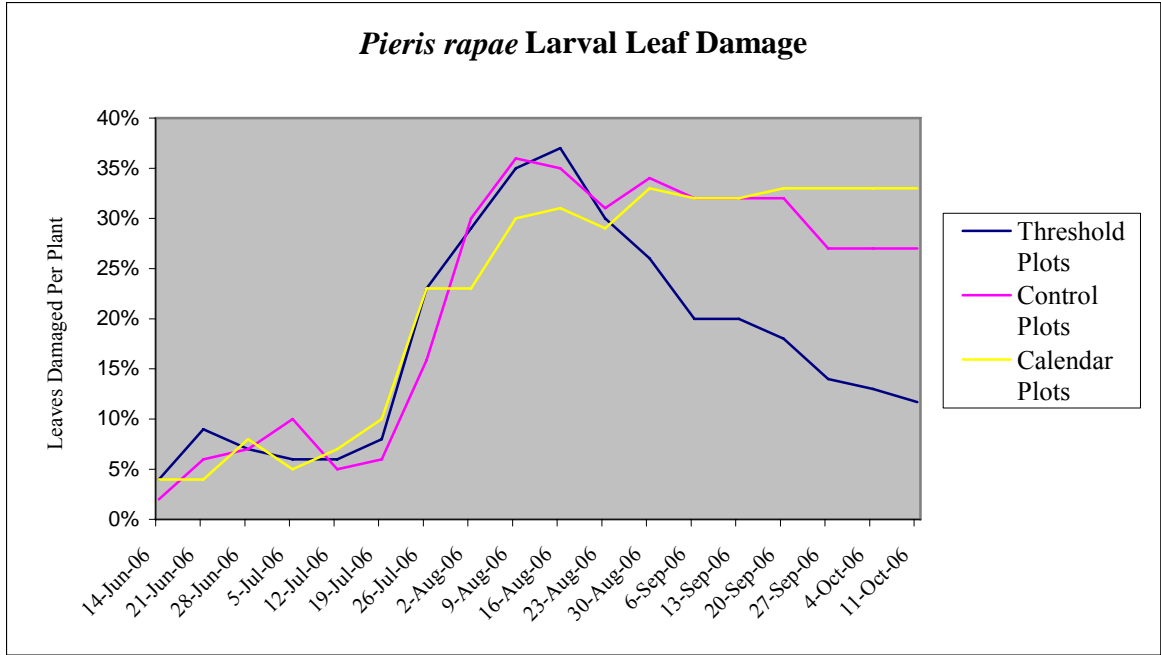
**Table 1.** Results of pitfall trap installations. Columns indicate the average number of spiders or carabid beetles captured during the 7-day trap installations.

### Larval Parasitism – Captive Larval Rearing

Of the 25 and 52 *P. rapae* larvae respectively collected on 28 June and 16 August, zero showed indication of parasitism. 25 of 25 larvae collected on 28 June reached the pupae stage, and all 25 emerged as live adults. 52 of 52 larvae collected on 16 August also pupated and emerged from the rearing chambers as adults. Of the 53 larvae collected on 6 September, 100% reached pupation as well. None of this group, however, emerged as adults. Each pupae in this group was examined with a 10x hand lens for signs of parasitoid exit holes or activity, yet no evidence of parasitism was found.

### Brussels Sprouts Leaf Damage

The larval damage to the Brussels sprouts foliage is shown in Figure 4. The percentage of damaged leaves in the two chemical treatment plots was similar to those in the “control” plots, and none exceeded 10% damage until the week of July 19. During the 4-week period that followed the week of July 19, the “calendar”, “threshold”, and “control” plots all displayed increases in foliar damage by more than 200%. The damage within all plots did not differ with any significance until August 23. At this point, the



**Figure 10.** Percentage of leaves damaged by *Pieris rapae* larval feeding – a comparison of the three treatments.

“control” and “calendar” treatments continued to demonstrate similar levels of damage while the “threshold” treatment diverged and exhibited a reduction in leaf injury.

The foliar-damage measurements taken at the point of plant harvest are displayed below in Table 2. “Threshold” treatment plots had the lowest percentage of leaves damaged with 11.7% of leaves showing some degree of larval feeding damage. These were followed by the “control” plots with 27.3%, while the “calendar” plots exhibited the highest percentage of leaves damaged at harvest with 33.3%.

	Foliar Damage	Sprout Damage Ratings						% of Crop Mass Unsalable
		1	2	3	4	5	6	
Threshold	11.7%	89.2%	5.9%	2.4%	1.3%	0.7%	0.4%	2.7%
Control	27.3%	68.1%	16.0%	6.7%	3.0%	2.7%	3.5%	11.5%
Calendar	33.3%	60.3%	17.5%	6.0%	4.1%	4.4%	7.6%	20.2%

**Table 2.** Brussels sprouts leaf and sprout damages, as measured at the time of harvest. Foliar damage is expressed as a percentage of the leaves on each plant that had some degree of larval feeding damage. 1-6 damage rating columns express the percentage of individual sprouts from the three treatments that received each particular damage rating. The “% of crop mass unsalable” column indicates the percentage of the total crop mass for each treatment that received a 4+ damage rating. Though considered “unsalable” by market standards, sprouts damaged by voles were excluded from measurement in all test plots.

One-Way ANOVA analysis indicated that the deviation between the three treatments was highly significant ( $p < .001$ ). Multiple comparison, post-hoc tests using Tukey HSD also indicated a highly significant variation between the “threshold” plots and the other two treatments ( $p < .001$ ), as well as a significant variation between the damage in the “control” plots and that of the “calendar plots” ( $p = .013$ ).

#### Brussels Sprouts Crop Damage

The results of implementing the 1-6 damage rating system on harvested Brussels sprouts are displayed in Table 2. Sprouts receiving a rating of 1, 2 or 3 were deemed to be a marketable crop, while those receiving a rating of 4, 5, or 6 were considered unsalable. The “threshold” treatment had the lowest percentage of unsalable material, with 2.7% of the total crop mass that was not fit for sale. “Control” plots had significantly ( $p < .001$ ) more unsalable sprouts than the “threshold” plots, averaging 11.5% of the total crop per plant. “calendar” treatment plots also differed significantly ( $p < .001$ ) from the “control” plots, exhibiting the highest recorded average of 20.2% of the crop being unfit for sale.



## DISCUSSION

### ICW Activity

The imported cabbage worm, *Pieris rapae*, had two full generations on Biodesign Farm during the summer of 2006. These are evident in Fig. 7 by the two spikes in adult ICW population figures, which were followed by peaks in egg and larval presence. Of these two, the first generation was much smaller than the second, consistent with Dempster's (1967) assertion that larval pressure is greatest during mid or late summer generations. The third small peak of *P. rapae* adult activity that occurred on 13 September may have two possible explanations. First, the peak may be attributed to a source-sink relationship between Biodesign Farm and surrounding areas, and not to a partial third generation. By planting several acres of brassica plants, the research site may have attracted these *P. rapae* adults from surrounding areas where generational phases were slightly out of sync with those at the research site. This would then lead a late season rise in butterfly numbers. An alternate explanation, however, arises from the larvae that were captured and reared for the parasitism investigation. The larvae captured on 16 August - which hatched from eggs laid by ICW adults during the upswing of the second generation population curve - all pupated and emerged as adults. The group of larvae captured on 6 September - which hatched several weeks after the first group of larvae (and on the downward side of the population curve) - remained in the pupae stage, presumably to over-winter. The small, third peak of adults observed in September may therefore have been due to the first group of early-pupating and emerging adults. Regardless, no corresponding increases in egg or larval presence followed the third peak, indicating that a full third generation did not occur.

The peaks and valleys of *P. rapae* larval populations during this research created periods of time when crop plants were relatively free from larval presence (note specifically the period prior to 19-July in Fig. 7). These periods of minimal larval activity correspond with periods of low crop damage, and thus producers may benefit from timing certain crops to be grown and harvested during these periods. Understanding these pest population fluctuations is an important piece of creating a viable IPM plan for *P. rapae* in western Montana.

### Beneficial Arthropods and Chemical Treatments

The sweep net sample data from the three treatment plots demonstrate the impact of the two chemical treatments upon the populations of arthropod predators (Fig. 9). The “control” and “threshold” plots expressed nearly identical arthropod populations, indicating that the Bt chemical treatment had little or no effect on any of the arthropods sampled. The “calendar” plot, however, showed highly significant variations in arthropod populations as compared to the “control”. Populations of nabid bugs, minute pirate bugs, lady beetle larvae, syrphid fly larvae, and spiders within the “calendar” plots were never recorded at levels equal to or greater than those of the “control” and “threshold” plots, and were often significantly lower (Fig. 9). Arthropod populations also regularly declined during the sampling period which followed the application of pyrethrin/rotenone insecticides, and often took two weeks or longer for recovery to pre-treatment numbers. These findings are consistent with Dempster (1968(2)), Clark et al. (1997), Elzen (2001), and other research indicating that broad spectrum insecticides such

as the pyrethrin/rotenone blend can significantly reduce populations of beneficial arthropods.

Pitfall traps are not necessarily an accurate indicator of population size, but rather an indicator of arthropod activity level. Some species of ground beetles may actually exhibit higher numbers in pitfall traps due to increased activity if applied pesticides reach the ground level (Dempster 1968(2)). For this reason, pitfall trap data should be used with caution, and only for comparison between treatments. Pitfall data indicated a much lower level of activity for carabid beetles and spiders in the “calendar” plots as compared to the other two treatments. While this does not necessarily indicate a large decrease in carabid *populations*, it does suggest a decrease in ground beetle and spider *activity* levels in the pyrethrin/rotenone treated plots. A more detailed examination of ground beetle and spider mortality and dispersal following treatment would be necessary to determine if this decrease resulted from arthropod evacuation or from death following insecticide application.

There was no significant difference between the spiders or carabid beetle activity in the “threshold” and “control” plots, indicating that Bt insecticide had little or no effect upon their activity levels.

#### Larval Presence and Chemical Treatments

Larval presence varied across the three treatments, with the greatest difference occurring after 26 July (Fig. 8). It was at this point that the “threshold” treatment diverged from the other two, and with the exception of one sampling day (16 August) it exhibited significantly ( $P < 0.05$ ) fewer larvae. Periods of time also occurred when zero

larvae were present in the “threshold” treatment, while larvae were found concurrently in the “control” and “calendar” plots. Presence of fewer larvae in “threshold” plots than in “calendar” plots indicates that some factor within the threshold-based Bt applications was more successful than the calendar-based pyrethrin/rotenone sprays for reducing *P. rapae* larvae populations. The effectiveness of Bt (or the ineffectiveness of pyrethrin/rotenone), however, cannot completely explain why *P. rapae* larval presence was *higher* in the “calendar” plots than it was in the “control” plots.

Bt appeared to be a more effective insecticide than the pyrethrin/rotenone on *P. rapae* in this study. This impact would be a *primary* effect of the insecticides, in which more larvae were directly killed in the “threshold” than in the “calendar” plots. This assertion is supported by the reduction of larvae that followed six of the seven applications of Bt insecticide (the exception was 16 August), which maintained larval numbers near or below those in the “control” plots (Fig. 8). Peaks in the larval populations of the “threshold” plots occurred only during population upswings as new larvae were hatching, and quickly leveled off as these larvae contacted the Bt. The larvae treated with the pyrethrin/rotenone contact-insecticide did not regularly decrease in number following application, and actually increased on several occasions when overall *P. rapae* larvae activity was actually decreasing.

It is likely that the “calendar” sprays removed/killed more predatory arthropods than did the Bt or “control” plots, which is evident when comparing the graphs in Fig. 9. This may have then triggered a trophic cascade – a *secondary* effect of the insecticide – that removed natural predatory controls and led to an increase in larval populations.

Since “control” plots and “threshold” plots contained nearly identical beneficial

arthropod populations, we can assume that levels of predation were similar between them. The level of larval reduction in “threshold” plots, which exceeded the reduction in “control” plots, may then be attributed to the positive primary effect of the Bt insecticide. The “calendar” plots, however, showed higher levels of larval presence and lower levels of beneficial arthropods. We can assume that there was no primary effect from the pyrethrin/rotenone that *increased* pest numbers, so we therefore look to a secondary effect in those plots. The drop in arthropod populations in “calendar” plots was the only other recorded difference between them and the “control” plots. It follows, therefore, that the reduction in arthropod populations might have been positively correlated to a reduction in pest predation, and therefore negatively correlated to *P. rapae* larval populations.

Other confounding factors may exist, of course, such as sampling effects, unaccounted-for predators (arthropods or otherwise), or other controlling factors that either limited *P. rapae* activity in the “threshold” and “control” plots, or that bolstered activity in “calendar” plots. Further research on these treatments - including several seasons of data by which to compare them - is necessary to draw conclusions as to true correlation between applied pyrethrin/rotenone insecticides, reduced arthropod predator populations, and increased *P. rapae* larval presence.

#### Brussels Sprouts Leaf Damage and Chemical Treatments

Brussels sprouts leaf damage increased throughout the season for all three treatments until mid-August, and showed the greatest gains during corresponding population increases in *P. rapae* larvae (note the period of 19 July and 16 August in Figs.

7 and 10). By mid-summer, Brussels sprouts plants were adding 2-5 leaves per plant, per week. This growth means that for damage percentages to *increase, more* leaves must have been damaged by larvae than were added each week on each plant. A damage percentage that held constant from one week to the next indicates that some leaves were still being damaged, while others were being added and left untouched (i.e., 5 leaves damaged out of 20 total leaves equals 25% damage; if four new leaves are added, and one more is damaged, 6 leaves damaged out of 24 also equals 25% damage). Decreases in leaf damage percentages indicate little or no larval feeding, as well as the addition of new, undamaged leaves on each plant.

All three treatments showed sharp increases in leaf damage and *P. rapae* larval presence, during which time all research plots far-exceeded accepted pest thresholds (Hines and Hutchison, 2001) established for chemical control. This indicated that neither of the chemical treatments, nor the “control” was able to prevent larval feeding during the outbreak. One explanation for this lack of pest control is that new larvae hatched daily during these pest outbreaks, and might have missed pyrethrin contact or the window of Bt effectiveness. This may have occurred because pyrethrin/rotenone and Bt insecticides break down rapidly in sunlight and water, usually within 24-48 hours (Casida and Quistad 1995). When new larvae are hatching every day and pesticides are applied every seven, there are at least four or five pesticide-free days during which larvae may feed.

A second explanation for the lack of *P. rapae* control during extreme population increases may be connected to the population size and/or feeding habits of the predatory arthropods within the system. First, it is possible that insufficient populations of predatory arthropods existed in any plot – including in the “control” plot - which could

consume the rapidly increasing pest numbers. If such were the case, the predators present would be consuming at their maximum level until new life-cycles of the current arthropods gave rise to new generations. Minute pirate bugs and syrphid flies, for instance, take 3-7+ days to pass from oviposition to a life stage where they can feed on prey. Carabid beetles and spiders, however, may take several weeks or more. Second, the very nature of the predatory arthropods sampled (i.e. *generalist* predators) may have led to predation of many prey species other than *P. rapae*. Population curves in classic one-predator/one-prey relationships often show a pair of offset curves (expressed by the Lotka-Volterra model of interspecific competition), in which prey organisms show peaks in their populations first. This is followed by a second peak, the predator population, which requires the augmentation in food (prey) resources to fuel its own population increase. Generalist predators, however, have many prey species to choose from, and thus may not exhibit any direct relationships to one species or another if many are present. While the diverse system in this study attracted many species of predatory arthropods, it also attracted many other insects to various degrees. Though at relatively low population levels, species of aphids (*Aphidoidea*:Hemiptera) lygus (*Lygus spp.*, *Miridae*:Hemiptera) and thrips (*Thysanoptera*) were observed within plots throughout the growing season. These species may have provided an alternative or more easily-accessible food source for the generalist arthropod predators as compared to *P. rapae* larvae.

More information is needed before connections can be made between these different arthropod species, though these findings can inform the direction of future research efforts. Several more seasons of researching and tracking *P. rapae* and predatory arthropod activity may yield important information about the interactions of

generalist predator/prey lifecycles, and may determine whether a complex of generalist arthropod predators can control a specific pest even when its populations are rapidly increasing.

After the spike in leaf damage, the three treatments diverged. By comparing larval presence data (Fig. 8) and damage data (Fig. 10), we can see that higher late-summer levels of larval presence in the “control” and “calendar” plots corresponded with higher levels of continued leaf damage than in “threshold” plots. Again, it is clear that leaf damage was reduced in the Bt treatment plots as compared to the increased damage in pyrethrin/rotenone treatment plots. It is, however, difficult to discern without further research which portion of this variation was truly correlated to the negative, secondary effect of the pyrethrin/rotenone on the multi-species assemblage of predatory arthropods.

#### Brussels Sprouts Damage and Chemical Treatments

Damage to the Brussels sprouts crop varied between treatments in a way similar to the end results of leaf damage assessments. “Threshold” plots had the least crop damage, followed by the “control” plots, with “calendar” plots having the greatest quantity of unmarketable crop. As evident in Table 2, “calendar” plots also yielded the highest percentage of total sprouts ranking 4-6 (in the unmarketable range) for damage. This indicates a greater percentage of sprouts within “calendar” plots that experienced heavy feeding damage as compared to the other plots.

Because individual Brussels sprouts were not assessed for damage until harvest, a progressive damage assessment cannot be made. For this reason, it is unknown *when* damage took place or which arthropod predators were most active at that particular time.



Instead, we may assert only that within this study the threshold-based Bt treatments yielded significantly less crop damage than “control” plots, while bi-weekly pyrethrin/rotenone treatments yielded significantly more damage than “control” plots. This would indicate that when the predatory arthropods in this diversified system were sprayed with the pyrethrin/rotenone (and thus significantly reducing their populations as compared to the other plots), that an increased level of crop damage occurred as a result. To determine which arthropod predators were most affected by the pyrethrin/rotenone spray, or which species were most effective in controlling *P. rapae* in the “threshold” or “control” plots will require future research.

### Parasitism

The recorded rate of parasitism at Biodesign Farm during the 2006 season was zero. While wasps were counted during sweep net sampling, their numbers varied widely, and did not pass homogeneity of variance tests for any treatment. No identifications were made of wasps captured during sweep netting, because it was anticipated that some fraction of larvae reared for parasitism rates would give rise to identifiable specimens. Therefore, there is no way to determine which species of wasps *were* being captured in sweep-net samples. Research has shown that the effectiveness of parasitoids can be greatly reduced if they are distracted by other pests in the system (Cardinale et al. 2003). It is unlikely in this case, however, due to the specialized nature of *C. glomeratus*, and because no other comparable larval pests shared the research area with the *P. rapae* larvae. It can only be determined, therefore, that specialized parasitoids had no measured effect on *P. rapae* larvae, or on crop damage.

## Other Considerations

Several issues that were not included in the initial research questions arose during the course of this research. These topics may provide insight into the practicality of this research, and shall lead us finally to the conclusion and suggestions that have come out of this study.

### *Chemical Treatment Practicality*

It is clear by looking at the harvest data for unmarketable Brussels sprouts mass that there was a significant difference between treatments (Table 2). The low level of damage in the “threshold” treatment plots was the result of natural predatory control, as well as seven applications of Bt insecticide over the course of the season. For this research, Bt was applied at a rate of 6.5 m<sup>2</sup> per minute, including mixing and cleanup, with a cost of \$0.0044 per m<sup>2</sup> (price of Bt alone, no other costs considered). If this were extrapolated out to a per-acre figure, it would cost \$17.81 (with Bt priced at \$3.85 per kilo) and would take over 10 hours to treat each acre. Even if mechanized application were used to save time (which would then cost more), this treatment would still represent a significant time input.

It will be important for producers to begin looking hard at the quality of the product that they wish to harvest, and how it matches their level of inputs. For example, if Brussels sprouts will sell at the local Farmers Market with a moderate level of damage, perhaps the added money and time spent applying Bt is not worth the 9% improvement in crop marketability. If, however, a grower can find a price premium for unblemished

sprouts that exceeds the level of input, perhaps this treatment does offer a financial incentive for implementation.

### *Threshold Levels*

This season, all of the treatment plots at Biodesign Farm greatly exceeded accepted pest-threshold levels for at least seven weeks. Again, a grower will have to consider the damage that results from a certain level of pest pressure, and determine whether or not that level of *presence* truly leads to a corresponding level of *damage*. Thresholds may be more effective as flexible guidelines to be modified for use in each individual production system, so that the action taken matches the goals of the grower and leads to the outputs that are desired.

### *No-Till Brussels Sprouts*

The minimum-tillage system implemented in this study exhibited good population sizes of beneficial arthropod predators. It is unknown how these arthropod counts would have compared to bare-soil sweeps if a portion of the plots had been tilled clean before planting, though prevailing literature would give a nod to the untilled plots (Thomas 1991, Clark et al. 1997, Kinnunen et al. 2001, Denys and Tscharnke 2002). Unfortunately, the per-plant harvest decreased by 35% in the minimum-till system as compared to tilled systems on the farm in previous years. This also agreed with published reports that cropping systems with certain living mulches can see decreased yields and lost crops that make increased arthropod populations a moot point in production systems (Dempster 1969, Masiunas 1997). Our research indicated yield

reductions in the no-till living-mulch plots may have been attributable to lower soil temperatures, slow mineralization of nitrogen, and slow root setting in the transplanted seedlings due to low N:P ratios (see Figs. 11-14 in Appendix B). It should be noted that test plots at the same research site which contained *minimum-tillage* management with the same living-mulch produced yields that met or exceeded those of tilled plots in other seasons.

The initial control of the *T. pretense* with the flaming device was successful, yet became difficult as the clover grew faster and taller than the Brussels sprouts seedlings. The red clover required large quantities of water to grow and be maintained, which ruled out the possibility of using drip irrigation. Voles were a significant pest in this system, as they found copious refuge from predators under the cover of the living mulch. Vole impact was the greatest early in the season, as entire vegetable seedlings were lost due to chewing at ground level. Later in the season, voles damaged the lower rows whorls of sprouts on each stem (which happen to be the largest ones), averaging 2.5 lost sprouts per plant. The negative effects in this system may be reduced by using a different species of living-mulch such as a lower growing white clover (*T. repens*), changing mulch management to reduce cover for pests such as voles, or .experimenting with *minimum-tillage* + a living mulch so that it is near the crop plants but not *under* them.

## CONCLUSION

We conclude here, at the terminus of this research, that the three treatments had varying effects upon the activity and presence of *P. rapae*, as well as on the populations of arthropod predators. Plots treated with Bt had the lowest level of crop and plant damage, and showed no negative impacts on the populations of beneficial arthropods. The unsprayed “control” plots also had low levels of crop damage and had predatory arthropod populations equivalent to those in Bt plots. Plots treated with a pyrethrin/rotenone spray, however, had the highest levels of plant and crop damage in the study, and also had the lowest levels of arthropods sampled. These results agree with suggestions by Dempster (1968(a), 1968(b)) that broad-spectrum insecticide applications aimed at *P. rapae* may significantly reduce populations of predatory arthropods within the system. There is a strong indication that the reduction in arthropods was a primary effect of the applied pyrethrin/rotenone sprays, which then led to the secondary effect of reduced predation and increased pest presence. From this we conclude that pyrethrin/rotenone sprays are less effective in controlling *P. rapae* larvae than Bt, and that our results confirm previously published assertions that the use of pyrethrin/rotenone sprays is incompatible with IPM programs that include arthropod predators. Calendar applications of pesticides did not reduce *P. rapae* larval populations as compared to unsprayed control plots, thus we conclude that this technique of chemical pest control is unadvisable for this crop pest.

All of the predators found in this study were generalists, and appear to have contributed a measurable amount of pest control. Parasitoids played no appreciable part

in control of *P. rapae* larvae in any of the areas sampled. Producers would do well to encourage *both* generalists and specialists in their systems, as generalists may help control multiple pest species while specialists may help to reduce the time-lag between the rapid increase in pest populations and the response of biological controls. More research must be undertaken on these complex systems if we are to understand the relationships between a pest and an assemblage of arthropod predators such as these. Multiple seasons of data collection may well highlight connections between the lifecycles of arthropod predators and a specific prey species.

The diverse farmscape examined here did, in fact, produce a varied population of arthropod predators that seem to have helped reduce *P. rapae* larval populations and damage in plots unsprayed by broad-spectrum insecticides. It is impossible to determine the exact cause and effect relationships between individual pieces of the diverse farmscape and arthropod predators studied here. However, by comparing published research on *individual portions* of the system - such as flowering hedgerows increasing arthropod populations (Thomas 1991, Denys and Tschamke 2002) and reduced tillage and grassy banks encouraging ground beetles (Clark et al. 1997, Kinnunen et al. 2001) – we can see that many different components of the farmscape may have played a role. Further research on *complete systems* such as this, as opposed to research on individual structures (i.e. one hedgerow, one grassy bank) in otherwise conventional fields, may broaden our understanding of the complex interactions that occur in real, diverse agroecosystems.

Farmers in western Montana may see benefits in reduced crop damage by timing their brassica crops according to generational peaks of crop pests. Prior to 26-July, pest

levels were insufficient to create any economic damage, while after that date the larvae of the pest was observed in high numbers. With an understanding of *when* the pest may exhibit the highest population levels, a producer may choose crops such as early season broccoli or other short-season brassica plants that will be harvested *before* the pest may damage them.

The successful pest control achieved in this study was countered by a 35% reduction in yield over tilled plots in previous years. Competition for resources between the crop plants and the living-mulch appears to have been an important limiting factor. Future research should focus on designing a system that affords the benefits of a dense no-till living-mulch, while allowing for the crop plants to acquire resources as readily as they can in bare-soil, conventionally tilled plots.

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## APPENDIX A

### Biology, Lifecycle, and IPM considerations for Predators and Parasitoids

#### Syrphid Fly Larvae (Family *Syrphidae*)

Syrphid flies, also known as hover flies, are most often seen in their adult stages during warm season months. The adults resemble bees or wasps – though lacking stingers - and often hover in the air around flowers and nectar sources in search of food.

#### *Life Cycle*

Female adult syrphid flies lay their eggs singly or in small groups on the leaves or shoots of plants. Often, these sites of oviposition are located among or very near aphid colonies. Eggs hatch quickly, usually within 48-72 hours, at which time the small, emergent maggots will range around plant surfaces in search of prey. The predacious larvae feed heavily for one or two weeks on aphids, early instars caterpillars, and other small insects. Ashby (1974) found that syrphid larvae will kill and consume ICW larvae, though only at prey sizes smaller than third-instar. The larvae will pupate on or near the plants where they feed, giving rise to adult syrphid flies in one or two more weeks. Depending upon climate and food sources, syrphids can have as few as two or as many as seven generations each year.

#### *Pesticides and Habitat Disturbance*

Syrphids are susceptible to broad-spectrum insecticides, especially in their larval stages when movement or escape is difficult or impossible. In a three year study of short-

term insecticide impacts, Jansen (2000) found that syrphid larvae numbers were reduced by several different insecticides. Spinosad, an insecticide gaining popularity in ICW control, is also toxic to syrphid fly larvae (Chaney 2003).

### Ground Beetle (Coleoptera, *Carabidae*)

Ground beetles (family *Carabidae*) collectively encompass over 2500 known species of North American terrestrial beetles. Often referred to simply as “Carabids” or “Carabid Beetles”, these beetles are some of the largest predatory insects found in agricultural systems (12-35mm in length) (Antonelli 1993). Though nearly all species are nocturnal, adults are often seen moving during daylight hours when their habitat is disturbed (Lyon).

### *Life Cycle*

The eggs of most ground beetles are laid singly or in small clusters on or slightly below the surface of the soil. Eggs hatch within 5-7 days, giving rise to slender, segmented larvae with powerful jaws. Depending upon the species, larvae will proceed through 2-4 instars while feeding underground. Pupation occurs within the soil as well, and gives rise to emergent adults in 5-7 days. Most species in North America will take a full year to complete the cycle from egg to adult, though conditions that are unusually harsh may extend this period to 2-3 years in length (Lyon, Antonelli 1993). Adult ground beetles are truly generalist predators, feeding on a wide array of insect eggs, larvae, and adults, as well as on some plant matter. Population levels of ground beetles have been positively associated with levels of pest control and prey removal (Lovei and Sunderland

1996; Menalled, Lee and Landis 1999, Lee, Menalled and Landis 2001), and have been shown to consume *P. rapae* larvae (Dempster 1968(2), Ashby 1974). Adults may live for one year or longer, depending upon species and environment (Lyon).

### *Pesticides and Habitat Disturbance*

Ground beetles are highly susceptible to broad spectrum insecticides. Dempster (1968(2)) in his study of the effects of the broad spectrum insecticide DDT, showed a reduction in the population of beetle larvae and adults following pesticide applications. The use of specific toxins such as *Bacillus thuringensis* (*Bt*) for caterpillar pests can prevent the unintentional killing of these beneficial predators (Antonelli 1993). In addition to pesticides, disturbance from agricultural practices such as tillage and mowing may also displace or reduce carabid populations (Clark et al. 1997). Kinnunen et al. (2001) have indicated that each of these populations of carabid beetles may be localized and unique in agricultural systems. Untilled or undisturbed areas along field borders or within fields can slow carabid dispersal, and may thus increase these populations in some areas by increasing shelter, food sources, and niches. To maximize the beneficial effects of carabid beetles, landscapes must then be varied so as to maintain areas of refuge while still allowing for dispersal (Frampton et al. 1994). This would maintain healthy populations and also allow carabids to search for prey (i.e. pests) across the agricultural landscape.

Nabid/Damsel Bug (*Nabis spp.*, Hemiptera: Nabidae)

The Nabid or “damsel” bug is a part of the family *Nabidae*, which includes 39 genera and 380 species worldwide, and approximately 34 species occurring in North America. Resembling a small preying mantis, they range in size from 0.25”-0.50”, and have fully-developed wings for flight (Lattin 1981). Nabids also possess long antennae, which play a large role in the search and acquisition of their prey (Freund 2000).

*Life Cycle*

Adult females oviposit eggs into the tissues of growing plants. Nymphs hatch quickly, and follow the simple metamorphosis that is characteristic of all Hemiptera. The wingless nymphs molt 3-5 times, ultimately emerging from their final instar as winged adults. This process takes approximately 50 days to complete, but will vary with temperature and resource availability. Adult or final instar stages are the most common for over-wintering (Lattin 1981). Adults are acknowledged as efficient generalist predators, feeding on the eggs, small larvae, and adults of aphids, *lygus*, and other known agricultural pests, including *P. rapae*. Using their strong forelegs - similar to those of the praying mantis - nabids grasp their prey securely while using their rostrum (a 4-segmented piercing, sucking mouthpiece) to consume the soft insides of their prey. They have been shown to be extremely effective in consuming ICW larvae (Dempster 1967, Schmaedick and Shelton 1999), and are one of the few effective predators when *P. rapae* larvae are of third-instar size and larger (Ashby 1974). Nabid activity is generally the highest during July and August in North American regions.

### *Pesticides and Habitat Disturbance*

Nabid bugs are sensitive to many broad spectrum insecticides, and have been shown to decline in population size after their application (Dempster 1968(2)). Further, these populations may take several weeks to rebuild, even when following applications of less toxic pesticides (Baird & Homan, 1996). The use of pest-specific insecticides such as Bt may have less direct impact upon nabid bugs, yet findings from Harwood et al. (2005) indicate that Bt endotoxins are showing up in the bodies of non-target arthropod predators such as nabid bugs. It is currently unknown if the source of these toxins is due to direct feeding on plant tissue containing Bt proteins, or from the consumption of prey that have in turn consumed Bt laden plant tissues.

As ambush predators, nabid bugs feed efficiently when afforded adequate cover. Vegetative buffers, untilled strips, and living mulches may all provide improved refuge and shelter, foraging areas, and access to prey.

### Lady Beetle (Coleoptera: *Coccinellidae*)

The lady beetle is also known commonly as the ladybug or ladybird beetle. It falls within the *Coccinellidae* family that has over 4500 named species, with over 450 of those native to North America. *Coccinellids* range from 2mm to 10 mm in length, and are usually identified quite easily by their characteristic orange/red background that is typically punctuated by dark spots.

### *Lifecycle*

Lady beetles over winter in the adult stage, often finding shelter in clusters under plant debris, rocks, loose bark, and sometimes inside buildings. In spring, adults will disperse – often over long distances – to find food and egg laying sites. The eggs, clusters of yellow/orange, barrel shaped eggs are laid on the surfaces of plant leaves, often near aphid colonies. Females may lay up to 1000 eggs throughout their life, which can span a three month period during ideal summer conditions. Upon hatching, larvae will range extensively (up to 12 meters away) in search of food, and will consume aphids, thrips, eggs of many insect species including other *Coccinellidae* eggs, and other small, soft bodied insects including the imported cabbage worm (Ashby 1974). Larvae pass through four instars over a 20-30 day period, and will then pupate. In approximately 3-12 days adults will emerge, and depending upon the climatic zone and resources available, they will feed, mate, or search for an over-wintering site. Most common *Coccinellidae* species have one or two generations in the U.S.

### *Pesticides and Habitat*

Lady beetles, like other beneficial arthropods, are susceptible to certain insecticides. Banken and Stark (1998) found that female *C. septempunctata* ceased all egg laying activity following exposure to neem insecticide, and that all individuals exposed to concentrations greater than 100 ppm died within 10 days. Insecticidal soaps can be injurious to several *Coccinellid* species (Smith and Krischik 2000), and predatory activity is reduced in adults and late-instar larvae when prey containing pesticide residues are encountered (Singh et al. 2004). Adult lady beetles do appear tolerant of spinosad

(Galvan et al. 2006), and strains of Bt do not negatively affect predation activity (Giroux et al. 1994).

### Minute Pirate Bug (*Orius spp.*)

The minute pirate bug – *Orius spp.* - is one of over 20 genera within the Anthrocoridae (Pirate Bug) family. These true bugs are small, with most measuring less than 0.2” in length, and having distinct black and white colorations on their wings.

### *Life Cycle*

Female minute pirate bugs lay eggs, either singly or in small groups, within the tissues of plants. Eggs hatch in 3-5 days, and give rise to the immature nymphs. Nymphs pass through five stages, growing larger with each one. Immature minute pirate bugs are generalist predators much like the adults, but lacking wings for flight. Both nymph and adult stages of *Orius spp.* will feed on a variety of insect prey, including those considered to be agricultural pests such as thrips, aphids, and the eggs and small larvae of caterpillars. Prey are grasped by strong forelegs, and the sharp beak-like mouthpiece (characteristic of all true bugs) is used to pierce skin or eggs and withdraw the soft insides. *Orius spp.* have life cycles that span 20-30 days, and may have 2-4+ generations within a season, depending upon resources and environmental conditions (Askari and Stern 1972).



### *Pesticides and Habitat*

*Orius* species are sensitive and susceptible to some broad spectrum insecticides. Pesticide residues of malathion, endosulfan, profenofos, fipronil, and cyfluthrin have been proven to be incompatible with *Orius insidiosus*, with some chemicals resulting directly in death and others resulting in reduced prey consumption (Elzen 2001). Insecticides with more novel modes of actions, most notably *Bacillus thuringensis* and spinosad, do not directly injure minute pirate bugs (Al-Deeb et al. 2001, Elzen 2001, Williams et al. 2003)

Minute pirate bugs require shelter and alternate food sources when insect prey are unavailable. Sources of nectar - such as flowering perennials or ground covers – may help retain *Orius spp.* populations in agricultural systems when pest numbers are low.

### Spiders (order Areneae)

Spiders are of the order *Areneae*, which falls within the larger class *Arachnidae*. There are many thousands of species within the families and genera of *Areneae*. All share a similar physiology, with 4 pairs of legs and a small mouth that is used to suck out the soft portions of their prey. Many species build characteristic webs for shelter or prey capture, while others tend towards nomadic hunting methods.

### *Life Cycle*

Spiders reproduce through eggs, which are bundled together in sacks. They will pass through a series of instars before reaching adulthood, feeding upon their yolks during early stages. Adult spiders can live for a year or more, and generally feed on a

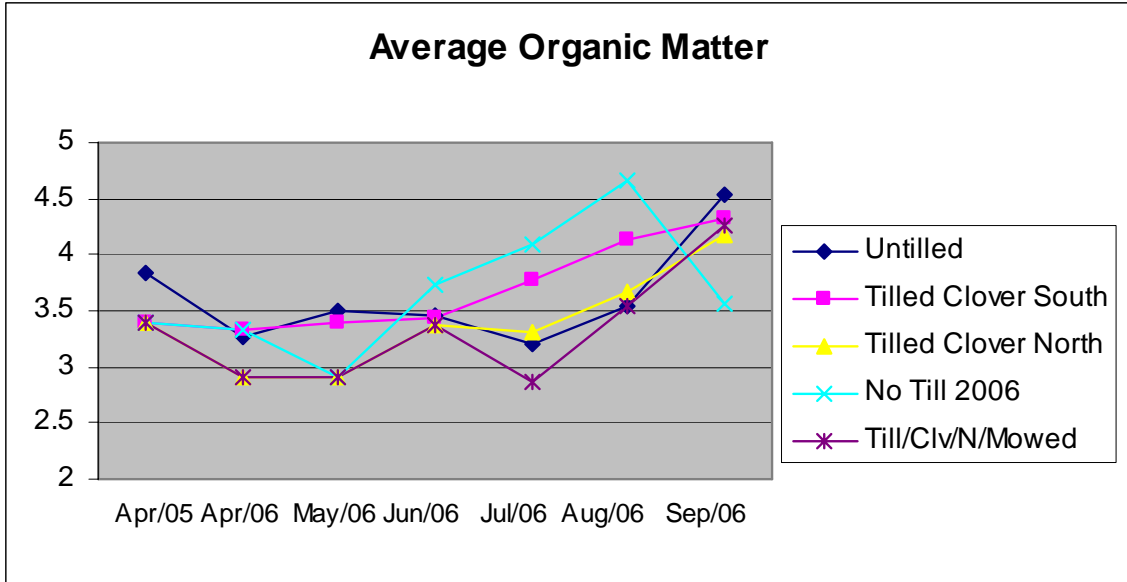
wide range of prey including aphids, thrips, larvae and caterpillars, other spiders, and more. Spiders cannot chew their food, but rather rely on a small, sucking mouth to withdraw the soft insides of their prey.

### *Pesticides and Habitat*

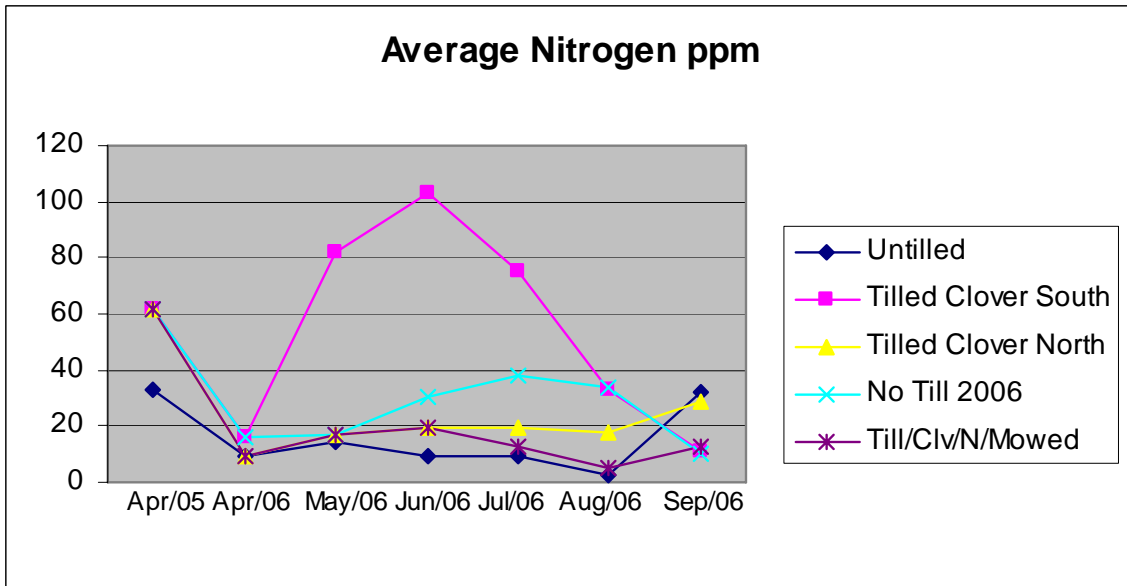
Spiders are extremely sensitive to broad spectrum insecticides. Wisniewska and Prokopy (1997) found spider population reductions of 200-300% in apple orchards that were under chemical management. Dempster (1967, 1968(1), 1968(2)), when measuring effects of the broad spectrum insecticide DDT upon *Pieris rapae* and arthropod predators, found that pesticides reduced spider populations significantly. These pesticide impacts were then compounded by the disturbance of tillage, which displaced the spiders that recolonized the plots following chemical application.

APPENDIX B

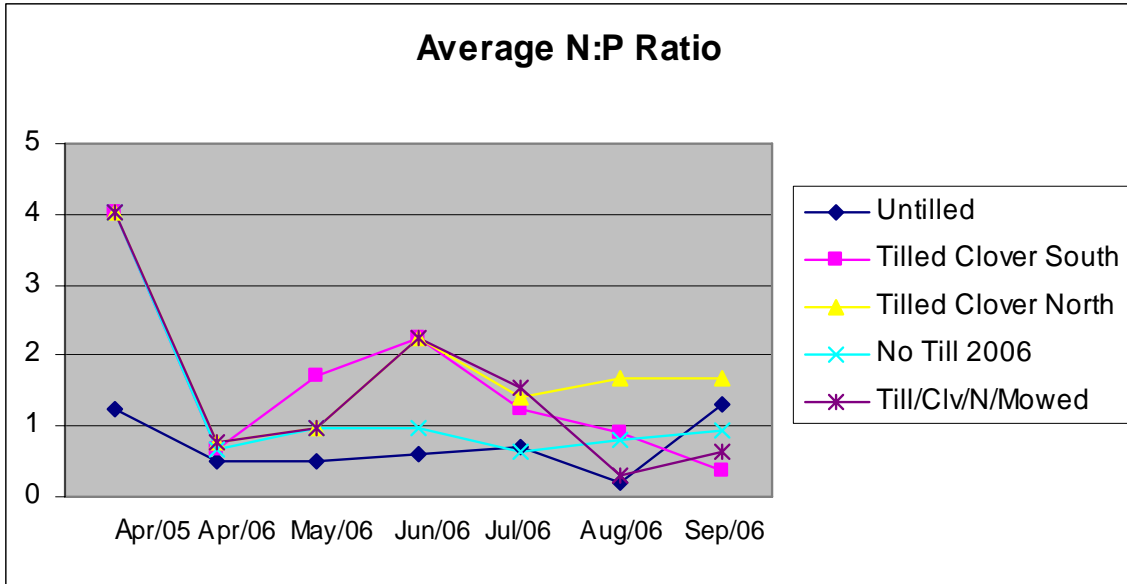
Supplemental Figures, Graphs, and Data



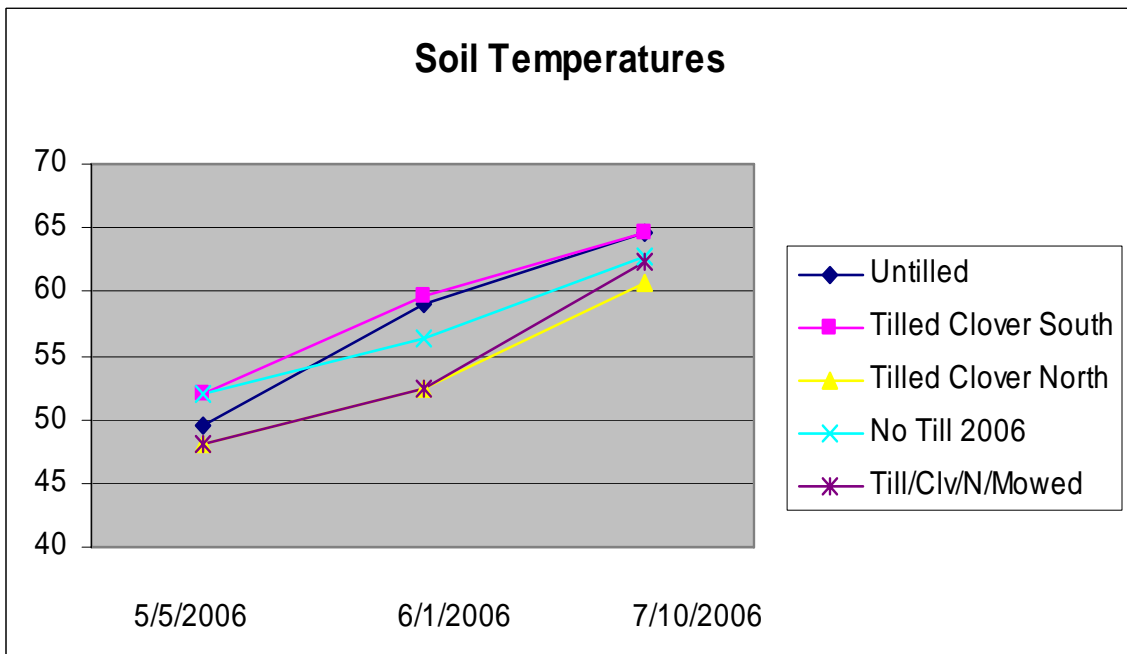
**Figure 11.** Average soil organic matter for field plots. All plots were located within the same field as where *P. rapae* studies were conducted. “No Till 2006” indicates plots of Brussels sprouts from *P. rapae* study.



**Figure 12.** Average nitrogen ppm in field plots. All plots were located within the same field as where *P. rapae* studies were conducted. “No Till 2006” indicates plots of Brussels sprouts from *P. rapae* study.



**Figure 13.** Average N:P ration for field plots. All plots were located within the same field as where *P. rapae* studies were conducted. “No Till 2006” indicates plots of Brussels sprouts from *P. rapae* study.



**Figure 14.** Average soil temperatures in field plots. All plots were located within the same field as where *P. rapae* studies were conducted. “No Till 2006” indicates plots of Brussels sprouts from *P. rapae* study.