THESIS

TWO ENTOMOLOGICAL STUDIES:

1. THE POTENTIAL OF METHYL JASMONATE APPLICATIONS AS A PEST MANAGEMENT METHOD ON CRUCIFEROUS CROPS

2. CONTRIBUTIONS TO THE BIOLOGY OF *DISHOLCASPIS QUERCUSMAMMA* (WALSH) (HYMENOPTERA: CYNIPIDAE)

Submitted by

Crystal Lee McEwen

Department of Bioagricultural Sciences and Pest Management

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Summer 2011

Master's Committee:

Advisor: Whitney Cranshaw

Boris Kondratieff
Frank Stonaker

ABSTRACT

TWO ENTOMOLOGICAL STUDIES:

1. POTENTIAL OF METHYL JASMONATE APPLICATIONS AS A PEST CONTROL METHOD ON CRUCIFEROUS CROPS

2. CONTRIBUTIONS TO THE BIOLOGY OF *DISHOLCASPIS QUERCUSMAMMA* (WALSH) (HYMENOPTERA: CYNIPIDAE)

Methyl jasmonate (MeJA) is known for the many physiological roles in plants, including induced resistance to herbivores. Treating plants with exogenous applications of MeJA has been shown to have various effects on the behavior of herbivores. This study sought out to quantify the effects of MeJA applications on field grown cruciferous crops in both pest response and crop response. The suitability of MeJA as a pest management tool depends on the tradeoff of costs and benefits of jasmonate-induced resistance.

MeJA applications were shown to reduce flea beetle (*Phyllotreta* spp.) feeding in a greenhouse setting. Feeding was reduced as early as the same day of treatment and feeding was further reduced over a period of 4 days. When applied in a field setting MeJA was effective at reducing the numbers of flea beetles, at least briefly, on broccoli, Chinese cabbage, Brussels sprouts, and rutabaga.

MeJA applications can affect lepidopterous pests by changing oviposition preferences or by affecting development. Brussels sprouts showed a reduction in *Pieris rapae* (Linnaeus) and *Trichoplusia ni* (Hübner) eggs found on MeJA treated foliage. Conversely multiple applications of MeJA on cabbage resulted in an increase in *P. rapae* eggs found on foliage. Trials where larvae were reared on field grown MeJA treated food showed that *P. rapae* larvae developed in

the same amount of time as those larvae that were reared on untreated food and that they weighed approximately the same. In that same trial, *T. ni* showed that male pupal periods were longer and pupae of both sexes weighed more when they were reared on MeJA treated food.

MeJA applications reduced plant size in broccoli, Chinese cabbage, and Brussels sprouts. Yield was also reduced in Chinese cabbage and rutabaga. During 2009 and 2010 MeJA applications were found to have no effect on yield in broccoli. MeJA treated broccoli showed a delay in maturity during both seasons. Protein levels on MeJA treated plants were only affected in rutabaga where there was a decrease in the protein levels in the roots of plants that were treated with both MeJA and insecticides.

Studies were done to clarify the biology of the cynipid gall wasp *Disholcaspis quercusmamma* (Walsh). This wasp was previously known only from its asexually reproducing females that develop inside conspicuous twig galls and the sexually reproducing generation has remained unidentified. Spring bud galls were identified and sexual generation adults were reared from these galls. A morphological description was developed for the sexual generation wasps and their galls.

The identity of the sexual generation of *D. quercusmamma* was confirmed by rearing trials ad DNA analysis. The sexual generation galls were found on both of the hosts that support the asexual generation; *Quercus macrocarpa* Michx. and *Quercus bicolor* Willd. While some trees were noted to be resistant to the formation of the asexual generation gall, those same trees were found to contain the sexual generation twig galls.

The sexual generation galls develop in buds and become visible in the spring after bud break. The sexual generation wasps emerge in the spring and oviposit in newly developing twigs. Parasitoids reared from the sexual generation galls that are shared with the asexual generation are *Torymus denticulatus* (Breland) (Torymidae) and *Sycophila dubia* (Walsh)

(Eurytomidae). Parasitoids that appear to be unique to the sexual generation are the pteromalid species *Lyrcus* nr. *nigroaeneus* (Ashmead), and the unidentified males of *Pteromalus* sp. and *Mesopolobus* sp. New records for the parasitoids associated with the asexual generation in northern Colorado include *Eurytoma querciglobuli* (Fitch), *T. denticulatus*, and *Baryscapus racemariae* (Ashmead).

ACKNOWLEDGMENTS

I am especially thankful for the loving support of my husband, Brady McEwen. Thank you for your understanding, participation, and motivation throughout the time we have been together. Your belief in me and your encouragement have kept me moving forward even in the hardest times. This endeavor, and the next, would not have been possible without your patience, support, and enthusiasm. You are my light at the end of the tunnel.

Special thanks are extended to my parents for their love, and encouragement. I would not have made it this far without your extensive generosity and support. To my sister Jenni, I am grateful for your support as well as your willingness to help me whenever I needed a shoulder to lean on. I am thankful for all of the sacrifices made by my great-grandfather, Big Daddy, and my grandfather, James J. Cooke, which have allowed their grandchildren the opportunities to pursue their passions.

I wish to express my deep sense of gratitude and appreciation to my advisor Dr. Whitney Cranshaw. His advice, teachings, and above-and-beyond support throughout my degree have been invaluable to my current and future careers. I would also like to thank my other committee members; Dr. Boris Kondratieff and Dr. Frank Stonaker, thank you both for the use of your equipment, motivation, and for your helpful insights in this study. I am also thankful for the help and knowledge that Dr. Paul Ode shared with me throughout my study. Special thanks are extended to the following Cranshaw lab field crew members for their help with the collections, rearing, counting, and every other sweat-filled or tedious task I required: Matt Camper, Mindy Hill, Kyle Conrad, Mike Dickinson, Peter Forrence, Matt Hudson, and Stephanie Szostek.

I wish to thank Stephanie Szostek, at Colorado State University, for her instruction and for the use of the Dr. Howard Schwartz lab during the DNA extractions. I would also like to thank the Colorado State University Proteomics and Metabolics Facility for sequencing our DNA samples and Dr. James Nicholls at the University of Edinburgh for analyzing the sequences. Special thanks to those who have performed insect identifications; Dr. Steve Heydon (UC Davis), Dr. Shawn Clark (BYU), and Dr. Mike Gates (SEL, Agricultural Research Service, USDA). I greatly appreciate Dr. Phillip Chapman's guidance in the statistical analyses performed in this study.

TABLE OF CONTENTS

CHAPTER ONE

THE RESPONSE OF FLEA BEETLES (*PHYLLOTRETA SPP*.) TO THE EFFECTS OF METHYL JASMONATE APPLICATIONS ON VARIOUS CRUCIFEROUS CROPS

Introduction

Phyllotreta spp. (Coleoptera: Chrysomelidae) flea beetles are key pests of cruciferous crops grown in the High Plains of North America (Al-Doghairi 2000, Chittenden & Marsh 1920, Demirel 2003). Flea beetle feeding produces distinctive pits in leaves that gouge deeply through the leaf surface, often cutting through thinner leaves leaving "shotholes". Flea beetles can be harmful to seedlings, edible greens, and in extreme infestations can reduce yield from established plants (Chittenden & Marsh 1920, Finch & Thompson 1992). In the Rocky Mountain region, flea beetles are most destructive early in the growth season, June through July (Al-Doghairi 2000, Chittenden & Marsh 1920). *Phyllotreta* spp. present in northern Colorado [*P. cruciferae* (Goeze), *P. pusilla* Horn] tend to prefer certain crucifers more than others (Al-Doghairi 2000) and the reasons behind these preferences have been one area of interest.

Cruciferous crops produce many distinctive secondary chemicals, particularly glucosinolates (Hicks 1974). While these chemicals are often thought of as defensive compounds, some specialist insects are thought use them for host recognition (Fernandez & Hilker 2007, Nielsen 1978, Nielsen et al. 2001) and feeding stimulants (Bartlet et al. 1994, Nielsen 1978, Nielsen et al. 2001, Tahvanainen 1983). Glucosinolates can be constitutive and their production can be induced in response to stress, wounding, or herbivory (Bodnaryk 1994, Doughty et al 1995). The qualitative and quantitative composition of the glucosinolates are

thought to be related to the specialization of the insect herbivores (Nielsen 1978 and Tahvanainen 1983). Methyl jasmonate (MeJA) and jasmonic acid (JA) can systemically induce the production of many secondary compounds in many types of plants (Bartlet et al. 1999, Bodnaryk 1994, Cheong & Choi 2003, Doughty et al. 1995, Thaler et al. 2001). Due to MeJA's ability to increase the production of secondary chemicals, MeJA applications have the potential to affect the feeding preferences of insect herbivores (Avdiushko et al 1997, Bartlet et al. 1999, Thaler et al. 2001, Zhang et al. 2008). Crucifer feeding flea beetles are thought to use secondary compounds as feeding stimulants (Hicks 1974, Nielsen 1978) and for this reason MeJA-induced increases in these compounds could potentially increase flea beetle feeding. However, some induced changes in the plant chemistry can potentially reduce feeding by both generalists and specialists (Bartlet 1999, Loivamäki et al. 2004, Nielsen 1978, Nielsen et al. 2001, Thaler et al. 2001). Thaler (2001) documented that MeJA applications have been shown to deter a wide range of insect pests on tomatoes, including flea beetles.

Numerous experiments have shown that MeJA induction increases the accumulation of glucosinolates in crucifers (Bartlet et al. 1999, Bodnaryk 1994, Doughty et al. 1995, Kubicka & Zadernowski 2007, Loivamäki et al. 2004) and can reduce herbivory (Aviushko et al. 1997, Bartlet et al. 1999, Thaler et al. 2001). Most of the studies that have investigated the effects of secondary chemical induction on flea beetle feeding have concentrated on the effects of MeJA treatments on oilseed or mustard crops (Bartlet et al. 1999, Bodnaryk & Palaniswamy 1990, Demirel 2003, Hicks 1974, Lamb 1988, Loivamäki et al. 2004, Palaniswamy & Lamb 1993) or have concentrated on plants in a lab setting (Avdiushko et al. 1997, Bartlet et al. 1994, Bartlet et al. 1999, Bodnaryk & Palaniswamy 1990, Demirel 2003, Hicks 1974, Loivamäki et al. 2004, Nielsen 1978, Nielsen et al. 2001 Palaniswamy & Lamb 1993). It is important to investigate how flea beetles respond to MeJA treated plants in the field because not only can the plants respond

differently in the field (Loivamäki 2004) but the flea beetles may also behave differently in the field compared to a lab setting (Tahvanainen 1983). The purpose of this chapter is to quantify the response of flea beetles to MeJA applications on various crucifer crops in a field setting in order to offer insight on its applicability as a pest management technique.

Materials and Methods

Studies were conducted during two growing seasons – 2009 and 2010. All trials that used methyl jasmonate treatments [cyclopentaneacetic acid, 3oxo-2-(2-pentenyl)-methylester Bedoukian Research Inc., 21 Finance Drive, Danbury, Connecticut 06810-4192] were applied at 14.2mM solutions. Separate solutions were made for each application date and involved 6.65 grams of 96% MeJA per 2000mL water. Solutions were mixed by agitation. This concentration of MeJA was shown to be effective at producing differences in flea beetle numbers in previous preliminary trials (Cranshaw, unpublished).

All field experiments were conducted at the Colorado State University Horticulture Field Research Center (CSU HFRC), north of Fort Collins, Colorado. Flea beetles used for field and greenhouse studies consisted of a mixture of *P. cruciferae* and *P. pusilla*. These two species are too similar in appearance to consistently distinguish in the field and present similar risk to cruciferous crops (Finch & Thompson 1992).

Experiment 1.1. Flea Beetle Feeding Preferences, Preliminary Greenhouse Study, 2009

This experiment was conducted at the Colorado State University Insectary Building. A sample of 24 cabbage (*Brassica oleracea* Linnaeus, Capitata group) seedlings were divided into two treatment groups: untreated; and MeJA treated. The MeJA treated plants were treated approximately one week prior to the experiment. The plants were randomly arranged in four

six-packs and placed in the middle of a cage. Approximately 40 flea beetles were collected in the field by sweep net from various crucifers at the CSU HFRC. The beetles were allowed to feed on the experiment plants for four days, 9 July to 13 July.

A similar experiment was performed immediately after the last evaluation and was adjusted in order to allow more feeding damage on the plants. The second trial involved a total of 12 plants with six being untreated and the remaining six MeJA treated approximately 1.5 weeks prior to exposure to beetles. For this trial, 40 beetles were confined with the plants from 13 July through 17 July.

The plant damage from flea beetle feeding was determined by tracing the leaves on graph paper and coloring in the areas that were damaged by feeding. The number of quarter squares on the graph paper colored in as damaged and the quarter squares within the whole leaf image were estimated. Percent leaf area damaged on each plant was calculated by dividing the number of quarter squares indicating damage by the total leaf area.

Experiment 1.2. Flea Beetle Feeding Preferences, Greenhouse Study, 2010

Five cages were housed in an outdoor, shaded screened house. The cages were set up as randomized complete block with each cage representing a block containing 4 treatment groups. Each cage had two six-packs with cabbage (cv. Tendersweet) seedlings containing 3 plants of each treatment.

Flea beetles were captured in the field using a sweep net from canola (*Brassica napus Linnaeus*) or flixweed (*Descurainia sophia* Linnaeus). Flea beetles were isolated from the sweep samples using an aspirator. Approximately 100 beetles, consisting of a mixture of *P. cruciferae* and *P. pusilla*, were put into large vials for transportation and release into cages.

There were 4 treatment groups: MeJA treatment on 5 July, MeJA treatment on 7 July, MeJA treatment on 9 July, and no MeJA treatment. The treatment groups provided a

graduation of time following MeJA treatment before the plants were exposed to the insects; 4 days, 2 days, and same day. The plants were placed in the center of the cage and the open vial of beetles was placed in the middle of the plants. The flea beetles were exposed to the plants from 9-12 July.

The plant damage was recorded by tracing and coloring in the leaf area damaged by feeding onto white paper on 12 July. The images were scanned with a reference ruler and saved as .jpeg files. The total damaged area was later determined using ImageJ software (Rasband 1997-2005). The total damaged area (mm^2) for each plant was recorded and the average for each treatment was used for evaluating differences between the treatments.

Experiment 1.3. Flea Beetle Feeding Preferences, Field Study, 2009

On 29 May, broccoli (*B. oleracea*, Botrytis group, cv. Windsor) and Chinese cabbage (*Brassica rapa Linnaeus,* Pekinensis group, cv. Tall Michihili) were transplanted in single rows with the plants spaced 46cm at the CSU HFRC. Each row was divided into a randomized complete block with four replications of three treatments. The treatment groups were: one MeJA treatment, two MeJA treatments, and untreated. All plots that were treated with MeJA were treated prior to transplanting, on 26 May. Those plots that had a second MeJA treatment were treated again, following transplanting on 23 June.

On 5 June, Brussels sprouts (*B. oleracea*, Gemmifera group, cv. Diablo) were transplanted in two rows with the plants in each row spaced 46cm apart and the rows were staggered. The Brussels sprouts were divided into four replications of two treatments with the plots spanning across both rows. The experimental set up as a completely random design. The treatment groups were: one MeJA treatment; and untreated. The single application of MeJA was performed on 15 June.

Evaluations of the broccoli and Chinese cabbage plots were made by counting the total number of flea beetles on the middle six plants in each plot. The flea beetle population in northern Colorado attacking cruciferous crops consists of a mixture of *P. cruciferae* and *P. pusilla*. These species are very similar in appearance and could not be separated without microscopic examination. Counts were performed on 1 June, 15 June, 22 June, 26 June, 30 June, and 8 July.

The Brussels sprouts plots were evaluated by counting the total number of flea beetles on the middle 4 plants in each row, for a total of 8 plants counted per plot. These counts were done on 17 June, 22 June, 26 June, 30 June, and 8 July. All counts started in the northwestern corner of the field, working southeast to minimize beetle disturbance by shadow cover and movement.

Experiment 1.4. Flea Beetle Feeding Preferences, Field Study, 2010

Flea Beetle Count Evaluation

Brassica carinata A. Braun, canola, broccoli (cv. Arcadia), the north row of Brussels sprouts (cv. Churchill), rutabaga (*B. napus*, Napobrassica group, cv. Helenor), and Chinese cabbage (cv. Bilko) were planted at 46cm spacing in single rows each at the CSU HFRC. Each row was set up as a randomized complete block design with four blocks and two treatments each. The treatment groups were: MeJA treated and untreated. The MeJA treatments were performed on 28 June and 21 July.

Plot evaluations for the flea beetles were done by counting the total number of flea beetles on the middle six plants of each plot. Flea beetle counts were performed on 13 July and 26 July. Counts were performed in the morning and started with the northwest corner of the field, working southeastward to minimize the disturbance of the plants via shadow cover and movement.

Insect Feeding Damage Evaluation

Broccoli (cv. Arcadia), rutabaga (cv. Helenor), a north row and a south row of Brussels sprouts (cv. Churchill) were planted at 46cm spacing in single rows each at the CSU HFRC. Each row was set up as a randomized complete block design with four blocks and two treatments each. The treatment groups for the broccoli, rutabaga, and the north row of Brussels sprouts were: MeJA treated; and untreated. The MeJA treatments were performed on 28 June and 21 July. The treatment groups for the south row of Brussels sprouts were: no treatment; a single application of MeJA; two applications of MeJA; and three applications of MeJA. The first or single MeJA was applied on 10 June, the second application on 30 June, and the third application was on 21 July.

Plots were evaluated by counting the total shotholes in four expanded leaves at cardinal points on the middle six plants of every plot. Counts were performed on 19 August.

Statistical Analysis

All data were analyzed using SAS software (version 9.2, copyright 2002-2008). Analyses using SNK for pairwise comparisons only report the overall ANOVA p value and significant differences between treatment groups are $p<0.05$. The data from the preliminary 2009 flea beetle feeding preference greenhouse experiment were analyzed using a two-sample t-test. Both of the 2009 preliminary greenhouse trials required a log transformation to control for variance and the first trial required the addition of 0.1 to the percent damage values prior to the transformation since some of the values were zero. The data from the 2010 flea beetle feeding preference greenhouse experiment were analyzed by calculating the average damaged area for each treatment within a block and those average values underwent a square root transformation, to control for variance, followed by an ANOVA test with a SNK comparison.

The data from the 2009 flea beetle counts on broccoli underwent a square root transformation followed by an ANOVA with a SNK analysis. The data from the Chinese cabbage and Brussels sprouts did not require a transformation and were analyzed using an ANOVA with a SNK comparison. The data from the 2010 flea beetle counts underwent a square root transformation to control for variance but the shothole evaluations did not require a transformation. These plots were analyzed using an ANOVA with a SNK comparison.

Results

Experiment 1.1. Flea Beetle Feeding Preferences, Preliminary Greenhouse Study, 2009

Both of the trials resulted in significant differences between treated and untreated groups. The first trial (Table 1.1), involving 24 plants, showed that the MeJA treated group had a significantly lower percentage of damage on the leaves from feeding ($t=-3.27$; $df=22$; $p=0.0035$), nearly by a factor of five. The second trial (Table 1.2), involving 12 plants, also showed that the MeJA treated plants had a significantly lower percentage of damage on the leaves ($t=-5.06$; $df=10$; $p=0.0005$), also nearly by a factor of five.

Table 1.1. Flea beetle feeding damage evaluation on cabbage seedlings treated with methyl jasmonate (14.2mM) prior to exposure to flea beetles. Experiment was performed in greenhouse cages and 24 plants were exposed to 40 flea beetles (*Phyllotreta* spp.) from 9-13 July, 2009.

Treatment	Average amount of feeding damage per plant (% leaf area)
MeJA	0.63 (a)
Untreated	3.02 (b)

*Plants were treated approximately one week prior to experiment.
**Flea beetles included *Phyllotreta cruciferae* (Goeze) and *Phyllotreta pusilla* Horn that were captured in the field from various cruciferous host plants.
***Numbers not followed by the same letter are significantly different ($p<0.05$) using a two-sample t-test following a log transformation. Since some of the original values were zero, 0.1 was added to all the original values prior to the log transformaiton.

Table 1.2. Feeding damage evaluation on cabbage seedlings treated with methyl jasmonate (14.2 mM) prior to exposure to flea beetles. Experiment was performed in a greenhouse cages and 12 plants were exposed to 40 flea beetles (*Phyllotreta* spp.) from 13-17 July, 2009.

Treatment	Average amount of feeding damage per plant (% leaf area)
MeJA	2.76 (a)
Untreated	11.44 (b)

*Plants were treated approximately 1.5 weeks prior to experiment.
** Flea beetles included *Phyllotreta cruciferae* (Goeze) and *Phyllotreta pusilla* Horn that were captured in the field from various cruciferous host plants.
***Numbers not followed by the same letter are significantly different ($p<0.05$) using a two-sample t-test following a log transformation.

Experiment 1.2. Flea Beetle Feeding Preferences, Greenhouse Study, 2010

The evaluation of the damaged area on the cabbage plants (Table 1.3) showed that the effects of MeJA applications were quickly expressed and could affect flea beetle injury. The untreated group had significantly more damaged leaf area compared to any of the MeJA treated groups ($F=46.77$; $df=3, 12$; $p<0.0001$). The plants that were treated on the same day as exposure to beetles had the same amount of damaged leaf area as the plants that were treated two days prior to exposure. The plants that were treated four days prior to exposure had significantly lower amounts of damaged leaf area compared to all the other treatment groups. The average damaged area on the untreated plants was approximately 75 times the damaged area on the plants treated with MeJA four days prior to exposure. The two days difference in treatment times between the plants treated four days prior and those treated two days prior to exposure produced a 28-fold effect in the leaf area damaged by flea beetles.

Table 1.3. Flea beetle feeding preferences on cabbage (cv. Tendersweet) seedlings receiving methyl jasmonate (14.2mM) application on various schedules. Experiments were performed in a greenhouse at the Insectary Building, Colorado State University, Fort Collins, Colorado, 2010.

Treatment	Average leaf area damaged (mm^2)[1]
Untreated	169.23 (a)
MeJA on same day as exposure (9 July)	100.41 (b)
MeJA 2 days prior to exposure (7 July)	62.66 (b)
MeJA 4 days prior to exposure (5 July)	2.26 (c)

*Plants caged with flea beetles from 9-12 July.
** Flea beetles included *Phyllotreta cruciferae* (Goeze) and *Phyllotreta pusilla* Horn that were captured in the field from various cruciferous host plants.
[1]Numbers not followed by the same letter are significantly different ($p<0.05$) after a square root transformation.

Experiment 1.3. Flea Beetle Feeding Preferences, Field Study, 2009

The 1 June flea beetle counts on broccoli (Table 1.4) indicated that the numbers of flea beetles counted on a plot was dependant on whether MeJA was applied ($F=17.06$; $df=2, 6$; $p=0.0033$). The untreated group had significantly more flea beetles present than the MeJA treated plots. Both sets of the MeJA treated plots had received only a single MeJA application by this counting date. This shows that the use of MeJA effectively reduced the flea beetles present on broccoli this early in the season.

The 15 June flea beetle counts on broccoli (Table 1.4) showed that the numbers of flea beetles counted on a plot was dependant on whether MeJA was applied ($F=8.49$; $df=2, 6$; $p=0.0178$). The number of beetles counted on the untreated plots was significantly higher than the MeJA treated plots and the number of beetles present was at least 2 fold the infestation on the same plots one week earlier. Both sets of the MeJA treated plots had received only a single MeJA application by this counting date. This shows that the effects of the 26 May MeJA applications were still proving effective at reducing the flea beetles present on broccoli as the growing season moved towards the peak of flea beetle infestation.

The 22 June flea beetle counts on broccoli (Table 1.4) showed that the numbers of flea beetles counted on a plot was dependant on whether MeJA was applied ($F=10.43$; $df=2, 6$; $p=0.0112$). The untreated group had significantly more flea beetles than the MeJA treated plots. Once again, this counting date was before the second MeJA applications so both of the sets of MeJA treated plots are the same. This data from this counting date shows that the MeJA treatment was effective at reducing the numbers of flea beetles during the peak of flea beetle infestation.

The 26 June flea beetle counts on broccoli (Table 1.4) showed that the numbers of flea beetles counted on a plot was dependant on the MeJA treatment schedule ($F=11.7$; $df=2, 6$;

p=0.0085). The untreated plots and the plots that had only a single MeJA application had more than 3-fold the number of flea beetles compared to the group with two MeJA. The plots with only a single MeJA application were not statistically different from the untreated plots at this point. This may have been because the effects from the MeJA application might have diminished. Alternately, since the infestation on the untreated plots had peaked the previous week the untreated plots may have been expressing an induced response due to flea beetle damage that was similar to the single MeJA treatment.

The 30 June flea beetle counts on broccoli (Table 1.4) showed that the numbers of flea beetles counted on a plot was not dependant on whether MeJA had been applied (F=2.08; df=2, 6; p=0.2065). While the plots that had been treated twice with MeJA still had lower numbers of flea beetles present, this difference was no longer significant.

The 8 July flea beetle counts on broccoli (Table 1.4) showed that the numbers of flea beetles counted on a plot was still not dependant on the treatments applied (F=3.26; df=2, 6; p=0.1101). Although not significantly different, the untreated plots had the fewest flea beetles counted. This also suggests that the reduction in feeding might be due to effects of previous flea beetle feeding on the untreated plots.

Table 1.4. Flea beetle counts on broccoli (cv. Windsor) treated with methyl jasmonate (14.2mM) at different treatment levels. Plots were established 29 May, 2009 by transplanting at the Colorado State University Horticulture Research Center.

	Average number of flea beetles per plot[1]					
Treatment[2]	1 June	15 June	22 June	26 June	30 June	8 July
Untreated	19.75 (a)	40.25 (a)	51.00 (a)	43.25 (a)	9.75 (a)	8.75 (a)
MeJA 1x	6.75 (b)	14.50 (b)	31.00 (b)	49.50 (a)	12.25 (a)	17.75 (a)
MeJA 2x	5.75 (b)[3]	21.00 (b)[3]	23.75 (b)[3]	13.25 (b)	4.00 (a)	11.50 (a)

[1] Numbers not followed by the same letter are significantly different by SNK analysis after a square root transformation ($p<0.05$).

[2] The first MeJA treatment was performed on 26 May and the second application was on 23 June.

[3]Only one MeJA application had been applied by this date so the MeJA 2x treated plots are the same as the MeJA 1x treated plots.

The 1 June flea beetle counts on Chinese cabbage (Table 1.5) showed that the numbers of flea beetles present on a plot were not dependant on whether MeJA had been applied ($F=0.08$; $df=2, 6$; $p=0.9209$). Both sets of the MeJA treated plots had received only a single MeJA application by this counting date.

The 15 June flea beetle counts on Chinese cabbage (Table 1.5) showed that the numbers of flea beetles counted on a plot was not dependant on whether MeJA was applied ($F=3.0$; $df=2, 6$; $p=0.1252$). Both sets of the MeJA treated plots had received only a single MeJA application by this counting date.

The data from the 22 June flea beetle counts on Chinese cabbage (Table 1.5) showed that the numbers of flea beetles counted on a plot was not dependant on whether MeJA had been applied ($F=2.92$; $df=2, 6$; $p=0.1301$). Both sets of the MeJA treated plots had received only a single MeJA application by this counting date.

The 26 June flea beetle counts on Chinese cabbage (Table 1.5) showed that the numbers of flea beetles counted on a plot was dependant on the MeJA treatment schedule ($F=14.17$; $df= 2, 6$; $p<0.0053$). The untreated plots and the plots that were treated once with MeJA had approximately 3 times more flea beetles compared to the treatment group that received two MeJA applications. The untreated plots were not significantly different from the plots that were treated once with MeJA. This shows that using only one MeJA treatment was not effective at controlling for flea beetles during the peak of the flea beetle infestation but using two applications was effective at reducing the number of flea beetles, at least briefly. This significant effect occurred one week after the second application of MeJA was applied.

The 30 June flea beetle counts on Chinese cabbage (Table 1.5) showed that there were no differences in the numbers of flea beetles counted on the plants between the different treatment groups ($F=0.06$; $df=2, 6$; $p=0.9453$). A drop in the number of flea beetles present was

noted for this date and the numbers increased again for the subsequent counting date suggesting that the flea beetle distributions might have been effected by external factors.

The 8 July flea beetle counts on Chinese cabbage (Table 1.5) showed that the numbers of flea beetles counted on a plot was not dependant on whether MeJA had been applied ($F=2.68$; $df=2, 6$; $p=0.147$). The flea beetle numbers were elevated again but no significant differences in beetle numbers were noted.

Table 1.5. Flea beetle counts on Chinese cabbage (cv. Tall Michihili) treated with methyl jasmonate (14.2mM) at different treatment levels. Plots were established 29 May, 2009 by transplanting at the Colorado State University Horticulture Research Center, Fort Collins, Colorado.

	Average number of flea beetles per plot[1]					
Treatment[2]	1 June	15 June	22 June	26 June	30 June	8 July
Untreated	6.50 (a)	51.25 (a)	83.25 (a)	97.00 (a)	35.25 (a)	70.25 (a)
MeJA 1x	5.75 (a)	45.75 (a)	80.25 (a)	90.50 (a)	37.25 (a)	60.75 (a)
MeJA 2x	6.50 (a)[3]	37.5 (a)[3]	60.25 (a)[3]	30.25 (b)	34.50 (a)	87.75 (a)

[1]Numbers from each date not followed by the same letter are significantly different by SNK analysis, $p<0.05$.

[2] The first MeJA treatment was performed on 26 May and the second application was on 23 June.

[3]Only one MeJA application had been applied by this date so the MeJA 2x treated plots are the same as the MeJA 1x treated plots.

The data from the 17 June flea beetle counts on Brussels sprouts (Table 1.6) showed that the number of flea beetles counted on a plot was not dependant on whether MeJA had been applied ($F=0.14$; $df=1, 6$; $p=0.7214$). The untreated group had similar numbers of beetles present compared to the MeJA treated group.

The 22 June flea beetle counts on Brussels sprouts (Table 1.6) showed that the number of flea beetles counted on a plot was not dependant on whether MeJA had been applied ($F=3.5$; $df=1, 6$; $p=0.1105$). The average number of beetles counted on the untreated plots appears to be higher than the MeJA treated plots but the difference is not significant since the numbers of flea beetles varied greatly within the treatment groups.

The data from the 26 June flea beetle counts on Brussels sprouts (Table 1.6) showed that the number of beetles counted on a plot depended on whether the plot had been treated with MeJA ($F=6.65$; $df=1, 6$; $p=0.0418$). The plots that were treated with MeJA had significantly fewer flea beetles compared to the untreated plots. The difference was more that 2-fold.

The 30 June flea beetle counts on Brussels sprouts (Table 1.6) showed that the number of beetles counted on a plot depended on whether the plot had been treated with MeJA ($F=16.19$; $df=1, 6$; $p=0.0069$). The untreated plots had significantly more flea beetles compared to the MeJA treated plots.

The data from the 8 July flea beetle counts on Brussels sprouts (Table 1.6) showed that the number of flea beetles counted on a plot was not dependant on whether MeJA had been applied ($F=0.55$; $df=1, 6$; $p=0.4879$). The average number of beetles counted on the untreated plots appears to be higher than the MeJA treated plots but the difference is not significant since the numbers of flea beetles varied greatly within the treatment groups.

.

Table 1.6. Flea beetle counts on Brussels sprouts (cv. Diablo) treated with methyl jasmonate (14.2mM) at different treatment levels. Plots were established 5 June, 2009 by transplanting at the Colorado State University Horticulture Research Center, Fort Collins, Colorado.

	Average number of flea beetles per plot[1]				
Treatment[2]	17 June	22 June	26 June	30 June	8 July
Untreated	17.75 (a)	20.50 (a)	50.75 (a)	22.00 (a)	40.50 (a)
MeJA 1x	20.00 (a)	9.25 (a)	23.75 (b)	13.25 (b)	27.00 (a)

[1]Numbers from each date not followed by the same letter are significantly different by SNK analysis, $p<0.05$.

[2] The MeJA treatment was performed on 15 June.

Experiment 1.4. Flea Beetle Feeding Preferences, Field Study, 2010

Flea Beetle Count Evaluation

The 13 July flea beetle counts on *B. carinata* (Table 1.7) showed that the MeJA applications did not significantly affect the number of beetles present (F=0.34; df=1, 3; p=0.6014). At this point in the season only a single treatment of MeJA had been applied. The evaluation of the 26 July flea beetle counts also showed that the MeJA applications did not significantly affect the numbers of beetles present (F=9.80; df=1, 3; p=0.0520). There was a significant block effect (F=13.98; df=3, 3; p=0.0287). It was noted that the fourth block had higher numbers of beetles than the other blocks and this variation may have obscured ability to determine significant differences. Both the MeJA treated plots and the untreated plots experienced a decrease in the number of beetles between the two counting dates (Table 1.7).

The evaluation of the 13 July flea beetle counts on canola (Table 1.7) showed that the MeJA applications did not significantly affect the number of beetles present (F=0.45; df=1, 3; p=0.5502). The lack of differences may be due to the fact that only a single treatment of MeJA had been applied by the time these initial counts were performed. The evaluation of the 26 July flea beetle counts showed that the MeJA applications also did not significantly affect the numbers of beetles present (F=3.98; df=1, 3; p=0.1404). As seen with the *B. carinata*, one replication had a count value that was much higher than the rest for that treatment group and this increase in variation may explain why there were no significant differences between the treatments. These plots that had the higher values were located next to each other. Both the MeJA treated plots and the untreated plots experienced a decrease in the number of beetles between the two counting dates (Table 1.7).

The evaluation of the 13 July flea beetle counts on broccoli (Table 1.7) showed that the MeJA applications did not significantly affect the number of beetles present (F= 0.11; df=1, 3;

p=0.7592). The data from the 26 July flea beetle counts showed that the number of beetles present on the MeJA treated plots was significantly lower than the untreated plots (F=27.35; df=1, 3; p=0.0136). Following a second application of MeJA applied 21 July more than a four-fold difference in flea beetle numbers were observed between the treatments. The number of beetles present on the MeJA treated plots decreased between the counting dates and the untreated plots had an increase in the number of flea beetles (Table 1.7).

The evaluation of the 13 July flea beetle counts on Brussels sprouts (Table 1.5) showed that the MeJA applications did not significantly affect the number of beetles present (F=0.05; df=1, 3; p=0.8394). The data from the 26 July flea beetle counts showed that the number of beetles present on the MeJA treated plots was significantly lower than the untreated plots (F=13.38; df=1, 3; p=0.0353). This drop occurred after the second application of MeJA to the treated plots. Within ten days the number of flea beetles went from being almost equal to a five-fold difference between the treatments. During those ten days, the beetles on the untreated plots increased while the MeJA treated plots saw a decrease in the number of beetles (Table 1.7).

The evaluation of the 13 July flea beetle counts on rutabaga (Table 1.7) showed that that the MeJA applications did not significantly affect the number of beetles present (F=1.21; df=1, 3; p=0.3525). The data from the 26 July flea beetle counts showed that the number of beetles present on the MeJA treated plots was significantly lower than the untreated plots (F=48.00; df=1, 3; p=0.0062). As with the Brussels sprouts, rutabaga had a five-fold difference in flea beetles between the treatments on that date. The number of beetles present on the MeJA treated plots decreased between the counting dates and the untreated plots had an increase in the number of flea beetles (Table 1.7).

The evaluation of the 13 July flea beetle counts on Chinese cabbage (Table 1.7) showed that the MeJA applications did not significantly affect the number of beetles present ($F=0.17$; $df=1, 3$; $p=0.7045$). The evaluation of the 26 July flea beetle counts showed that the MeJA applications did not significantly affect the numbers of beetles present ($F=2.56$; $df=1, 3$; $p=0.2081$). This was the only vegetable crop that did not see a significant effect on the number of flea beetles present after a second application of MeJA was applied. Both the MeJA treated and the untreated plots experienced an increase in the numbers of beetles between the two counting dates.

Table 1.7. Flea beetle counts on various cruciferous crops that were treated with methyl jasmonate (14.2mM). Plots were planted at the Colorado State University Horticulture Research Center, Fort Collins, Colorado, 2010.

		Average number of flea beetles per plot	
Crop	Treatment	13 July	26 July
B. carinata[1]	Untreated	36.75 (a)	11.75 (a)
	MeJA	32.50 (a)	29.25 (a)
Canola[1]	Untreated	34.50 (a)	24.00 (a)
	MeJA	30.50 (a)	8.75 (a)
Broccoli[1]	Untreated	18.50 (a)	37.50 (a)
	MeJA	17.50 (a)	8.25 (b)
Brussels Sprouts[1]	Untreated	13.00 (a)	24.00 (a)
	MeJA	12.50 (a)	4.75 (b)
Rutabaga[2]	Untreated	24.50 (a)	73.50 (a)
	MeJA	21.50 (a)	14.76 (b)
Chinese Cabbage[1]	Untreated	52.50 (a)	89.00 (a)
	MeJA	43.50 (a)	52.00 (a)

[1]Within each variety, numbers within a column not followed by the same letter are significantly different ($p<0.05$) by SNK after a square root transformation.
[2]Numbers not followed by the same letter are significantly different ($p<0.05$) after a log transformation.
*MeJA treatments were performed on 28 June and 21 July.
**The averages are based on the total beetles counted within each plot (four replications). The total consists of the beetles counted on the middle six plants in that plot.

Flea Beetle Feeding Damage Evaluation

The shothole damage evaluation on broccoli (Table 1.8) showed that there were no significant differences between the numbers of shotholes counted on the untreated and MeJA treated plants (F=5.93; df=1, 3; p=0.0929). The average number of shotholes counted on the untreated plots seems higher than the average for the MeJA treated plots; however, due to the high numbers of shotholes counted in the first block, the variation prevented any statistical significance.

The shothole damage evaluation on rutabaga (Table 1.8) showed that the untreated plots had significantly more shotholes than the MeJA treated plots (F=3.01, df=1, 3; p= 0.0004). The MeJA treated plants had less than half the number of shotholes found on the untreated plants and this difference in damage was reflected in the lower numbers of beetles present on the MeJA treated plots on 26 July (Table 1.7). On that date there was nearly a five-fold difference in the numbers of flea beetles counted and this highly significant difference in the numbers of beetles produced a 3-fold difference in the amount of feeding damage on the plants.

The shothole damage evaluation on the north row of Brussels sprouts (Table 1.8) showed that the untreated plants had 3 times more shotholes than the MeJA treated group (F=72.17; df=1, 3; p=0.0034). This difference in the feeding damage between the treatment groups could be expected since there was a five-fold difference in the numbers of beetles counted on untreated and MeJA treated plants on 26 July (Table 1.7).

The shothole damage evaluation on the south row of Brussels sprouts (Table 1.8) showed no significant differences between the treatment groups (F=1.15; df=3, 9; p=0.3805). While the average number of shotholes counted on the plots that were treated three times appears to be smaller than the other averages, a high amount of variation between replications

prevented establishing significant differences. The differences between the rows of Brussels sprouts may have been due to the maturity of the plants since the south row was planted before the north row. Another factor to consider is that this row received damage early in the field season from a tractor cultivation and this wounding may have induced a similar response to a MeJA treatment.

Two of the four sets of plots (rutabaga and the north row of Brussels sprouts) showed that the MeJA induced response had a significant effect on shothole damage seen on plants. While only two of the crop sets showed significant differences all had a similar trend, with the untreated plots having the highest numbers of shotholes and the MeJA treated plots lower numbers (Figure 1.8).

Table 1.8. Flea beetle feeding damage on various cruciferous crops that were treated with methyl jasmonate (14.2mM). Plots were transplanted at the Colorado State University Horticulture Research Center, Fort Collins, Colorado. Plots were evaluated on 19 August, 2010.

Crop	Treatment	Average number of holes in leaves from flea beetle feeding[1]
Broccoli[2]	Untreated	880.50 (a)
	MeJA 2x	616.50 (a)
Rutabaga[2]	Untreated	1034.75 (a)
	MeJA 2x	430.00 (b)
Brussels Sprouts (North row)[2]	Untreated	543.75 (a)
	MeJA 2x	180.25 (b)
Brussels Sprouts (South row)[3]	Untreated	874.25 (a)
	MeJA 1x	821.75 (a)
	MeJA 2x	805.00 (a)
	MeJA 3x	698.00 (a)

[1]Numbers not followed by the same letter are significantly different ($p<0.05$).
[2]MeJA applications were done on 28 June and 21 July.
[3]MeJA was applied on 10 June, 30 June, and 21 July.

Discussion

The results from the greenhouse experiments (2009 and 2010) indicated that MeJA applications can reduce the feeding damage inflicted by flea beetles. Bartlet et al. (1999) showed that the induced effects changed flea beetle feeding preferences 4 days after treatment. In this experiment the effects of MeJA were immediate and also showed that the induced response grew over a period of a week.

Past research has shown mixed results for how flea beetles respond to wound-induced host plants. Some studies have showed that flea beetle response to wounding varies by cultivar (Demirel 2003, Palaniswamy & Lamb 1993) and others have found that flea beetle response to wounding can similar across cultivars (Demirel 2003, Peng et al. 1992, Vaughn & Hoy 1993, Zhang et al 2008). The data from this experiment showed that flea beetle response to MeJA applications varies across crop type. The induced resistance to flea beetle feeding occurred in all the food crops except Chinese cabbage. Both broccoli and Brussels sprouts appeared to show induced resistance for at least a short time after MeJA treatment. The MeJA-induced response in broccoli was expressed soon after MeJA applications but the response of Brussels sprouts seemed to be delayed by a 2-3 weeks. Rutabaga also showed an induced resistance that resulted in a dramatic difference in damaged leaf area and in the numbers of flea beetles present during the second count date in 2010. In the case of Chinese cabbage, MeJA had a minimal effect on flea beetles. In this study Chinese cabbage also had the highest flea beetle numbers, consistent with the findings of Al-Doghairi (2000).

The differences observed between the treatments during 2009 were temporary suggesting that either the plants recovered from the effects of MeJA or that the untreated plants sustaining flea beetle damage were being induced to a similar extent as the MeJA treatments. The field grown plants were exposed to other herbivores as well as non-chemical

factors, such as microclimatic factors and physical features of the habitat, and these stressors can affect feeding choice in flea beetles (Tahvanainen 1983). The untreated plants in the field would have been stressed to some extent and this gradual induction may explain the loss in effect seen in the 2009 field experiments. The environmental stresses of the field may also lend explanation for the differences between the results from the field and greenhouse experiments.

MeJA is known to be involved in many physiological processes, including the selective induction of glucosinolates (Doughty et al. 1995) and promotion of other secondary compound production (Bodnaryk & Palaniswamy 1990, Doughty et al. 1995, Cheong & Choi 2003, Neilsen et al. 2001). Wound-induced plants have been shown to produce volatile chemicals that have been found to be attractive to flea beetles (Fernandez & Hilker 2007) but flea beetles are also known to avoid feeding on plants that have been wounded (Fernandez & Hilker 2007, Palaniswamy & Lamb 1993). These studies suggest that glucosinolates may not be the chemicals involved in flea beetle feeding preferences.

Early work on the chemical properties of cruciferous plants found that volatile products of glucosinolates can attract flea beetles and that glucosinolates can act as feeding stimulants. Feeny et al. (1970) tested the host range of *P. cruciferae* and noted that there was a strong correlation between the plants attacked and the presence of glucosinolates. Field traps containing isothiocyanates, volatile glucosinolate products, were found to be very attractive to adult flea beetles (Feeny et al. 1992, Pivinick et al. 1992, Vincent & Stewart 1984). Hicks (1974) found that culturing bean leaves in glucosinolates would make the plants attractive and the beetles would feed on the leaves even though beans are not usually suitable hosts. She also found that increasing the concentration of the glucosinolates would increase feeding. These results involving glucosinolates, and their products, as feeding/olfactory stimulants coupled with

the studies that have shown that jasmonates can induce glucosinolate production (Bartlet et al. 1999, Bodnaryk 1994, Cheong & Choi 2003, Doughty et al. 1995, Thaler et al. 2001) suggest that MeJA-induction would produce higher levels of flea beetle feeding.

To further investigate the role of glucosinolates in the behavior of flea beetles, Bodnaryk and Palaniswamy (1990) explored how using crops with low glucosinolates would affect flea beetle feeding damage. The basis for their experiment was that since glucosinolates were thought of as feeding stimulants to crucifer specialists, then removing the glucosinolates would prevent flea beetle attack. However, their results showed that a considerable amount of flea beetle feeding occurred on naturally suitable host species, even though they had been depleted of glucosinolates. Because of these results they suggested that *P. cruciferae* would likely choose host plants based on the absence of feeding deterrents rather than the presence of glucosinolates. Other studies (Bartlet et al. 1994, Lamb 1988, Nielsen 1978) also found that the presence of stimulating glucosinolates are not always indicative of host suitability and are not solely responsible for the beetles' ability to discriminate between glucosinolate containing host plants.

Nielsen (1988) suggested that oligophagous species would discriminate between glucosinolate containing host plants by the occurrence of feeding deterrents and that monophagous species would discriminate based on feeding stimulants other than glucosinolates. Both *P. cruciferae* and *P. pusilla* are oligophagous, feeding on many types of plants in Brassicaceae and less commonly a few other plant families (Al-Doghairi 2000, Feeny et al. 1970). This would mean that the reduction in flea beetle feeding seen on the MeJA treated plants would be due to the induction of a feeding deterrent rather than changes in the levels of glucosinolates or other feeding stimulants. MeJA has been shown to induce the production of

other feeding deterrents, including proteinase inhibitors (Avdiushko et al. 1997, Casaretto et al 2004, Farmer et al. 1992) and increase lipoxygenase activity (Avdiushko et al. 1997).

Various crops can differ in their induced resistance, whether it result from wounding or jasmonate induction (Lu et al. 2004, Palaniswamy & Lamb 1993). Lu et al. 2004 reported that the more susceptible Chinese cabbage gained resistance to the diamondback moth, *Plutella xylostella* (Linnaeus), with JA applications but the more resistant cabbage became more susceptible after JA treatments. The current study also found that crops types responded differently but also found a different pattern in induced resistance. In this study the plants that seemed more resistant to flea beetle infestation during the earlier count date (broccoli, Brussels sprouts, and rutabaga) were made more resistant after the second MeJA application and the crops that seemed more susceptible during the first count date (canola, *B. carinata*, and Chinese cabbage) experienced no change in resistance from the MeJA applications. While Lu et al. (2004) found that Chinese cabbage developed a resistance to one moth they did note that the JA treatments did not seem to make the plants resistant to another moth, which was not the focus of their experiment.

The reduced numbers of flea beetles in field trials did not necessarily mean that the MeJA treated plants were fed on any less. Broccoli showed a reduction in flea beetles during both field seasons but the shothole counts in 2010 showed roughly similar amounts of feeding damage between the treatments. Brussels sprouts also showed a reduction in flea beetles numbers but variable differences in feeding damage. Rutabaga had a large difference in the number of flea beetles counted on the treatment groups and this was consistent with the difference in damage seen on the plants.

Overall, MeJA showed a reduction in flea beetle feeding on broccoli, Brussels sprouts, and rutabaga. The oilseed crops *B. carinata* and canola, as well as Chinese cabbage did not

show consistent reductions in feeding. The potential for MeJA use in pest management is also dependant on induced physiological changes that may be detrimental for the crop (Chapter 3). Crops like canola are bred for their low glucosinolate content (Bodnaryk & Palaniswamy 1990, Lamb 1988) in their seeds and the effects from MeJA may not be desirable. MeJA is also known to reduce photosynthesis and can be involved in senescence (Cheong & Choi 2003) and these effects may interfere with production. The applicability of MeJA treatments in crop production will ultimately depend on the tradeoffs between the reduction in flea beetle feeding and losses in yield or crop quality (Chapter 3).

References Cited

Al-Doghairi M.A., 2000. Pest management tactics for the western cabbage flea beetle (*Phyllotreta pusilla* Horn) on brassica crops. Doctoral dissertation, Colorado State University, Fort Collins, Colorado.

Anderson J.M., Spilatro S.R., Klauer S.F., and Franceschi V.R., 1989. Jasmonic acid-dependent increase in the level of vegetative storage proteins in soybean. Plant Science 62: 45-52.

Avdiushko S.A., Brown G.C., Dahlman D.L., and Hildebrand D.F. 1997. Methyl jasmonate exposure induces insect resistance in cabbage and tobacco. Environmental Entomology 26(3): 642-654.

Bartlet E., Kiddle, G., Williams I., and Wallsgrove R. 1999. Wound-induced increases in the glucosinolate content of oilseed rape and their effect on subsequent herbivory by a crucifer specialist. Entomologia Experimentalis et Applicata 91: 163-167.

Bartlet E., Parsons D., Williams I.H., and Clark S.J. 1994. The influence of glucosinolates and sugars on feeding by the cabbage stem flea beetle, *Psylliodes chrysocephala*. Entomologia Experimentalis et Applicata 73: 77-83.

Bodnaryk R.P. 1994. Potent effect of jasmonates on indole glucosinolates in oilseed rape and mustard. Phytochemistry 35(2): 301-305.

Bodnaryk R.P. and Palaniswamy P. 1990. Glucosinolate levels in cotyledons of mustard, *Brassica juncea* L. and rape *B. napus* L. do not determine feeding rates of flea beetle, *Phyllotreta cruciferae* (Goeze). Journal of Chemical Ecology 16(9): 2735-2746.

Bruinsma M., Van Dam N.M., Van Loon J.J.A., and Dicke M., 2007. Jasmonic acid-induced changes in *Brassica oleracea* affect oviposition preferences of two specialist Herbivores. Journal of Chemical Ecology, 33: 655-668.

Casaretto J.A., Zúñiga G.E., and Corcuera L.J., 2004. Abscisic acid and jasmonic acid affect proteinase inhibitor activities in barley leaves. Journal of Plant Physiology 161: 389-396.

Cheong J.-J. and Choi Y.D., 2003. Methyl jasmonate as a vital substance in plants. Trends in Genetics 19(7): 409-413.

Chittenden F.H. and Marsh H.O., 1920. The western cabbage flea beetle. USDA, Bulletin No. 902. pp.21

Demirel N., 2003. Intgrated pest management studies of the insects affecting oilseed brassicas in Colorado. Doctoral dissertation, Colorado State University, Fort Collins, Colorado.

Doughty K.J., Kiddle G.A., Pye B.J., Wallsgrove R.M., and Pickett J.A. 1995. Selective induction of glucosinolates in oilseed rape leaves by methyl jasmonate. Phytochemistry 38(2): 347-350.

Farmer E.E., Johnson R.R., Ryan C.A., 1992. Regulation of expression of proteinase inhibitor genes by methyl jasmonate and jasmonic acid. Plant Physiology 98: 995-1002.

Feeny P., Paauwe K.L., and Demong N.J., 1970. Flea beetles and mustard oils: Host plant specificity of *Phyllotreta cruciferae* and *P. stiolata* adults (Coleoptera: Chrysomelidae). Annals of the Entomological Society of America 63(3): 832-841.

Fernandez P. and Hilker M., 2007. Host plant location by Chrysomelidae. Basic and Applied Ecology 8: 97-116.

Finch S. and Thompson A.R., 1992. Pests of cruciferous crops, *In* McKinlay R.G. (Ed.) "Vegetable Crop Pests." CRC Press, Boca Raton, FL, pp. 87-138.

Hicks K.L., 1974. Mustard oil glucosides: Feeding stimulants for adult cabbage flea beetles, *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). Annals of the Entomological Society of America 67(2): 261-264.

Kubicka E. and Zadernowski R., 2007. Enhanced jasmonate biosynthesis in plants and possible implications for food quality, a review. Acta Alimentaria 36(4): 455-469.

Lamb R.J., 1988. Susceptibility of low- and high-glucosinolate oilseed rapes to damage by flea beetles, *Phyllotreta* spp. (Coleoptera: Chrysomelidae). The Canadian Entomologist 120: 195-196.

Loivamäki M., **Holopainen J.K., and Nerg A.** 2004. Chemical changes induced by methyl jasmonate in oilseed rape grown in the laboratory and in the field. Journal Agricultural and Food Chemistry 52: 7607-7613.

Lu Y., Liu S., Liu Y., Furlong M.J., and Zalucki M.P., 2004. Contrary effects of jasmonate treatment of two closely related plant species on attraction of and oviposition by a specialist herbivore. Ecology Letters 7:337-345.

Meuriot F., Noquet C., Avice J.-C., Volenec J.J., Cunningham S.M., Sors T.G., Caillot S., and Ourry A., 2004. Methyl jasmonate alters N partitioning, N reserves accumulation and induces gene expression of a 32-kDa vegetative storage protein that possesses chitinase activity in *Medicago sativa* taproots. Physiologia Plantarum 120: 113-123.

Nielsen J.K. 1978. Host plant selection of monophagous and oligophagous flea beetles feeding on crucifers. Ent. exp. & appl. 24: 362-369.

Nielsen J.K., Hansen M.L., Agerbirk N., Petersen B.L., Halkier B.A. 2001. Responses of the flea beetles *Phyllotreta nemorum* and *P. cruciferae* to metabolically engineered *Arabidopsis thaliana* with an altered glucosinolate profile. Chemoecology 11: 75-83.

Palaniswamy P. and Lamb R.J., 1993. Wound-induced antixenotic resistance to flea beetles, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae) in crucifers. The Canadian Entomologist 125: 903-912.

Peng C., Weiss M.J., and Anderson M.D., 1992. Flea beetle (Coleoptera: Chrysomelidae) response, feeding, and longevity on oilseed rape and crambe. Environmental Entomology 21(3): 604-609.

Pivnick K.A., Lamb R.J., and Reed D. 1992. Response of flea beetles, *Phyllotreta* spp., to mustard oils and nitriles in field trapping experiments. Journal of Chemical Ecology 18(6): 863-873.

Rasband, W.S. ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2011.

SAS software, version 9.2 of the SAS System for windows, copyright 2002-2008, SAS Institute Inc., Cary, North Carolina.

Staswick P.E., Su W., and Howell S.H., 1992. Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an *Arabidopsis thaliana* mutant. Proceedings of the National Academy of Sciences of the United States of America 89: 6837-6840.

Tahvanainen J. 1983. The relationship between flea beetles and their cruciferous host plants: the role of plant and habitat characteristics. OIKOS 40: 433-437.

Thaler J.S., Stout M.J., Karban R., and Duffey S.S. 2001. Jasmonate-mediated induced plant resistance affects a community of herbivores. Ecological Entomology. 26: 312-324.

Ueda J. and Kato J., 1982. Inhibition of cytokinin-induced plant growth by jasmonic acid and its methyl ester. Physiologia Plantarum 54: 249-252.

Vaughn T.T. and Hoy C.W., 1993. Effects of leaf age, injury, morphology, and cultivars on feeding behavior of *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). Environmental Entomology 22(2): 418-424.

Vincent C. and Stewart R.K., 1984. Effect of allyl isothiocyanate on field behavior of crucifer-feeding flea beetles (Coleoptera: Chrysomelidae). Journal of Chemical Ecology 10(1): 33-39.

Zhang P., Shu J., Fu C., Zhou Y., Hu Y., Zalucki M.P., Liu S. 2008. Trade-offs between constitutive and induced resistance in wild crucifers shown by a natural, but not an artificial, elicitor. Oecologia 157: 83-92.

CHAPTER TWO

RESPONSE OF LEPIDOPTERA LARVAE TO THE INDUCED EFFECTS OF METHYL JASMONATE APPLICATIONS ON VARIOUS CRUCIFEROUS CROPS

Introduction

Some of the more notable pests on cruciferous crops are lepidopteran pests such as the imported cabbageworm, *Pieris rapae* (Linnaeus) (Lepidoptera: Pieridae), and the cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae). Lepidopteran pests are defoliators that reduce yields indirectly by means of leaf loss. In late stages, Lepidoptera larvae can directly reduce yields by damaging marketable heads (Hern et al. 1996, Finch & Thompson 1992).

Methyl jasmonate (MeJA) and jasmonic acid (JA) are involved in the upregulation and downregulation of various processes within many types of plants. Their roles in the systemic induction of secondary compound production are of particular interest (Cheong & Choi 2003, Bartlet et al. 1999, Doughty et al. 1995, Lu et al. 2004, Thaler et al. 2001). Cruciferous crops produce a variety of distinctive secondary chemicals, particularly glucosinolates (Agrawal & Sheriffs 2001, Ciska et al. 2000, Doughty et al. 1995, Finch & Thompson 1992). Some Lepidopteran specialists have been documented to use these secondary compounds for host location (Bruce et al. 2005, Hern et al. 1996, Renwick & Chew 1994, van Loon et al. 1992) and as oviposition stimulants (Bruinsma et al. 2007, Hern et al. 1996, Renwick & Chew 1994, Renwick & Radke 1988).

These studies suggest that MeJA applications could have an effect on Lepidoptera host selection and oviposition preferences. It is thought that *P. rapae* and *T. ni*

respond to specific ratios of common plant volatiles rather than species-specific plant kairomones for host location (Bruce et al. 2005, Landolt 1989, van Loon et al. 1992). Therefore, MeJA applications could affect host location by altering the ratio of volatile chemicals given off by the host plants. Lu et al. (2004) demonstrated that the plant volatiles from cabbage and Chinese cabbage changed due to JA treatment and that this change affected the attraction of the crucifer specialist the diamondback moth *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae). However, as shown in the study by Landolt (1993) the plants to which moths are first attracted to are not necessarily the preferred host for oviposition. In that study, *T. ni* females were first attracted to injury-induced cotton plants but then preferred to oviposit on the untreated plants. Females were also more attracted to the untreated cabbage plants but there were no differences in the oviposition choices between the damaged and untreated plants. It is has been proposed that avoiding wound or jasmonate induced host plants would be advantageous to the development of the offspring since the larvae growth was slower on JA induced food (Bruinsma et al. 2007).

Lepidoptera larvae are generally restricted to the plant on which they hatched on due to limited dispersal capacity (Bruinsma et al. 2007, Hern et al. 1996, Renwick & Chew 1994) and host selection by the mother largely determines where the larvae will develop. For this reason, there has been interest in how MeJA treated hosts affect the development of Lepidoptera larvae. Lu and Liu (2005) found that JA treatments on cabbage had negative effects on the development and fecundity of *P. xylostella*. Bruinsma et al. (2007) found that rearing *Pieris rapae* larvae on JA treated Brussels sprouts increased the time between hatching and pupation compared to those reared on untreated Brussels sprouts.

Jasmonate-mediated plant responses can potentially increase feeding by some herbivores (Agrawall & Sherriffs 2001) while reducing feeding by other herbivores (Avdiushko et

al. 1997, Bartlet et al. 1999, Thaler et al. 2001). Cruciferous food crop production could potentially benefit from MeJA applications by means of deterred oviposition and reduced health of the Lepidopteran pest. The purpose of this study is to investigate how MeJA treatments on various cruciferous crops affect the oviposition preferences for a specialist and a generalist Lepidopteran pest (*P. rapae* and *T. ni*, respectively) and to compare any effects on the development of those species in the lab.

Methods and Materials

Studies were conducted during two growing seasons – 2009 and 2010. All trials that used methyl jasmonate treatments [cyclopentaneacetic acid, 3oxo-2-(2-pentenyl)-methylester Bedoukian Research Inc., 21 Finance Drive, Danbury, Connecticut 06810-4192] were applied at 14.2mM solutions. Separate solutions were made for each application date and involved 6.65 grams of 96% MeJA per 2000mL water. Solutions were mixed by agitation. This concentration of MeJA was shown to be effective at producing differences in flea beetle numbers in previous preliminary trials (Cranshaw, unpublished). All field experiments were conducted at the Colorado State University Horticulture Field Research Center (CSU HFRC), north of Fort Collins, Colorado.

Experiment 2.1. Lepidoptera Oviposition Preference, Field Study, 2010

At the CSU HFRC, cabbage (*Brassica oleracea*, Capitata group, cv. Tendersweet), a south row of Brussels sprouts (*B. oleracea*, Gemmifera group, cv. Churchill), a north row of Brussels sprouts (*B. oleracea*, Gemmifera group, cv. Churchill), broccoli (*B. oleracea,* Botrytis group, cv. Arcadia), and rutabaga (*B. napus*, Napobrassica group, cv. Helenor) were planted in single rows each. Each row was planted with 46cm spacing between plants and set up as a randomized complete block design with 4 blocks, each having 4 treatments.

There were three treatment groups in the row of cabbage: untreated, one MeJA treatment, and two MeJA treatments. There were four treatment groups for the south row of Brussels sprouts: untreated, one MeJA treatment, two MeJA treatments, and three MeJA treatments. For these two crops the first or single MeJA was applied on 10 June, the second application on 30 June, and the third application was on 21 July.

The two treatment groups for the north row of Brussels sprouts, broccoli, and rutabaga were MeJA treated and untreated. The MeJA treatments were performed on 28 June and 21 July, 2010.

All plots were evaluated by observing the presence of eggs. Evaluations of the cabbage plots were done on the 26 July and evaluations of the south row of Brussels sprout plots were done on 13 July, 26 July, and 19 August. The north row of Brussels sprouts, broccoli, and rutabaga were evaluated on the 19 August. The broccoli was also evaluated on 27 July. The evaluations on the 13 July, 26 July, and 27 July involved counting eggs on the middle 4 plants of each plot. The evaluation on the 19 August were made by counting the total number of eggs on 4 cardinal, expanded leaves on the middle 6 plants of each plot.

Experiment 2.2. Lepidoptera Development, Preliminary Lab Experiment, 2009

This experiment was conducted at the Colorado State University Insectary building. Forty (40) *P. rapae*, eggs were harvested from *Brassica* spp. at the CSU HFRC. Each egg was placed in a 100mm petri dish with moistened filter paper. The dishes were labeled with the treatment group and identification number. Petri dishes were stored in a Biotronette plant growth chamber (Lab-line Instruments Inc.) that was programmed for 14 hours of light, 10 hours of dark, and a constant 22°C.

Upon hatching, the larvae were then divided into two treatment groups and fed foliage from either untreated or MeJA treated plants. The plants used were broccoli (cv. Windsor) and

Brussels sprouts (cv. Diablo) planted at the CSU HFRC. The broccoli and Brussels sprouts were grown in single rows each with plants spaced 46cm apart. Both rows contained untreated and MeJA treated plots. MeJA applications were applied 26 May for the broccoli and 15 June for the Brussels sprouts. A variety of leaves were picked as food, ranging from newly forming leaves to more expanded leaves. Food leaves were stored in plastic bags in a refrigerator and small portions were torn off as needed.

The experiment ran from 31 August to 5 October. The dishes were checked daily for hatching, pupation, and emergences. The dishes were also regularly cleared of frass and the food was changed out as needed. Each pupa was weighed within 24 hours of pupation. Evaluations were made of time spent in the larval stage, time spent in the pupal stage, and pupal weight. Sex was not determined.

Experiment 2.3. Lepidoptera Development, Lab Experiment, 2010

An expanded rearing experiment was conducted the following season in the Colorado State University Plant Sciences building. For this trial 300 eggs from each of two species of Lepidoptera, *Trichoplusia ni* and *P. rapae,* were harvested from *Brassica* spp. at the CSU HFRC. Each egg was placed in a 100mm petri dish with moistened filter paper. Petri dishes were stored in a Sanyo MIR-554 growth chamber that was programmed for 14 hours of light and held a constant 22°C.

A row of cabbage (cv. Tendersweet) was set up as a randomized complete block design with 4 blocks, each with three treatments and the plants were spaced 46cm apart. The treatment groups were untreated, treated with MeJA once, and treated twice. The first treatment of MeJA was performed on 10 June and the second treatment was done on 30 June.

The majority of the food in this trial was harvested on 13 July due to there being some damage done by a tractor pass. As wounding is known to induce jasmonic acid production

undamaged young leaves were harvested within 15 minutes of the injury to possible wound effects. Leaves from individual plots were refrigerated in plastic bags marked with the treatment group and block.

During rearing the dishes containing individual caterpillars were checked daily. Small pieces of leaves were torn off for feeding ass needed, the size of the leaf fragments used being based on the amount of food needed by the caterpillar in a single day. The dishes were also cleared of frass and the old food was changed out as needed. A second harvest of leaves needed to be done on 17 August to allow completion of development for the remaining *T. ni* larvae.

Twenty-five individuals from each species were assigned to each treatment group within each block. The experiment ran from 13 July to 30 August. The dates for hatching, pupation, and emergence were recorded for each individual to determine differences in time spent as larvae or pupae. The *P. rapae* were sexed as adults based on the presence (females) of two black spots near the center of the dorsal side of the forewing or just a single spot (male) (Kolyer 1966). The *T. ni* were sexed as pupae following Shorey et al. (1962). Data used for evaluation included time spent in the larval stage, time during pupation, pupa weight, and sex.

Statistical Analyses

All data were analyzed using SAS software (version 9.2, copyright 2002-2008). The data from the 2010 oviposition preference trial was analyzed using an ANOVA with a SNK comparison. The data from *T. ni* on Brussels sprouts and broccoli had some evidence of unequal variances but the original data was used for the evaluation. Neither square root nor log transformations controlled the variance. When both transformations were attempted, they did not change the results compared to the analysis using the original data so Dr. Phillip Chapman (Colorado State University) recommended that the original data be used.

Data from the 2009 preliminary Lepidoptera development experiment was evaluated using a two-sample t-test without a transformation. Where there was evidence of unequal variances the values from the Satterthwaite method were reported, though it should be noted that the outcome from this method was not different from the pooled method.

Each sex of each species in the 2010 Lepidoptera development experiment was evaluated separately. Analysis composed of an unbalanced design ANOVA followed by lsmeans to compare individual differences.

Results

Experiment 2.1. Lepidoptera Oviposition Preference, Field Study, 2010

The data from the *P. rapae* egg counts on cabbage (Table 2.1), from 26 July, showed that there were significantly fewer eggs laid on cabbage plants that were untreated compared to plants treated twice with MeJA ($F=7.34$; $df=3, 6$; $p=0.0244$). Multiple applications of MeJA attracted oviposition by *P. rapae* similar to the findings of Agrawal and Sherriffs (2001).

Results for the *P. rapae* egg counts (Table 2.1) on the south row of Brussels sprouts on 13 July showed that there were no significant treatment differences in total eggs present on each plant ($F=1.35$; $df=3, 15$; $p=0.3190$). The *P. rapae* egg counts from 26 July showed that the treatment group that had three applications of MeJA had significantly fewer eggs present than both the non-treated group and the group with only one MeJA application ($F=5.24$; $df=3, 15$; $p=0.0230$). The *P. rapae* egg counts from the south row of Brussels sprouts on 19 August showed that the plots that had two MeJA applications had significantly fewer eggs present than the plots receiving either one or three applications ($F= 5.13$; $df=3, 15$; $p=0.0244$). The data from

the *P. rapae* egg counts from the north row of Brussels sprouts on 19 August showed that the number of eggs counted on the untreated plots was not different from the MeJA treated plots (F=0.76; df=1, 7; p=0.4478).

Results for the *P. rapae* egg counts (Table 2.1) on 27 July from broccoli showed that the number of eggs counted on the untreated plants was the same as the MeJA treated plants (F=3.79; df=1, 7; p=0.1468). This was also the case on 19 August (F=0.22; df=1, 7; p=0.6714).

The results from the *P. rapae* egg counts (Table 2.1) on 19 August from rutabaga showed that the number of eggs counted on the untreated plants was the same as the MeJA treated plants (F=4.12; df=;1, 7 p=0.1353). These results are only representative of one count date and more data is needed to know how *P. rapae* responds to MeJA induced rutabaga.

Table 2.1. *Pieris rapae* eggs present on various cruciferous crops treated with methyl jasmonate (14.2mM). Plots were established at the Colorado State University Horticulture Field Research Center, Fort Collins, Colorado, 2010.

		Average number of eggs per plot			
Crop	MeJA	13 July[1]	26 July[1]	27 July[1]	19 August[2]
Cabbage	No		13.25 (a)		
	1x		17.75 (a)		
	2x		24.50 (b)		
Brussels Sprouts	No	16.25 (a)	21.00 (a)		6.25 (ab)
(South row)	1x	9.25 (a)	19.00 (a)		11.00 (a)
	2x	10.50 (a)	13.75 (ab)		5.25 (b)
	3x	9.00 (a) [3]	8.50 (b)		10.50 (a)
Brussels Sprouts	No				8.75 (a)
(North row)	2x				13.50 (a)
Broccoli	No			16.25 (a)	10.25 (a)
	2x			10.50 (a)	8.75 (a)
Rutabaga	No				4.75 (a)
	2x				1.50 (a)

[1]Averages are based on the total number of eggs counted on four whole plants in each plot.
[2]Averages based on the total number of eggs counted on four expanded, cardinal leaves on the middle six plants of each plot.
[3]Counts for this date were performed before the third application of MeJA.
*Within each crop type, numbers in each column not followed by the same letter are significantly different ($p<0.05$).
**Cabbage and south row of Brussels sprouts had the first MeJA treatments on 10 June and the second treatment on 30 June. The south row of Brussels sprouts had the third application on 21 July.
***The treatment dates for the north row of Brussels sprouts, broccoli, and rutabaga were 28 June and 21 July.

The data from the 26 July *T. ni* eggs counted on cabbage (Table 2.2) showed that there were no significant differences in the number of eggs laid across all treatment groups (F=0.94; df=3, 6; p=0.4406). This suggests that multiple MeJA applications affected the choice of oviposition for the specialist species (*P. rapae*) but not the generalist (*T. ni*).

The results for the *T. ni* egg counts (Table 2.2) on 13 July from the south row of Brussels sprouts showed no significant differences in the eggs present across the treatment groups (F=0.27; df=3, 15; p=0.8460). The *T. ni* egg counts on 26 July showed that the treatment group that received three applications of MeJA had significantly fewer eggs than the untreated group (F=5.92; df=3, 15; p=0.0163). The *T. ni* egg counts on Brussels sprouts from 19 August showed that all of the treatment groups had similar numbers of eggs present for both the south row of Brussels sprouts (F=0.67; df=3, 15; p=0.5889) and the north row (F=0.33; df=1, 7; p=0.6042).

The *T. ni* egg counts (Table 2.2) on 27 July from broccoli showed that the number of eggs counted on the untreated plants was the same as the MeJA treated plants (F=3.69; df=1, 7; p=0.1505). The *T. ni* egg counts from the 19 August on broccoli also indicated that there were no differences between the untreated and MeJA treated plots (F=0.22; df=1, 7; p=0.6714).

The results from the *T. ni* egg counts (Table 2.2) on 19 August from rutabaga showed no significant difference in the number of eggs laid on MeJA treated and untreated plants (F=1.26; df=1, 7; p=0.3441). However, no eggs were noted on MeJA treated rutabaga suggesting that it may be of interest to make additional observations of MeJA treatment effects on rutabaga.

Table 2.2. *Trichoplusia ni* eggs present on various cruciferous crops treated with methyl jasmonate (14.2mM). Plots were established at the Colorado State University Horticulture Field Research Center, Fort Collins, Colorado, 2010.

		Average number of eggs per plot			
Crop	MeJA	13 July[1]	26 July[1]	27 July[1]	19 August[2]
Cabbage	No		14.25 (a)		
	1x		12.25 (a)		
	2x		8.75 (a)		
Brussels Sprouts	No	10.00 (a)	12.75 (a)		2.50 (a)
(South row)	1x	8.00 (a)	5.00 (ab)		0.50 (a)
	2x	13.00 (a)	6.75 (ab)		0.75 (a)
	3x	8.50 (a) [3]	0.75 (b)		1.00 (a)
Brussels Sprouts	No				0.75 (a)
(North row)	2x				0.25 (a)
Broccoli	No			5.00 (a)	0.00 (a)
	2x			0.75 (a)	1.75 (a)
Rutabaga	No				3.00 (a)
	2x				0.00 (a)

[1]Averages are based on the total number of eggs counted on four whole plants in each plot.
[2]Averages based on the total number of eggs counted on four expanded, cardinal leaves on the middle six plants of each plot.
[3]Counts for this date were performed before the third application of MeJA.
*Within each crop type, numbers in each column not followed by the same letter are significantly different ($p<0.05$).
**Cabbage and south row of Brussels sprouts had the first MeJA treatments on 10 June and the second treatment on 30 June. The south row of Brussels sprouts had the third application on 21 July.
***The treatment dates for the north row of Brussels sprouts, broccoli, and rutabaga were 28 June and 21 July.

Experiment 2.2. Lepidoptera Development, Preliminary Lab Experiment, 2009

The preliminary trial (Table 2.3) showed that the mean larval time for larvae reared on MeJA treated food was not significantly different from that of larvae reared on untreated food ($t=0.53$, $df=22.107$; $p=0.6038$). The mean pupal times between the larvae reared on either diet were not significantly different ($t=-0.85$; $df=33$; $p=0.4032$). However, mean pupal weight for larvae reared on the MeJA treated food was over 13% more than mean pupal weight for larvae reared on the untreated food ($t=-2.62$; $df=27.828$; $p=0.0140$).

Table 2.3. *Pieris rapae* development on field grown broccoli (cv. Windsor) and Brussels sprouts (cv. Diablo) leaves treated with methyl jasmonate (14.2mM) or untreated. Plants were grown at Colorado State University Horticulture Field Research Center, Fort Collins, Colorado, during 2009.

Food Treatment	Average larval time (days)[1]	Average pupal time (days)[1]	Average pupal weight (grams)[1]
Untreated	16.52 (a)	10.42 (a)	0.150 (a)
MeJA treated	16.19 (a)	10.88 (a)	0.171 (b)

[1]Numbers in each column not followed by the same letter are significantly different by t-test ($p<0.05$).

*Pupal weight was measured within 24 hours of pupation.

**MeJA applications were applied to the broccoli plants on 26 May and 15 June for the Brussels sprouts.

****P. rapae* eggs were collected from *Brassica* spp. at the Colorado State University Horticultural Field Research Center, Fort Collins, CO.

****Larvae were reared in petri dishes stored a Biotronette plant growth chamber, from Lab-line Instruments Inc., that was programmed for 14 hours of light, 10 hours of dark, and a constant 22°C.

Experiment 2.3. Lepidoptera Development, Lab Experiment, 2010

The data from the rearing of *P. rapae* females (Table 2.4) showed that larvae fed MeJA treated food did not produce any significant differences in the time spent in the larval stage compared to the larvae reared on untreated leaves($F= 1.59$; $df=2, 139$; $p=0.2078$). They also did not differ in time spent in the pupal stage ($F=0.46$; $df=2, 139$; $p=0.6315$) or in pupal weight ($F=0.72$; $df=2, 139$; $p=0.4895$).

The *P. rapae* males (Table 2.4) showed that being reared on MeJA treated food did not affect the overall development of the larvae. There were no significant differences in the time spent in the larval stage ($F= 1.47$; $df=2, 104$; $p=0.2337$), pupal stage ($F=1.41$; $df=2, 104$; $p=0.2482$), or in pupal weight ($F=1.90$; $df=2, 104$; $p=0.1545$) between the treatment groups.

Table 2.4. *Pieris rapae* development on field grown cabbage (cv. Tendersweet) leaves treated with methyl jasmonate (14.2mM) or untreated. Plants were grown at Colorado State University Horticulture Field Research Center, Fort Collins, Colorado, during 2010 and foliage was harvested on 13 July.

Sex	Food Treatment	Larval Time (days) [1]	Pupal time (days) [1]	Average Pupal Weight (grams) [1]
Female				
	Non-treated	16.00 (a)	8.78 (a)	0.172 (a)
	MeJA 1x	16.42 (a)	8.69 (a)	0.172 (a)
	MeJA 2x	16.32 (a)	8.80 (a)	0.168 (a)
Male				
	Non-treated	16.29 (a)	9.00 (a)	0.185 (a)
	MeJA 1x	16.48 (a)	9.09 (a)	0.183 (a)
	MeJA 2x	16.77 (a)	9.24 (a)	0.191 (a)

[1]Within each sex, numbers in the same column not followed by the same letter are significantly different ($p<0.05$) based on lsmeans.

*Pupal weight was measured within 24 hours of pupation.

**MeJA applications were applied 10 June and those plots receiving a second application were also treated on 30 June.

*** *Pieris rapae* eggs were collected from *Brassica* spp. at the Colorado State University Horticultural Field Research Center.

**** Larvae were reared in petri dishes that were stored in a Sanyo MIR-554 growth chamber that was programmed for 14 hours of light and held a constant 22°C.

The *T. ni*, females (Table 2.5) showed that there were significant differences in the length of the larval stage between the treatment groups (F=9.79; df=2, 117; p=0.0001). Larvae reared on food treated once with MeJA had larval stages lasting over one day less than the larvae reared on untreated leaves (p=0.0009) and almost 1.5 days less than those larvae reared on the leaves treated twice with MeJA (p<0.0001). The treatment applied to the food did not affect the time spent in the pupal stage (F=0.15; df=2, 117; p=0.8629). The *T. ni* females did differ in pupal weight between the treatment groups (F=7.72; df=2, 117; p=0.0007). Larvae reared on food that was treated once with MeJA weighed 7.6% more than the larvae reared on untreated food (p=0.0002). The individuals reared on food that was treated twice with MeJA weighed 4.6% more than those reared on untreated food (p=0.0117).

The *T. ni* males (Table 2.5) showed that there were significant differences in the length of the larval stage between the treatment groups (F=10.24; df=2, 135; p<0.0001). Larvae reared on untreated food spent more time in the larval stage than those reared on leaves treated once with MeJA (p=0.0305) but less than those reared on leaves treated twice with MeJA (p=0.0125). The larvae reared on leaves treated once with MeJA also had shorter larval periods compared to the larvae reared on leaves treated twice with MeJA (p<0.0001). The treatment applied to the food had a significant effect on the length of the pupal stage (F=4.08; df=2, 135; p=0.0191). Larvae reared on leaves treated twice with MeJA had significantly longer pupal stages compared to those reared on untreated leaves (p=0.005). Males did differ in pupal weight based on the treatment applied to the food (F=5.97; df=2, 135; p=0.0033). The larvae reared on the untreated weighed significantly less than the larvae reared on leaves treated with MeJA once (p=0.0111) and twice (p=0.0016). The individuals reared on food that was treated

once with MeJA weighed 4.6% more than the larvae reared on untreated food. The individuals reared on food that was treated twice with MeJA weighed 5.7% more than those reared on untreated food.

Table 2.5. *Trichoplusia ni* development on field grown cabbage (cv. Tendersweet) leaves treated with methyl jasmonate (14.2mM) or untreated. Plants were grown at Colorado State University Horticulture Field Research Center, Fort Collins, Colorado, during 2010 and foliage was harvested on 13 July and 17 August.

Sex	Food Treatment	Larval Time (days) [1]	Pupal time (days) [1]	Average Pupal Weight (grams) [1]
Female				
	Non-treated	21.09 (a)	10.78 (a)	0.237 (a)
	1x MeJA-treated	19.95 (b)	10.73 (a)	0.255 (b)
	2x MeJA-treated	21.34 (a)	10.73 (a)	0.248 (b)
Male				
	Non-treated	21.10 (a)	11.46 (a)	0.262 (a)
	1x MeJA-treated	20.32 (b)	11.58 (ab)	0.274 (b)
	2x MeJA-treated	22.01 (c)	11.75 (b)	0.277 (b)

[1]Within each sex, numbers in the same column not followed by the same letter are significantly different ($p<0.05$) based on lsmeans.
*Pupal weight was measured within 24 hours of pupation.
**MeJA applications were applied 10 June and those plots receiving a second application were also treated on 30 June.
*** *T. ni* eggs were collected from *Brassica* spp. at the Colorado State University Horticultural Field Research Center.
**** Larvae were reared in petri dishes that were stored in a Sanyo MIR-554 growth chamber that was programmed for 14 hours of light and held a constant 22°C.

Discussion

Oviposition preferences can depend on inducible chemical properties of the host plant. Plant chemicals not only act as oviposition stimulants (Bruinsma et al. 2007, Landolt 1993, Renwick & Radke 1988) but they can also express whether the plant has been damaged (Bartlet et al. 1999, Doughty et al. 1995, Thaler et al. 2001) or whether other females have oviposited on that plant (Bruinsma et al. 2007, Rothschild & Schoonhoven 1977). Agrawal and Sherriffs (2001), Bruinsma et al. (2007) and Lu et al. (2004) have shown that jasmonate-mediated induction in cruciferous crops can affect oviposition preferences. Agrawal and Sherriffs (2001) showed that feeding-induced wild radish plants, *Raphanus raphanistrum* (Linnaeus), were preferred for oviposition by *P. rapae* compared to the untreated and scissor clipped plants. The study by Bruinsma et al. (2007) was performed on a crop type that was more relevant to the current study, and they found that *P. rapae* laid fewer eggs on Brussels sprouts plants that had been treated with JA compared to the untreated plants. Lu et al. (2004) showed that *P. rapae* oviposition preferences were not affected by JA-induction in cabbage or Chinese cabbage but their results for *P. xylostella* showed that the MeJA treatments had a positive effect on the number of eggs laid on cabbage and a negative effect on the number of eggs laid on Chinese cabbage.

In the current study, *P. rapae* responded more to induced changes in the MeJA treated plants compared *T. ni*. *Trichoplusia ni* only responded to the induced changes in Brussels sprouts. Neither species showed a difference in egg numbers across any of the treatment groups for broccoli or rutabaga. MeJA treatments did affect the numbers of *P. rapae* eggs found on cabbage and Brussels sprouts. Fewer eggs were found on MeJA treated Brussels sprouts on two dates, consistent with Bruinsma et al. (2007). More *P. rapae* eggs were found on MeJA treated cabbage. This contrary to the result found in Lu et al. (2004) who reported no

differences in *P. rapae* oviposition preferences on cabbage. This discrepancy might be due to differences in methods. In their experiment the greenhouse grown plants were only exposed to herbivores in the field for four days immediately following JA treatment, whereas the current study had field grown plants exposed to herbivores for over three weeks after the last MeJA application. However, their results for *P. xylostella* were similar to the findings for *P. rapae* in the current study.

In the current study the only significant differences in eggs numbers seen for *T. ni* was higher numbers found untreated Brussels sprouts. This was consistent with a previous study that investigated the oviposition preferences involving plants with conspecific larvae. Landolt (1993) found that *T. ni* preferred to not oviposit on plants that contained conspecific larvae and also found that there were no preferences between damaged plants and plants containing conspecific larvae. Both studies show that *T. ni* avoids ovipositing on plants that have induced defenses.

The current study supports the findings from Lu et al. (2004) in that inducible plant reactions could make some plants more susceptible to oviposition while making other plants more resistant. This is likely because different crop types have differing levels of constitutive chemicals (Ciska et al. 2000, Lu et al. 2004) and MeJA selectively induces certain secondary chemicals that can play a role in host selection (Doughty et al. 1995). The value of MeJA applications for deterring oviposition by particular Lepidopteran pests is dependent on the species you are trying to defend against and the crop type you are treating.

MeJA applications on cabbage have been shown to attract one Lepidopteran pest while deterring another and therefore may not be an appealing pest management technique. On Brussels sprouts, there were generally more eggs laid on the untreated plants for both pests and

therefore MeJA may have potential on this crop. Data from earlier in the season needs to be collected for broccoli and rutabaga in order understand the effects of MeJA applications on these crops.

Another aspect that might have influenced host selection but was not specifically investigated in the current study is the effects of MeJA on the photosynthetic ability of the plant. Jasmonates decrease expression of certain genes involved in photosynthesis and can reduce the amount of chlorophyll in the leaves of plants (Cheong & Choi 2003, Creelman & Mullet 1997). Color and reflectance have been shown to be important to *P. rapae* females when deciding whether to land on a potential host plant (Renwick and Radke 1988).

Bruinsma et al. (2007) found that *P. rapae* larvae reared on JA-treated Brussels sprouts had longer larval stages without any increase in pupal weight. This means that the insect took longer to develop without experiencing any advantages of being able to feed for a longer period. In 2009, *P. rapae* larvae reared on MeJA treated food experienced higher pupal weights without longer larval periods. This could result from increased food consumption or perhaps from differing nutritional value in the MeJA treated plants such as protein (Anderson et al. 1989). In 2010, *P. rapae* larvae reared on untreated and MeJA treated food experienced no differences in development. The main difference between Bruinsma et al. (2007) and the current study is that the plants used (2007) were greenhouse grown versus field grown respectively. The field grown plants used in the current study would have been exposed to herbivores and other stressors, while the untreated plants in the Bruinsma et al. (2007) study would likely have been less induced by external factors. This would result in both food types (untreated and MeJA treated) in the current study having greater induction of defensive compounds compared to the untreated plants in Bruinsma et al. (2007).

The 2010 rearing experiment did not replicate the results from the 2009 preliminary experiment (Table 2.3). This may have been due to differences in the consistency of the quality of food provided to the developing larvae. Food provided in 2009 was picked on multiple dates and was comprised of two crop types rather than the single picking of a single crop.

While jasmonates can induce a response similar to herbivory, JA is an endogenous regulator involved in plant growth and development (Lu et al. 2004), therefore it may be inducing physiological changes that herbivory does not. This might allow differences to arise between the herbivory-induced plants (the untreated group) and the herbivory and MeJA-induced plants. The results from the current study, particularly those from 2010, are more consistent with what could be predicted from the results from the Broadway (1995) study. In her study, *P. rapae* showed resistance to the effects of proteinase inhibitors in cabbage, which are thought to protect plants from herbivores and have also been shown to be induced by jasmonates (Casaretto et al. 2004). This would suggest that *P. rapae* would be resistant to some jasmonate-induced defenses and this would explain why the larvae were able to develop on MeJA treated food without any adverse effects.

In the current study, *T. ni* larvae had a much different developmental response to the MeJA treated food compared to *P. rapae*. When reared on MeJA treated cabbage leaves *T. ni* showed evidence for longer larval periods, males experienced longer pupal periods, and the pupal weights were higher in the treatment groups that had 2 MeJA applications. This would suggest that *T. ni* experienced an increase in growth in response to rearing on induced food. This finding in not consistent with the findings from Broadway (1995) that found that *T. ni* larvae weighed less when reared on an artificial diet containing trypsin proteinase inhibitors, compounds found to be jasmonate-inducible in some plants (Casaretto et al. 2004, Farmer et al. 1992). The current findings are also not consistent with Avdiushko et al. (1997) that found that

treating cabbage with MeJA reduced the feeding of *T. ni*. Differences in methods may help explain the discrepancies in the results between these studies and the present study. Broadway (1995) used an artificial diet in their experiment and Avdiushko et al. (1997) used plants grown in a greenhouse, as opposed to the field grown plants used in the current study. This means that the other experiments had untreated checks that were not exposed to herbivores and environmental stresses found under file conditions. In the current study, both the untreated plants and the MeJA treated plants may have had some level of jasmonate-induction that could have been affecting the developing larvae.

The MeJA treatments may have also induced an increase in the production of other nutrients that herbivory could not, such as an increase in vegetative storage proteins (Anderson et al 1989). If this was the case, then both groups of larvae may have experienced negative effects from the induced toxins but the group that was reared on MeJA treated food may have been eating other induced nutrients that allowed them to weigh more. Another aspect that differed was that Avdiushko et al (1997) only allowed the larvae to feed on the experimental diet for 4 days. In contrast the current study had full rearing (hatching through pupation) of the larvae on MeJA treated leaves.

Although there were differences in the numbers of *P. rapae* eggs found on MeJA induced plants (Table 2.1) there were no differences in the development of the larvae. This differs from the findings of Bruinsma et al. (2007) that suggested avoiding jasmonate induced food is adaptive since they documented an increase in the larval period when larvae were reared on jasmonate-induced cabbage. In contrast, *T. ni* demonstrated diminished reaction to induced plant changes during oviposition but did experience potential benefits during development.

Overall, MeJA in field applications to brassicas had various effects on the oviposition preferences of *P. rapae* and *T. ni*. Its capacity to deter oviposition was dependant on crop type and may even make the crop more attractive to oviposition. While in previous studies, jasmonate-induced food produced negative effects on the development of *P. rapae* when the plants were grown in a lab setting, these results were not attained when the plants were grown in the field. Previous studies showed that lab grown food treated with MeJA reduced the feeding but when the food was grown in the field *T. ni* experienced a potential benefit from the MeJA treated diet in that they weighed more at the time of pupation. Exogenously applied MeJA in the field had negligible effects on Lepidoptera; however it may be worth pursuing its use for other pests or other crop varieties. The value of MeJA for managing lepidopterous pests in cruciferous crops was found to be marginal in this study.

References Cited

Agrawal A.A., Sherriffs M.F., 2001. Induced plant resistance and susceptibility to late-season herbivores of wild radish. Annals of the Entomological Society of America, 94(1): 71-75.

Anderson J.M., Spilatro S.R., Klauer S.F., and Franceschi V.R., 1989. Jasmonic acid-dependent increases in the level of vegetative storage proteins in soybean. Plant Science, 62: 45-52.

Avdiushko S.A., Brown G.C., Dahlman D.L., and Hildebrand D.F., 1997. Methyl jasmonate exposure induces insect resistance in cabbage and tobacco. Environmental Entomology, 26(3): 642-654.

Bartlet E., Kiddle, G., Williams I., and Wallsgrove R. 1999. Wound-induced increases in the glucosinolate content of oilseed rape and their effect on subsequent herbivory by a crucifer specialist. Entomologia Experimentalis et Applicata. 91: 163-167.

Broadway R.M., 1995. Are insects resistant to plant proteinase inhibitors? Journal of Insect Physiology, 41(2): 107-116

Bruce T.J.A., Wadhams L.J., and Woodcock C.M., 2005. Insect host location: a volatile situation. Trends in Plant Science, 10(6): 269-274.

Bruinsma M., Van Dam N.M., Van Loon J.J.A., and Dicke M., 2007. Jasmonic acid-induced changes in *Brassica oleracea* affect oviposition preferences of two specialist Herbivores. Journal of Chemical Ecology, 33: 655-668.

Capinera J.L., October, 1999. "Featured Creatures." University of Florida, revised November 2005. Retrieved from http://entnemdept.ufl.edu/creatures/veg/leaf/cabbage_looper.htm.

Casaretto J.A., Zúñiga G.E., and Corcuera L.J., 2004. Abscisic acid and jasmonic acid affect proteinase inhibitor activities in barley leaves. Journal of Plant Physiology, 161: 389-396.

Cheong J.-J. and Choi Y.D., 2003. Methyl jasmonate as a vital substance in plants. Trends in Genetics 19(7): 409-413.

Ciska E., Martyniak-Przybyszewska B., and Kozlowska H., 2000. Content of glucosinolates in cruciferous vegetables grown at the same site for two years under different climatic conditions. Journal of Agricultural and Food Chemistry. 48: 2862-2867.

Creelman R.A. and Mullet J.E., 1997. Biosynthesis and action of jasmonates in plants. Annual Review of Plant Physiology and Plant Molecular Biology, 48: 355-381.

Doughty K.J., Kiddle G.A., Pye B.J., Wallsgrove R.M., and Pickett J.A. 1995. Selective induction of glucosinolates in oilseed rape leaves by methyl jasmonate. Phytochemistry 38(2): 347-350.

Farmer E.E., Johnson R.R., Ryan C.A., 1992. Regulation of expression of proteinase inhibitor genes by methyl jasmonate and jasmonic acid. Plant Physiology 98: 995-1002.

Finch S. and Thompson A.R. 1992. Pests of cruciferous crops, In McKinlay R.G. (Ed.) Vegetable Crop Pests. MacMillan Press, London, England, pp. 87-138.

Hern A., Edwards-Jones G., and McKinlay R.D., 1996. A review of the pre-oviposition behavior of the small cabbage white butterfly, *Pieris rapae* (Lepidoptera: Pieridae). Annals of Applied Biology, 128(2): 349-370.

Kolyer J.M., 1966. The effect of certain environmental factors and chemicals on the markings of *Pieris rapae* (Pieridae). Journal of the Lepidopterists' Society, 20(1): 13-27.

Landolt P.J., 1989. Attraction of the cabbage looper to host plants and host plant odor in the laboratory. Entomologia Experimentalis et Applicata 53: 117-124.

Landolt P.J., 1993. Effects of host plant leaf damage on cabbage looper moth attraction and oviposition. Entomologia Experimentalis et Applicata 67: 79-85.

Lu Y., Liu S., Liu Y., Furlong M.J., and Zalucki, M.P., 2004. Contrary effects of jasmonate treatment of two closely related plant species on attraction of and oviposition by a specialist herbivore. Ecology Letters 7:337-345.

Lu Y. and Liu S., 2005. Effects of exogenous jasmonic acid induced plant responses on development and growth of *Plutella xylostella* . Chinese Journal of Applied Ecology, 16(1): 193-195.

Meuriot F., Noquet C., Avice J.-C., Volenec J.J., Cunningham S.M., Sors T.G., Caillot S., and Ourry A., 2004. Methyl jasmonate alters N partitioning, N reserves accumulation and induces gene expression of a 32-kDa vegetative storage protein that possesses chitinase activity in *Medicago sativa* taproots. Physiologia Plantarum 120: 113-123.

Renwick J.A.A. and Chew F.S., 1994. Oviposition behavior in Lepidoptera. Annual Review of Entomology, 39: 377-400.

Renwick J.A.A. and Radke C.D., 1988. Sensory cues in host selection for oviposition by the cabbage butterfly, *Pieris rapae*. Journal of Insect Physiology, 34(3): 251-257.

Rothschild M. and Schoonhoven L.M., 1977. Assessment of egg load by *Pieris brassicae* (Lepidoptera: Pieridae). Nature, 266: 352-355.

SAS software, version 9.2 of the SAS System for windows, copyright 2002-2008, SAS Institute Inc., Cary, North Carolina.

Shorey H.H., Andres L.A., and Hale Jr. R.L., 1962. The biology of *Trichoplusia ni* (Lepidoptera: Noctuidae). I. Life history and behavior. Annals of the Entomological Society of America. 55: 591-597.

Staswick P.E., Su W., and Howell S.H., 1992. Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an Arabidopsis thaliana mutant. Proceedings of the National Academy of Sciences of the United States of America 89: 6837-6840.

Thaler J.S., Stout M.J., Karban R., and Duffey S.S. 2001. Jasmonate-mediated induced plant resistance affects a community of herbivores. Ecological Entomology 26: 312-324.

Ueda J. and Kato J., 1982. Inhibition of cytokinin-induced plant growth by jasmonic acid and its methyl ester. Physiologia Plantarum 54: 249-252.

van Loon J.J.A., Frentz W.H., and van Eeuwijk F.A. 1992. Electroantennogram responses to plant volatiles in two species of *Pieris* butterflies. Entomologia Experimentalis et Applicata 62: 253-260.

CHAPTER THREE

VARIOUS RESPONSES OF CRUCIFER CROPS TO THE EFFECTS OF METHYL JASMONATE APPLICATIONS BEING APPLIED TO DETER PESTS

Introduction

Cruciferous crops (e.g. canola, mustards, cabbages, collards, and broccoli) are characterized by their secondary chemicals, mainly glucosinolates, which give this group their characteristic flavors (Finch & Thompson 1992, Macleod 1976). While these compounds deter some insects from feeding, other insects specialize on plants that contain these compounds, often using them for host selection (Finch & Thompson 1992, Fernandez & Hilker 2007, Nielsen 1988, Renwick & Chew 1994).

Flea beetles (*Phyllotreta* spp.) (Coleoptera: Chrysomelidae) and several species of Lepidoptera are some of the more notable pests of crucifers grown in northern Colorado (Al-Doghairi 2000, Chittenden & Marsh 1920, Demirel 2003). Pest management methods vary based on the target pests, though chemical control is most commonly applied (Finch & Thompson 1992). Host plant resistance is another area of interest and has had various levels of success against aphids, lepidopterous pests, and flea beetles (Al-Doghairi 2000, Anderson et al. 1992, Demirel 2003, Finch & Thompson 1992, Lamb 1988, Putnam 1977).

Methyl jasmonate (MeJA) and jasmonic acid (JA) can systemically induce the production of secondary chemicals in a way similar to wounding, thereby inducing plant resistance to various herbivores (Avdiushko et al. 1997, Bartlet et al. 1999, Bodnaryk 1994, Doughty et al. 1995, Thaler et al. 2001). For this reason, MeJA applications may have potential for use as a

pest management technique. However, MeJA is also known to be involved in many other physiological processes within plants and therefore could induce other changes on the plants that may or may not be beneficial to crop production.

Jasmonates have been shown to be involved in seed germination, growth, senescence, secondary metabolism, cell wall formation, nitrogen accumulation, and the production of proteins involved in photosynthesis (Cheong & Choi 2003, Creelman & Mullet 1997, Koda 1992, Ueda & Kato 1982). There is also evidence that various cultivars respond differently to jasmonate induction (Zhang et al. 2008). Therefore it is important to research the applicability of MeJA as a pest control method on a case by case basis. The purpose of this study is to quantify various effects of jasmonate induction on crop growth in a field study on a variety of commonly grown cruciferous crops.

Methods and Materials

Studies were conducted during two growing seasons – 2009 and 2010. All trials that used methyl jasmonate treatments [cyclopentaneacetic acid, 3oxo-2-(2-pentenyl)-methylester, Bedoukian Research Inc., 21 Finance Drive, Danbury, Connecticut 06810-4192] were applied at 14.2mM solutions. Separate solutions were made for each application date and involved 6.65 grams of 96% MeJA per 2000mL water. Solutions were mixed by agitation. This concentration of MeJA was shown to be effective at producing differences in flea beetle numbers in previous preliminary trials (Cranshaw, unpublished). All field experiments were conducted at the Colorado State University Horticulture Field Research Center (CSU HFRC), north of Fort Collins, Colorado.

Experiment 3.1. Plant Growth and Production Field Study, 2009

On 29 May, broccoli (*Brassica oleracea*, Botrytis group, cv. Windsor) and Chinese cabbage (*B. rapa,* Pekinensis group, cv. Tall Michihili) were transplanted in single rows with the

plants spaced 46cm. Each row was divided into a randomized complete block with four replications of six treatments: one MeJA application; two MeJA applications; insecticide treated; insecticide and one MeJA treatment; insecticide and two MeJA treatments; and untreated. All plots that were treated with MeJA were treated prior to transplanting, on 26 May. Those plots that had a second MeJA treatment were treated again on 23 June. Insecticide treatments were a drench application of Admire Pro (imidacloprid) on 26 May and a foliar application of Leverage (cyfluthrin, imidacloprid) on 15 June.

On 5 June, Brussels sprouts (*B. oleracea*, Gemmifera group, cv. Diablo) were transplanted in two rows with the plants in each row spaced 46cm apart and the rows were staggered. The Brussels sprouts were divided into a randomized design with four replications of six treatments. The treatments were: one MeJA treatment; two MeJA treatments; insecticide treated; insecticides and one MeJA treatment; insecticide and two MeJA treatments; and untreated. The plots extended across both rows. The first or single application of MeJA and insecticide were performed on 15 June. Those plots receiving a second treatment of MeJA dwere also treated on 12 July. Foliar insecticide applications (imidacloprid) were applied to the Brussels sprouts prior to transplanting and on 12 July.

Plant size evaluations of the broccoli and Chinese cabbage plots were made by measuring the height of the plants from soil level to the highest leaf on the middle four plants in each plot and by measuring the leaf to leaf diameter of the plants at its widest point. These measurements were performed on 8 July. The Brussels sprouts plots were evaluated on 8 July by measuring the height of the plants from soil level to the highest leaf and by measuring the leaf to leaf diameter of the plants at its widest point on the middle two plants in each row, for a total of four plants measured per plot.

Yield evaluations were only performed on broccoli and Chinese cabbage. Evaluations on the broccoli were made by measuring the average weight of all harvested heads from the each plot on a rolling harvest, only harvested once the head was ready. To standardize harvest throughout the trial the same person determined when each head was ready for harvest based on the tightness of the florets. Some of the plants had bolted before harvest began and therefore a standard number of heads could not be sampled from each plot. The harvest dates for broccoli were 28 July, 31 July, 4 August, 10 August, and 21 August. Evaluations on the Chinese cabbage were made by measuring the total weight of five harvested head from the middle five plants in each plot. These measurements were performed on 31 July.

Experiment 3.2. Harvest Field Study, 2010

On 24 June, broccoli (cv. Arcadia), rutabaga (*B. napus*, Napobrassica group, cv. Helenor), and Chinese cabbage (cv. Bilko) were transplanted at a 46cm spacing in single rows each. Each row was set up as a randomized complete block design with 4 blocks and 4 treatments. The treatment groups were: MeJA treated; MeJA and insecticide treated; insecticide treated; and untreated. The MeJA treatments were performed on 28 June and 21 July, 2010. The imidacloprid applications were done on 14 July.

Evaluations on the broccoli were made by measuring the average weight of five heads harvested from healthy plants in the middle of each plot. Plants adversely affected by infestations of the cabbage aphid were excluded. The broccoli heads were harvested biweekly ranging from 8 September to 4 October each head was cut by the same person to standardize harvest. The harvester cut the stalks just below the base of all the florets that were part of the crown of the head. The average number of days from the start of harvest to the date that the heads were actually harvested was also used to evaluate any delay in harvest time relating to treatment.

Evaluations on the rutabaga were made by measuring the average weight of five rutabagas harvested from the middle five plants in each plot. On 20 September, a single person determined where to cut off the plant tops and how much of the roots needed to be removed for each harvested rutabaga. They were cleared of mud and washed to prevent the soil from adding weight to the rutabagas.

Evaluations on the Chinese cabbage were made by measuring the average weight of five heads harvested from the middle five plants in each plot. On 8 September, a single person harvested all of the heads by cutting them at the base and removing loose wrapper leaves considered not to belong to the head.

Experiment 3.4. Protein and Antioxidant Content Study, 2010

Broccoli (cv. Arcadia), rutabaga (cv. Helenor), and Chinese cabbage (cv. Bilko) were planted at a 46cm spacing in single rows each. Each row was set up as a randomized complete block design with 4 blocks and 4 treatments each. The treatment groups were: MeJA treated; MeJA and insecticide treated; insecticide treated; and no treatment. The MeJA treatments were performed on 28 June and 21 July, 2010. The imidacloprid applications were done on 14 July, 2010.

The broccoli was harvested on a biweekly, rolling harvest from 8 September to 4 October. Florets from at least three of the heads from each plot were trimmed to make a combined sample. Rutabagas were harvested on 20 September. A square core, approximately two cm wide, was cut from each harvested rutabaga and sliced to make a combined sample for each plot. Chinese cabbage heads were harvested on 8 September. The top 8 cm of each harvested head was cut off and chopped in to smaller pieces to make a combined sample for each plot. Samples were placed in a cooler immediately after harvest.

A portion of each sample was taken to the Colorado State University Soil, Water and Plant Testing Laboratory to determine the percent protein in each sample. Evaluations were made by comparing the percent protein of each sample.

For antioxidant analysis the samples were weighed, freeze dried, weighed again, ground with a pestle, and transferred to a 15ml centrifuge tube. The samples were analyzed by Tatianna Zuber and Dr. Cecil Stushnoff's lab at Colorado State University. The total phenolics were used to represent the antioxidant levels and were determined using the Folin-Ciocalteu method (Singelton et al. 1999). Folin-Ciocalteu reagent was used to oxidize the extracts and sodium carbonate was used to neutralize the reaction. The absorbance was measured at 760nm. Galic acid (GAE) was used as a standard (concentration 2.5-50 mg/L) to determine the mg GAE/L of extract and a standard curve was obtained. Total levels of phenolics were given as mgGAE/100g fresh weight.

Statistical Analysis

All data were analyzed using SAS software (version 9.2, copyright 2002-2008). Data from the 2009 plant size experiment were evaluated using an ANOVA followed by an SNK comparison. The measurements for the four plants from each plot were averaged for the analysis.

The data from the 2009 broccoli harvest experiment were evaluated using two different ANOVA tests each followed by a SNK pairwise comparison. The first analysis was used to determine the effect of treatment on weight. The second analysis was used to determine the effects of treatment on the harvest date. The data from the 2009 Chinese cabbage harvest experiment were evaluated with an ANOVA followed by a SNK pairwise comparison.

The data from the 2010 harvest experiment for were evaluated with an ANOVA and a SNK comparison. The broccoli had two analyses performed to test for the effect of treatment

on both harvest weight and harvest date. The data from the 2010 protein content and antioxidant experiments were evaluated with individual ANOVA and a SNK tests to determine differences between treatments.

Results

Experiment 3.1. Plant Growth and Production Field Study, 2009

The broccoli plant size measurements (Table 3.1) showed that treatment was a significant factor in both height ($F=21.13$; $df=5,15$; $p<0.0001$) and diameter ($F=16.32$; $df=5,15$; $p<0.0001$). The smallest plants were those treated with two applications of MeJA and they were 44% smaller than the plants treated only with insecticide. The plants treated with two applications of MeJA were 36% smaller than the untreated plants.

The Chinese cabbage plant size measurements (Table 3.1) showed that treatment was a significant factor in both height ($F=9.82$; $df=5,15$; $p=0.0003$) and diameter ($F=9.33$; $df=5,15$; $p=0.0003$). The general trend observed in the plant size of Chinese cabbage was that the plots treated with insecticides were larger, in both height and diameter, than the plots that were not treated with insecticides. The smallest plants, in both height and diameter, were those that received two applications of MeJA. These plants were 28% shorter than the pesticide treated plants and 16% narrower. They were also 14% shorter than the untreated plants and 9% narrower.

The Brussels sprouts plant size measurements (Table 3.1) showed that treatment was a significant factor in both height ($F=4.41$; $df=5,18$; $p=0.0085$) and diameter ($F=17.97$; $df=5,18$; $p<0.0001$). Plant diameter seemed to be more affected by treatment than plant height. For plant height, the plants that were only treated with insecticides were significantly taller than any of the other plants and 31% taller than the plants that were treated twice with MeJA. There

were no other significant differences between plant heights. Plant diameter showed the plants that received two MeJA applications were 41% narrower than the insecticide only treatment group and 27% narrower than untreated plants.

Table 3.1. Plant size measurements taken on 8 July, 2009 on various cruciferous crops treated with methyl jasmonate and/or insecticides. Plots were transplanted at the Colorado State University Horticultural Research Center, Fort Collins, Colorado, 2009.

Crop Type	Treatment	Average Plant Height (cm)	Average Plant Diameter (cm)
Broccoli[1]	Untreated	27.94 (a)	46.75 (b)
	Insecticide	31.50 (a)	57.88 (a)
	Insecticide + MeJA 1x	30.19 (a)	50.13 (b)
	Insecticide + MeJA 2x	23.38 (b)	45.19 (b)
	MeJAx1	22.00 (b)	37.38 (c)
	MeJAx2	17.63 (c)	29.81 (d)
Chinese Cabbage[1]	Untreated	28.69 (c)	58.00 (b)
	Insecticide	34.25 (a)	63.19(ab)
	Insecticide + MeJA 1x	33.06 (ab)	65.44 (a)
	Insecticide + MeJA 2x	29.94 (bc)	63.25 (ab)
	MeJAx1	27.81 (cd)	61.56 (ab)
	MeJAx2	24.44 (d)	52.56 (c)
Brussels Sprouts[2]	Untreated	20.31 (b)	35.75 (b)
	Insecticide	24.00 (a)	44.19 (a)
	Insecticide + MeJA 1x	19.63 (b)	31.75 (b)
	Insecticide + MeJA 2x	21.00 (b)	35.88 (b)
	MeJAx1	19.69 (b)	31.13 (b)
	MeJAx2	18.19 (b)	25.81 (c)

*****Within each crop type, numbers in each column not followed by the same letter are significantly different ($p<0.05$) by SNK.

**Insecticide applications consisted of Admire Pro prior to transplanting in the field and then a foliar application of Leverage 15 June.

[1] Methyl jasmonate (14.2mM) applications were performed on 26 May and the second application on 23 June.

[2] Methyl jasmonate (14.2mM) applications were performed on 15 June and the second application on 12 July.

The 2009 broccoli harvest data (Table 3.2) showed that treatment did not affect the weight of the harvested heads ($F=0.89$; $df=5,15$; $p=0.5098$). The data (Table 3.2) also showed that treatment had a significant effect on the average length of time it took, from the start of harvest on 28 July, for the heads from each treatment group to be ready for harvest ($F=162.65$; $df=5,15$; $p<0.0001$). All treatment groups were significantly different from each of the other treatment groups. The plants that were treated with MeJA only took on average 18 days longer to reach harvest maturity than those treated with insecticides. Overall, this shows that the broccoli heads from the MeJA treated plants ended up weighing the same at the time of harvest though they took longer to reach a harvestable quality compared to the untreated and insecticide treated plants.

Table 3.2. Broccoli (cv. Windsor) harvest measurements taken on a rolling harvest from plants that had been treated with methyl jasmonate and/or insecticides. Plots were transplanted at the Colorado State University Horticultural Research Center, 2009.

Treatment	Average Weight Per Head (kg)	Average Number of Days Since the Start of Harvest[1]
Untreated	1.07 (a)	13.74 (d)
Insecticide	1.53 (a)	3.34 (f)
Insecticide + MeJA 1x	1.01 (a)	9.68 (e)
Insecticide + MeJA 2x	1.23 (a)	15.78 (c)
MeJA 1x	1.41 (a)	21.54 (b)
MeJA 2x	1.30 (a)	24.00 (a)

[1]Harvest started on 28 July and ran through 21 August. Heads were harvested at maturity.
*Numbers within a column not followed by the same letter are significantly different ($p<0.05$) by SNK.
**Insecticide applications consisted of Admire Pro prior to transplanting in the field and then a foliar application of Leverage 15 June.
***Methyl jasmonate (14.2mM) applications were performed on 26 May and the second application on 23 June.

The 2009 Chinese cabbage data (Table 3.3) showed that treatment had a significant effect on the weight of the harvested heads ($F=4.97$; $df=5,15$; $p=0.007$). The plants that had been treated with insecticide only produced significantly heavier heads compared to any other treatment group. Overall, the heads harvested from the plots that received insecticide applications tended to be heavier. When insecticides were coupled with MeJA the MeJA appeared to reduce the growth or resources allocated toward the head. The plants that received a single MeJA treatment produced heads that were 31% smaller than those produced by the insecticide treated plants and 9.9% smaller than those from the untreated plants.

Table 3.3. Chinese cabbage (cv. Tall Michihili) harvest measurements taken 31 July from plants that had been treated with methyl jasmonate and/or insecticides. Plots were transplanted at the Colorado State University Horticultural Research Center, Fort Collins, Colorado, 2009.

Treatment	Average Total Weight Per 5 Heads (kg)
Untreated	8.84 (b)
Insecticide	11.57 (a)
Insecticide + MeJA 1x	9.69 (b)
Insecticide + MeJA 2x	8.97 (b)
MeJA 1x	7.96 (b)
MeJA 2x	8.12 (b)

*Numbers not followed by the same letter are significantly different ($p<0.05$) by SNK.
**Insecticide applications consisted of Admire Pro prior to transplanting in the field and then a foliar application of Leverage 15 June.
***Methyl jasmonate (14.2mM) applications were performed on 26 May and the second application on 23 June.

Experiment 3.2. Harvest Field Study, 2010

The 2010 data from the broccoli harvest (Table 3.4) showed that treatment had a significant effect on the weight of harvested broccoli heads ($F=3.94$; $df=3,9$; $p=0.0476$). The insecticide treated plants produced significantly heavier heads than the MeJA treated plants. The heads from the MeJA treated plants were 28.9% lighter than the heads grown on the insecticide treated plants. While the heads from the MeJA treated plants tended to be smaller than the heads from the untreated group, they were not significantly different in weight. These results differ from the 2009 results in that all the averages from 2010 were lower than any of the average weights from 2009 and that MeJA treatments produced smaller, rather than larger, heads than the untreated and insecticide treated groups.

The data from the 2010 broccoli harvest (Table 3.4) also showed that treatment had a significant effect on the length of time it took, from the start of harvest on 8 September, for the heads to be ready for harvest ($F=63.67$; $df=3,9$; $p<0.0001$). The insecticide treated plants and untreated heads produced mature heads the earliest. The heads from the plants that were treated with both insecticide and MeJA matured an average of 12 days after the untreated and insecticide treated plants. Plants treated only with MeJA took the longest, at an average of 19 days after the untreated and insecticide treated plants. Overall, MeJA applications significantly delayed harvest and pairing MeJA with insecticide produced heads sooner than treating with MeJA alone.

Table 3.4. Broccoli (cv. Arcadia) harvest measurements taken on a rolling harvest from plants that had been treated with methyl jasmonate and/or insecticides. Plots were transplanted at the Colorado State University Horticultural Research Center, Fort Collins, Colorado, 2010.

Treatment	Average Weight Per Head (kg)	Average Number of Days Since the Start of Harvest[1]
Untreated	0.770 (ab)	3.65 (c)
Insecticide	0.987 (a)	0.50 (c)
Insecticide + MeJA	0.768 (ab)	15.7 (b)
MeJA	0.702 (b)	22.1 (a)

[1] Harvest started on 8 September, 2010 when the first head became mature enough for harvest.
* Numbers within a column not followed by the same letter are significantly different ($p<0.05$) by SNK.
**Insecticide applications consisted of imidacloprid applications performed on 14 July.
***Methyl jasmonate (14.2mM) applications were performed on 28 June and 21 July.

The 2010 harvest data from rutabaga (Table 3.5) showed that treatment had a significant effect on the harvest weight ($F=44.88$; $df=3,9$; $p<0.0001$). Each treatment group was significantly different from each of the other groups. The insecticide treated group produced the heaviest rutabaga harvest followed by the untreated plots, followed by the insecticide and MeJA treated plots, and the MeJA treated plots producing the lightest harvest. Treating the plants with MeJA reduced the average total weight of rutabagas harvested from a plot by 58.2% from the insecticide treated plots and 44.3% compared to the untreated plots. The used of MeJA paired with insecticide applications produced significantly larger harvest than MeJA alone, but overall the use of MeJA dramatically reduced harvest in rutabaga.

Table 3.5. Rutabaga (cv. Helenor) harvest measurements taken on 20 September, 2010 from plants that had been treated with treated with methyl jasmonate and/or insecticides. Plots were transplanted at the Colorado State University Horticultural Research Center, Fort Collins, Colorado, 2010.

Treatment	Average Total Weight Per 5 Rutabagas (kg)
Untreated	5.52 (b)
Insecticide	7.35 (a)
Insecticide + MeJA	4.44 (c)
MeJA	3.08 (d)

* Numbers within a column not followed by the same letter are significantly different ($p<0.05$) by SNK.
**Insecticide applications consisted of imidacloprid applications performed on 14 July.
***Methyl jasmonate (14.2mM) applications were performed on 28 June and 21 July.

The 2010 harvest data from Chinese cabbage (Table 3.6) showed that treatment had a significant effect on the harvest weight ($F=21.49$; $df=3,9$; $p=0.0002$). The plots that received MeJA applications produced significantly less than the plots that did not receive MeJA applications, regardless of insecticide treatments. The MeJA treated plots had a total harvest weighing 33.8-47.3% less than the plots that were not treated with MeJA.

Overall, in 2010 the insecticide treated plots produced significantly more than the MeJA treated plots, across all crops. The untreated plots produced significantly more than the MeJA treated plots in rutabaga and Chinese cabbage but not in broccoli. The data from broccoli differed highly between 2009 and 2010 so more research is needed to investigate how MeJA affects production in that crop.

Table 3.6. Chinese cabbage (cv. Bilko) harvest measurements taken on 8 September, 2010 from plants that had been treated with methyl jasmonate and/or insecticides. Plots were transplanted at the Colorado State University Horticultural Research Center, Fort Collins, Colorado, 2010.

Treatment	Average Total Weight Per 5 Heads (kg)
Untreated	8.36(a)
Insecticide	9.01 (a)
Insecticide + MeJA	5.53 (b)
MeJA	4.75 (b)

* Numbers within a column not followed by the same letter are significantly different ($p<0.05$) by SNK.
**Insecticide applications consisted of imidacloprid applications performed on 14 July.
***Methyl jasmonate (14.2mM) applications were performed on 28 June and 21 July.

Experiment 3.3. Protein and Antioxidant Content Study, 2010

The 2010 protein content data from broccoli (Table 3.7) showed that treatment did not have a significant influence on the protein present in the broccoli florets ($F=2.01$; $df=3,9$; $p=0.1826$). High variation may have obscured differences. The 2010 protein content data from rutabaga (Table 3.7) showed that treatment had a significant effect on the protein levels ($F=6.55$; $df=3,9$; $p=0.0121$). The untreated plants produced roots with significantly higher levels of protein than the plants that were treated with both MeJA and insecticides. The 2010 protein content data from Chinese cabbage (Table 3.7) showed that treatment did not have a significant influence on the protein present in the cabbage head ($F=2.01$; $df=3,9$; $p=0.1826$).

Table 3.7. Protein content in harvested cruciferous crops that had been treated with methyl jasmonate and/or insecticides. Plots were transplanted at the Colorado State University Horticultural Research Center, 2010. Protein content analyses were performed by the Colorado State University Soil, Water and Plant Testing Laboratory, Fort Collins, Colorado.

Crop Type	Treatment	Protein content per sample (percent)
Broccoli[1]	Untreated	41.50 (a)
	Insecticide	41.83 (a)
	Insecticide + MeJA	37.09 (a)
	MeJA	37.51 (a)
Rutabaga[2]	Untreated	15.43 (a)
	Insecticide	13.83 (ab)
	Insecticide + MeJA	12.75 (ab)
	MeJA	14.20 (b)
Chinese Cabbage[3]	Untreated	34.35 (a)
	Insecticide	34.73 (a)
	Insecticide + MeJA	32.48 (a)
	MeJA	34.85 (a)

[1] Broccoli samples were taken as a rolling harvest (as heads matured enough for harvest) starting on 8 September. Sample for analysis consisted of florets cut from a head.
[2] Rutabaga samples were taken from on 20 September, at the time of harvest. Sample for analysis consisted of a core sample cut from a rutabaga that was sliced.
[3] Chinese cabbage samples were taken on 8 September. Sample for analysis consisted of a chopped section of the top portion of the cabbage head.
* Numbers within a column not followed by the same letter are significantly different ($p<0.05$) by SNK.
**Insecticide applications consisted of imidacloprid applications performed on 14 July.
***Methyl jasmonate (14.2mM) applications were performed on 28 June and 21 July.

The data for the antioxidant content in broccoli florets (Table 3.8) did not vary due to treatment effects based on level of GAE ($F=1.33$; $df=3,9$; $p=0.3241$). The data for the antioxidant content in rutabaga (Table 3.8) shows that treatment did not have a significant effect on the levels of GAE ($F=0.45$; $df=3,9$; $p=0.7236$). The 2010 data for the antioxidant content in Chinese cabbage heads, (Table 3.8) shows that treatment did not have a significant effect on the levels of GAE ($F=1.33$; $df=3,9$; $p=0.3241$). Variation and tissue type may have contributed to the lack of significant differences.

Table 3.8. Antioxidant content in harvested cruciferous crops that had been treated with methyl jasmonate and/or insecticides. Plots were transplanted at the Colorado State University Horticultural Research Center, 2010. Phenolic content was analyzed by Dr. Stushnoff's lab at Colorado State University, Fort Collins, Colorado.

Crop Type	Treatment	Galic Acid (mg)/ 100g Fresh Weight
Broccoli[1]	Untreated	42.11 (a)
	Insecticide	39.41 (a)
	Insecticide + MeJA	38.38 (a)
	MeJA	37.81 (a)
Rutabaga[2]	Untreated	13.58 (a)
	Insecticide	15.05 (a)
	Insecticide + MeJA	13.35 (a)
	MeJA	15.54 (a)
Chinese Cabbage[3]	Untreated	15.38 (a)
	Insecticide	16.89 (a)
	Insecticide + MeJA	18.26 (a)
	MeJA	18.18 (a)

[1] Broccoli samples were taken as a rolling harvest (as heads matured enough for harvest) starting on 8 September. Sample for analysis consisted of florets cut from a head.
[2] Rutabaga samples were taken from on 20 September, at the time of harvest. Sample for analysis consisted of a core sample cut from a rutabaga that was sliced.
[3] Chinese cabbage samples were taken on 8 September. Sample for analysis consisted of a chopped section of the top portion of the cabbage head.
* Numbers within a column not followed by the same letter are significantly different ($p<0.05$) by SNK.
**Insecticide applications consisted of imidacloprid applications performed on 14 July.
***Methyl jasmonate (14.2mM) applications were performed on 28 June and 21 July.

Discussion

Jasmonates have been reported to affect various physiological processes (Cheong & Choi 2003, Koda 1992). While their abilities to upregulate the production of secondary chemicals could make them attractive tools in pest management, their affect on plant growth is an area of concern. Dathe et al. (1981) found that JA, isolated from the pericarp of *Vicia faba* Linnaeus, inhibited growth of wheat (*Triticum aestivum* Linnaeus, cv. Hatri) seedlings. Their bioassays showed that the seedlings reached their maximum length at the same time as the control plants and therefore growth was not delayed, only reduced. This contrasts with the current study's findings that MeJA treated broccoli experienced a delay in crop maturity along with a decrease in plant size. Ueda and Kato (1982) also found that MeJA and JA at concentrations above 4.5 µM inhibited growth in radish (*Raphanus sativus* Linnaeus) cotyledons by about 10-30%. The findings of the current study showed that the size of mature plants reflected a similar decrease in growth.

Yamane et al. (1981) found that JA inhibited sheath elongation in rice seedlings (*Oryza sativa* Linnaeus). Their bioassays showed that JA also inhibited pollen germination in *Camellia sinensis* Linnaeus, as well as inhibited hypocotyl and root growth in lettuce (*Lactuca sativa* Linnaeus). Other studies have supported the finding that jasmonates inhibit root growth (Corbineau et al. 1988, Staswick et al. 1992). The current study may lend some support as well since rutabaga experienced such a large reduction in harvest weight compared to the other crops grown in the current study.

The ability of field applications of MeJA to reduce plant size was demonstrated in broccoli, Chinese cabbage, and Brussels sprouts. Chinese cabbage seemed to have the smallest reduction in plant size. Overall, the various crops responded differently to MeJA applications. MeJA was also shown to reduce yield during at least one season when applied to field grown

rutabaga, and Chinese cabbage. The data from broccoli harvest weight showed that yield was either not affected in MeJA treated plants compared to the untreated plants in both years. Broccoli also showed that the MeJA treated plants experienced a delay in harvest time.

Jasmonates have been shown to increase the accumulation of vegetative storage proteins (VSPs) in plants (Anderson 1988, Anderson et al 1989, Cheong & Choi 2003, Creelman & Mullet 1997, Koda 1992, Staswick et al. 1992). This accumulation has been shown in various plant tissues and has been considered as nitrogen storage and a potential partitioning of nitrogen under stressful conditions (Anderson 1989, Staswick 1990). The current study experienced a decrease in total protein content in rutabaga. This was not consistent with the findings from Meuriot et al. 2004 and Avice et al. 1997, that observed an increase in VSPs in taproots of jasmonate and stress induced plants. No significant differences were seen in broccoli florets and Chinese cabbage.

Another way in which MeJA could affect the quality of harvested vegetables is by changing the levels of antioxidants (Kubicka & Zadernowski 2007). Comparot et al. (2002) found that MeJA applications to canola (*Brassica napus* Linnaeus, cv. Westar) increased the enzymatic activity of antioxidants. They observed that roots and shoots differentially induced specific compounds. The three crops measured in the current study concentrated on three different parts of the plant. However, no significant differences were observed in the antioxidant levels found in the vegetable tissue from broccoli, rutabaga, or Chinese cabbage. Comparot et al. (2002) noted that there was a large increase in the protein levels and when protein content was factored into calculating the antioxidant activity, only the roots showed an increase in antioxidant activity. The factors leading to the decrease in protein content observed in rutabaga may have impeded any increase in antioxidant activity in that crop.

Overall, MeJA treated broccoli experienced no reduction in yield compared to the untreated plants and experienced a relatively consistent reduction in flea beetle infestations (Chapter 1). This would suggest that MeJA could offer some pest control without a significant yield loss in cases when pesticides are not used. Yield reduction in broccoli was only observed when compared to the pesticide treated plots (2010), not the untreated plots. MeJA could have potential as an integrated pest management technique in areas where flea beetle infestations are severe enough to reduce broccoli yield to an extent greater than the yield loss expected from MeJA applications.

Another aspect that may be worth pursuing is whether MeJA-induced increases in secondary chemicals can aid in biofumigation. The products from the breakdown of glucosinolates can have fungistatic qualities. Broccoli is an attractive option as a rotation crop due to its resistance to Verticillium wilt (Bhat & Subbararao 2001). MeJA could increase the quantities of glucosinolates in broccoli foliage, potentially making the broccoli plants a more effective biofumigant while providing pest management for broccoli production.

While rutabaga experienced a large reduction in flea beetle infestations after MeJA treatment (Chapter 1), MeJA is not an attractive option for pest management due to the severe reduction in yield following its use. MeJA is also not likely to be considered in the case of Chinese cabbage as this crop experienced did not experience significant a reduction in flea beetle feeding (Chapter 1) and there was some evidence that it may reduce yield.

References Cited

Al-Doghairi M.A., 2000. Pest management tactics for the western cabbage flea beetle (*Phyllotreta pusilla* Horn) on brassica crops. Doctoral dissertation, Colorado State University, Fort Collins, Colorado.

Anderson J.M., 1988. Jasmonic acid-dependent increases in the level of specific polypeptides in soybean suspension cultures and seedlings. Journal of Plant Growth Regulation 7: 203-211.

Anderson J.M., Spilatro S.R., Klauer S.F., and Franceschi V.R., 1989. Jasmonic acid-dependent increase in the level of vegetative storage proteins in soybean. Plant Science 62: 45-52.

Avdiushko S.A., Brown G.C., Dahlman D.L., and Hildebrand D.F. 1997. Methyl jasmonate exposure induces insect resistance in cabbage and tobacco. Environmental Entomology 26(3): 642-654.

Avice J.C., Ourry A., Lemaire G., Volenec J.J., and Boucaud J., 1997. Root protein and vegetative storage protein are key organic nutrients for alfalfa shoot regrowth. Crop Science 37(4): 1187-1193.

Bartlet E., Kiddle, G., Williams I., and Wallsgrove R. 1999. Wound-induced increases in the glucosinolate content of oilseed rape and their effect on subsequent herbivory by a crucifer specialist. Entomologia Experimentalis et Applicata 91: 163-167.

Bhat R.G. and Subbarao K.V., 2001. Reaction of broccoli to isolates of *Verticillium dahliae* from various hosts. Plant Disease 85(2): 141-146.

Bodnaryk R.P. 1994. Potent effect of jasmonates on indole glucosinolates in oilseed rape and mustard. Phytochemistry 35(2): 301-305.

Cheong J.-J. and Choi Y.D., 2003. Methyl jasmonate as a vital substance in plants. Trends in Genetics 19(7): 409-413.

Chittenden F.H. and Marsh H.O., 1920. The western cabbage flea beetle. USDA, Bulletin No. 902. pp.21.

Comparot S.M., Graham C.M., and Reid D.M., 2002. Methyl jasmonate eleicits a differential antioxidant response in light- and dark-grown canola (*Brassica napus*) roots and shoots. Plant Growth Regulation 38: 21-30.

Corbineau F., Rudnicki R.M., and Côme D., 1988. The effects of methyl jasmonate on sunflower (*Helianthus annuus* L.) seed germination and seedling development. Plant Growth Regulation 7: 157-169.

Creelman R.A. and Mullet J.E., 1997. Biosynthesis and action of jasmonates in plants. Annual Review of Plant Physiology and Plant Molecular Biology, 48: 355-381.

Dathe W., Ronsch H., Preiss A., Schade W., Sembdner G., and Schreiber K., 1981. Endogenous plant hormones of the broad bean, Vicia faba L. (-)-jasmonic acid, a plant growth inhibitor in pericarp. Planta 153: 530-535.

Demirel N., 2003. Intgrated pest management studies of the insects affecting oilseed brassicas in Colorado. Doctoral dissertation, Colorado State University, Fort Collins, Colorado.

Doughty K.J., Kiddle G.A., Pye B.J., Wallsgrove R.M., and Pickett J.A. 1995. Selective induction of glucosinolates in oilseed rape leaves by methyl jasmonate. Phytochemistry 38(2): 347-350.

Fernandez P. and Hilker M., 2007. Host plant location by Chrysomelidae. Basic and Applied Ecology 8: 97-116.

Finch S. and Thompson A.R., 1992. Pests of Cruciferous Crops, *In* McKinlay R.G. (Ed.) "Vegetable Crop Pests." CRC Press, Boca Raton, Florida, pp. 87-138.

Koda Y., 1992. The role of jasmonic acid and related compounds in the regulation of plant development. International Review of Cytology 135: 155-199

Kubicka E. and Zadernowski R., 2007. Enhanced jasmonate biosynthesis in plants and possible implications for food quality, a review. Acta Alimentaria 36(4): 455-469.

Lamb R.J., 1988. Susceptibility of low- and high-glucosinolate oilseed rapes to damage by flea beetles, *Phyllotreta* spp. (Coleoptera: Chrysomelidae). The Canadian Entomologist 120: 195-196.

Macleod A.J., 1976. Volatile flavour compounds of the cruciferae. *In* Vaughn J.G., Macleod A.J., and Jones B.M.G. Eds. "The biology and chemistry of the Cruciferae." Academic Press, New York, New York, pp. 307-340.

Meuriot F., Noquet C., Avice J.-C., Volenec J.J., Cunningham S.M., Sors T.G., Caillot S., and Ourry A., 2004. Methyl jasmonate alters N partitioning, N reserves accumulation and induces gene expression of a 32-kDa vegetative storage protein that possesses chitinase activity in *Medicago sativa* taproots. Physiologia Plantarum 120: 113-123.

Putnam L.G., 1977. Response of four Brassica seed crop species to attack by the crucifer flea beetle, *Phyllotreta cruciferae*. Canadian Journal of Plant Science 57: 987-989.

Renwick J.A.A. and Chew F.S., 1994. Oviposition behavior in Lepidoptera. Annual Review of Entomology, 39: 377-400.

SAS software, version 9.2 of the SAS System for windows, copyright 2002-2008, SAS Institute Inc., Cary, North Carolina.

Singleton V.L., Orthofer R., Lamuela-Raventos R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In Oxidants and Antioxidants, Pt A, 299:152-178. Academic Press Inc., San Diego, California.

Staswick P.E., 1990. Novel regulation of vegetative storage protein genes. The Plant Cell 2: 1-6.

Staswick P.E., Su W., and Howell S.H., 1992. Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an Arabidopsis thaliana mutant. Proceedings of the National Academy of Sciences of the United States of America 89: 6837-6840.

Thaler J.S., Stout M.J., Karban R., and Duffey S.S. 2001. Jasmonate-mediated induced plant resistance affects a community of herbivores. Ecological Entomology. 26: 312-324.

Ueda J. and Kato J., 1982. Inhibition of cytokinin-induced plant growth by jasmonic acid and its methyl ester. Physiologia Plantarum 54: 249-252.

Yamane H., **Takagi H., Abe H., Yokota T., and Takahashi N.,** 1981. Identification of jasmonic acid in three species of higher plants and its biological activities. Plant and Cell Physiology 22(4): 689-697.

Zhang P., **Shu J., Fu C., Zhou Y., Hu Y., Zalucki M.P., Liu S.** 2008. Trade-offs between constitutive and induced resistance in wild crucifers shown by a natural, but not an artificial, elicitor. Oecologia 157: 83-92.

CHAPTER FOUR

FORMATION OF A MORPHOLOGICAL DESCRIPTION OF THE SEXUAL GENERATION OF *DISHOLCASPIS QUERCUSMAMMA* (WALSH)

Introduction

The rough bulletgall wasp, *Disholcaspis quercusmamma* (Walsh), is a cynipid wasp (Hymenoptera: Cynipidae: Cynipini) that parasitizes bur oak, *Quercus macrocarpa* Michx, and swamp white oak, *Quercus bicolor* Willd (Basset 1890, Beutenmüller 1909, Eckberg 1993, Krombein et al. 1979). This species was originally described by Walsh (1869) within the genus *Cynips* Linnaeus. Previous work on this species has concentrated on the agamic females (Krombein et al. 1979) that are known to produce conspicuous twig galls in autumn. The asexual generation wasps form inside single larval cell galls that are rounded, pointed at the apex, and mature to have a woody texture. The galls often occur in clusters and in dense infestations can completely encompass newly maturing twigs (Basset 1890, Beutenmüller 1909, Eckberg 1993).

In 1881, Adler found that some cynipids alternate between asexual and sexual generations (Melika & Abrahamson 2000). This discovery was found after the original description of *D. quercusmamma* and until now a formal description of the sexual generation has not been published. *Disholcaspis eldoradensis* (Beutenmueller) was the first species in the genus known to have a formal morphological description of the sexual generation (Evans 1972) and subsequently sexual generations of *D. quercusvirens* (Ashmead) and D *cinerosa* (Bassett)

have been identified and their biologies described (Platt 2009, Morgan & Frankie 1982). Morphological descriptions have not been published for sexual forms of the latter two species.

In 1996, Melika and Abrahamson (2002) collected bud galls that were similar in appearance to the sexual generation galls of *D. eldoradensis* from the Bucknell Natural Area, Northumberland County, Pennsylvania. The host trees, *Q. bicolor*, were heavily infested with *D. quercusmamma* asexual generation galls and they hypothesized that the wasps reared from the spring bud galls were the sexual generation of *D. quercusmamma*. In 2006, Scott Digweed also noticed similar bud galls on *Q. macrocarpa* in Riding Mountain National Park, Manitoba, Canada (personal communications, 2010-2011).

In the spring of 2009, similar bud galls were found on *Q. macrocarpa* in northern Colorado and the wasps were reared for identification. The wasps from these galls were noted to differ substantially in morphological features and included males. To better describe *D. quercusmamma* as a species, it is important to define the morphological characters of the sexual generation as well as the asexual generation. The characters found in both generations are also important for assessing generic limitations (Melika & Abrahamson 2002). The purpose of this study is to generate a morphological description of the sexual generation of *D. quercusmamma* wasps and their galls.

Methods and Materials

Sexual Generation Specimen Collection

Sexual generation galls were collected from bur oak trees at seven Fort Collins, Colorado sites: the Colorado State University (CSU) Forest Service Nursery; CSU Horticultural Research Center; street-side trees at Shields Street and Stuart Street; street-side trees at Prospect Road

and Yount Street; the north side of Harmony Road and Starflower Drive; and the west side of the Fossil Ridge High School campus. Collections were made during May through June, 2009, and May through June, 2010.

The small portion of the shoot were the gall was attached was collected with the gall to prevent damaging the gall and desiccation. Galls were placed in 100x15 mm polystyrene petri dishes until emergence. Emerged wasps were either killed for preservation or were used for the rearing trial (Chapter 5). Wasps were point mounted with the galls point mounted on the same pin as the wasp.

Formation Morphological Description

Since this is one of the first morphological descriptions of a sexual generation in the genus *Disholcaspis* there was little guidance as to which characters could be important for distinguishing between species. Special attention was paid to the traits listed by Melika and Abrahamson (2000, 2002) as being important in differentiating cynipid genera or being potentially important to the sexual generation specimens in the genus *Disholcaspis.* Terminology follows Gibson (1985), Huber & Sharkey (1993), Melika and Abrahamson (2000, 2002), and the Hymenoptera Anatomy Consortium.

A Nikon SMZ800 with a mounted InfinityX-21C camera was used for viewing traits. A Wild M5A with a reticle micrometer was used to measure lengths. Photographs were taken using an InfinityX-21C camera on the Nikon SMZ800 and used to make linear drawings. The picture of the gall was taken using the InfinityX camera using Infinity Analyze software v.5.0.3 (Lumenera Corporation 2002-2009). Other pictures were taken by Matt Buffington at the USDA-ARS Systematic Entomology Laboratory. Images were acquired using the Ento Vision micro-imaging system coupled with a Leica M16 zoom lens on and JVC KY-75U 3-CCD digital video camera attached to a Wild M-5 stereomicroscope (Buffington & van Noort 2007). Images were

merged using Cartograph 5.6.0. Halo effects from the multi-focus composition pictures were touched up using Paint.NET software (Brewster 2011). An expanded version of this description will be published in collaboration with Scott Digweed.

Material Examined

The description of the sexual female and male was based on 7 female and 7 male specimens. These specimens were reared from bud galls and were labeled with the following information: one female labeled as "USA, CO, Larimer Co., Fort Collins, CSU Horticultural Research Ctr., 40.6109,-104.9961, 27 May, 2010, C. McEwen et al.; Ex Bud gall on bur oak, *Quercus macrocarpa* Michx., emerged 30-31 May, 2010"; one female labeled as "USA, CO, Larimer Co., Fort Collins, CSU Horticultural Research Ctr., 40.6109,-104.9961, 1 June, 2009; Ex Bud gall on bur oak, *Quercus macrocarpa* Michx."; one female and two males labeled as "USA, CO, Larimer Co., Fort Collins, CSU Horticultural Research Ctr., 40.6109,-104.9961, 24 May, 2010; Ex Bud gall on bur oak, *Quercus macrocarpa* Michx., emerged 30-31 May, 2010"; two females and three males were labeled as "USA, CO, Larimer Co., Fort Collins, Fossil Ridge High School, 40.5115,-105.0107, 27 May, 2010; Ex Bud gall on bur oak, *Quercus macrocarpa* Michx., emerged 30-31 May, 2010"; one female labeled as "USA, CO, Larimer Co., Fort Collins, Fossil Ridge High School, 40.5115,-105.0107, 27 May, 2010; Ex Bud gall on bur oak, *Quercus macrocarpa* Michx., emerged 5-7 June,2010"; one female and one male labeled as "USA, CO, Larimer Co., Fort Collins, Shields St. & Stuart St., 40.5631,-105.0961, 27 May, 2010; Ex Bud gall on bur oak, *Quercus macrocarpa* Michx., emerged 30-31 May, 2010"; and one male labeled as "USA, CO, Larimer Co., Fort Collins, CSU Forest Serv. Nursery, 40.5851-105.1409, 21 May, 2010; Ex Bud gall on bur oak, *Quercus macrocarpa* Michx., emerged 30-31 May, 2010." All specimens used for this description were deposited at the C.P. Gillette Museum of Arthropod Diverstiy, Colorado State University, Fort Collins, Colorado.

Description

Sexual Generation Female (Figure 4.8). Length 2.0-2.6 mm. Head, except mouth parts, mostly black with dark brown along inner margin of eyes and lower genae; mouth parts brown; antennae brown at base and darker toward the tips. Mesopleuron dark brown; propodeum dark brown to nearly black; mesoscutum black; legs yellow brown with last tarsal segment black at tip. Metasoma black to dark brown; ventral spine of hypopygium brown.

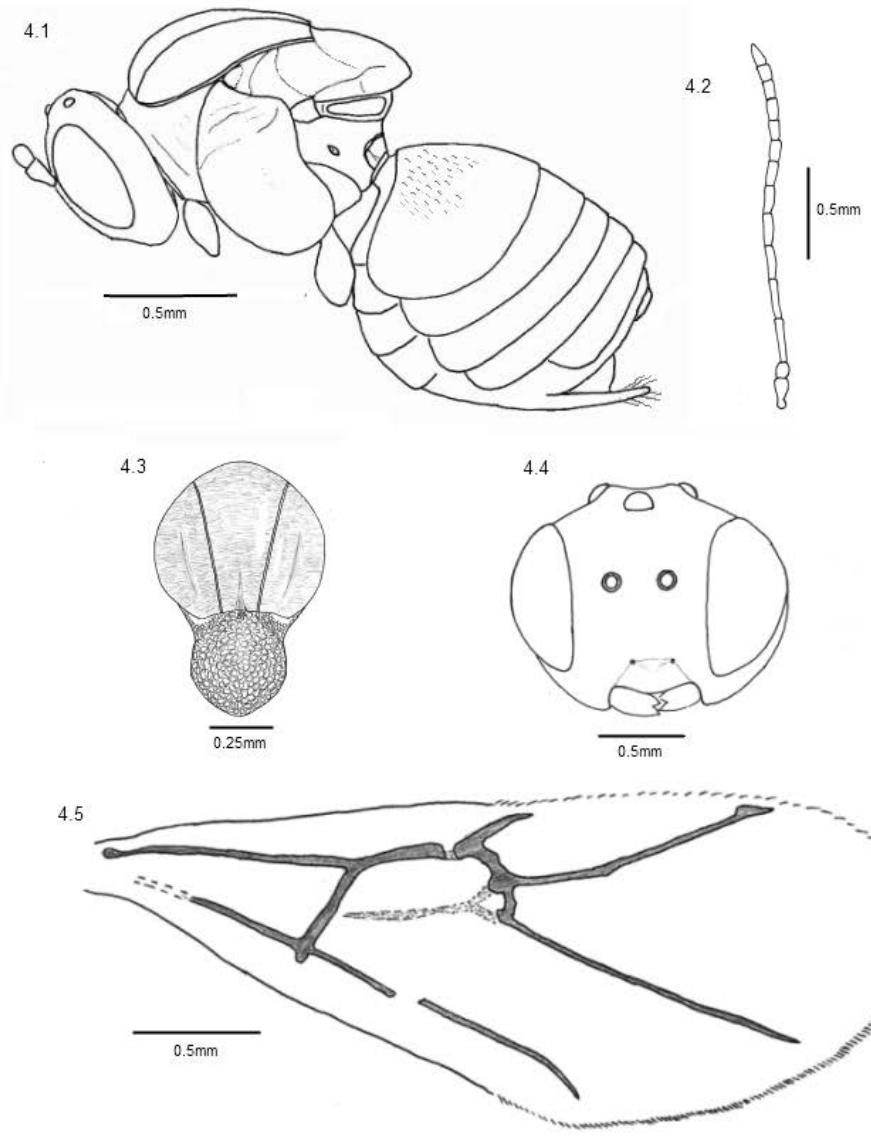
Head. Head reticulate to coriaceous and covered in fine setae. Head, in dorsal view, as broad as mesosoma; ocellar diameter about the same or slightly shorter than anterior ocellar-posterior ocellar distance; post-ocellar length much shorter than ocular-ocellar length; ocellar plate raised. In frontal view (Figure 4.4), head 1.2 times broader high; antennal sockets situated at or slightly above mid-height of compound eyes; distance between antennal sockets slightly longer than diameter, shorter than distance to inner eye margin; lower face slight bulging between clypeus and antennal sockets; malar space with no sulcus, about 0.2 times eye height; tentorial pits small, epistomal sulcus lightly impressed with small elevation just below the line, clypeus subtrapazoid. Antennae 14-segmented, approximately 0.9 times the length of the body; flagellomeres (F) filiform, F3 and F4 slightly broadened at center; base of scape about half as wide as apex, 1.4 times as long as pedicel; F1 is the longest flagellomere, 1.1 to 1.3 times the length of scape and pedicel, 1.1 times the length of F2; F3 and F4 equal in length, 0.8 times the length of F2 (Figure 4.2, Figure 4.6).

Mesosoma. In lateral view (Figure 4.1), mesosoma approximately 1.2 times longer than high; mesopleuron is 0.7 times the height of the mesosoma; pronotum smooth with rugulae extending medially from mesopleuron margin; mesopleuron smooth except mesopleural triangle, scattered setae mostly restricted to margins. In dorsal view (Figure 4.3, Figure 4.7), scutum nearly equal in length and width, mostly smooth to finely coriaceous or microreticulate,

shiny but pruinose; notauli nearly complete; anterior parallel lines absent; parapsidal lines mostly absent or present as faint grooves, incomplete; median mesoscutal sulcus present only as a slight indent or absent; scutellum longer than width, coarsely rugose; scutellar foveae absent, but scutellum often with smooth depressions laterally near transscutal line. Forewing 1.2-1.4 times the length of body, pubescent, cilia on margin; veins dark brown, distinct except spectral basal one-third of M+Cu_1, and Cu_1 with short spectral section one-third total length from base; areolet present; radial cell open, 2.8 times as long as broad (Figure 4.5). Tarsal claws with strong, moderately pointed basal lobe. Propodeum pubescent except center portion, which is dull and rugose, delimited by distinct lateral longitudinal carinae.

Metasoma. In lateral view (Figure 4.1), metasoma smooth, shiny but pruinose; nearly as high as long; ventral spine of hypopygium 3.0-3.2 times as long as broad; ventral spine of hypopygium with long brown setae, some reaching beyond tip of spine.

Male (Figure 4.10). Length 2.1-2.3 mm. Differ from females in being overall less pubescent, and uniformly black. Antennae 15-segmented, slightly longer than body length; scape barely longer than pedicel; F1 1.2-1.4 times longer than scape and pedicel; subsequent flagellomeres decreasing in size. In dorsal view (Figure 4.9) parapsidal lines absent; median musoscutal sulcus absent; scutellum is coriaceous, punctate, lightly pubescent with short setae. Mesosoma, in lateral view, 1.3 times as long as high in; mesopleuron is 0.6 times the height of the mesosoma. Propodeum has reduced sculpturing, smooth except faint lateral longitudinal carinae. Metasoma 1.3 times as long as high in lateral view.



Figures 4.1-4.5. *Disholcaspis quercusmamma* (Walsh), sexual generation female. 4.1: Body, lateral view. 4.2: Antenna, 4.3: Scutum and scutellum, dorsal view, 4.4: Head, front view, 4.5: Forewing

Figure 4.6

Figure 4.7

Figure 4.8

Figure 4.9

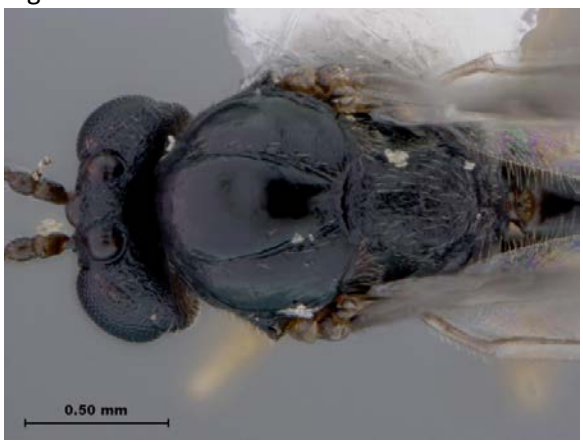


Figure 4.10

Figures 4.6-4.8: *Disholcaspis quercusmamma* (Walsh) sexual generation female; 4.6: Antenna, scape through flagellomere four; 4.7: Mesosoma, dorsal view; 4.8: Full body, lateral view; **Figures 4.9-4.10**: Sexual generation male; 4.9: Mesosoma, dorsal view; 4.10: Full body, lateral view. Photos taken by Matt Buffington.

Gall. Galls are formed in buds of *Q. macrocarpa* and *Q. bicolor*. Galls become conspicuous shortly after bud break and usually form in the center of the buds or outside the bud around the base of new leaves. Length of gall 2.7-3.2 mm long (Figure 4.11). Gall is ovoid with a thin, fragile wall. Gall surface is smooth. The gall is light brown or buff in color, sometimes with green or pink coloration near the tip during gall development. At gall maturation, the pink or green coloration sometimes turns brown or fades. Each leafing bud can have numerous galls but the galls are usually not clustered together. Emergence holes are produced on the side of the galls near the apex and are rough-edged.

Fig. 4.11. Gall of the sexual generation of *Disholcaspis quercusmamma* (Walsh) taken from bur oak, *Quercus macrocarpa* Michx., in the spring 2010.

References Cited

Basset H.F., 1890. New Species of North American Cynipidae. Transactions of the American Entomological Society 17(1): 59-92.

Beutenmuller W., 1909. Article V. The species of *Holcaspis* and their galls. Bulletin of the American Museum of Natural History 26: 29-45.

Brewster R., Paint.NET v3.5.8, © 2011, dotPDN LLC. Accessed multiple dates 2010-2011.

Buffington M.L. and van Noort S., 2007. A world revision of the Pycnostigminae (Cynipoidea: Figitidae) with descriptions of seven new species. Zootaxa 1392: 1-30.

Eckberg T.B., 1993. Observations on the biology and control of four landscape pests of the Colorado front range. Master's Thesis, Colorado State University, Fort Collins, Colorado.

Evans D., 1972. Alternate generations of gall cynipids (Hymenoptera: Cynipidae) on garry oak. The Canadian Entomologist 104: 1805-1818.

Gibson G.A.P., 1985. Some pro- and mesothoracic structures important for phylogenetic analysis of Hymenoptera, with a review of terms used for the structures. Canadian Entomologist 117: 1395-1443.

Huber J.T. and Sharkey M.J., 1993. Chapter 3 Structure. *In* Goulet H. and Huber J.T. (eds.), *Hymenoptera of the World: An identification guide to families*. Centre for Land and Biological Resources Research, Ottawa, pp. 11-59.

Hymenoptera Anatomy Consortium. Accessed on multiple occasions 2010-2011. Available at http://glossary.hymao.org.

Krombein K.V., Hurd Jr. P.D., Smith D.R., and Burks, B.D., 1979. Catalog of Hymenoptera in America North of Mexico, Vol. 1 Symphyta and Apocrita (Parasitica). Washington, D.C., Smithsonian Institution Press. pp. 1091-1092.

Lumenera Corporation, © 2002-2009. Infinity Analyze software, v.5.0.3. Ottawa, Ontario, Canada.

Melika G. and Abrahamson W.G., 2000. Historical review and current state of the world generic classification of oak gall wasps (Hymenoptera: Cynipidae: Cynipini). *In* Austin A.D. and Dowton M. (Eds.), *Hymenoptera, Evolution, Biodiversity and Biological Control*. CSIRO Publishing, Melbourne, pp. 218-230.

Melika G. and Abrahamson W.G., 2002. Review of the World General of Oak Cynipid Wasps (Hymenoptera: Cynipidae: Cynipini). *In* Melika G. and Thuroczy C.S. (Eds.), Parasitic Wasps: Evolution, Systematics, Biodiversity and Biological Control, Agroinform, Budapest, Hungary, pp. 150-190.

Morgan D.L. and Frankie G.W., 1982. Biology and control of the mealy-oak gall. Journal of Arboriculture 8(9): 230-233.

Platt J.E., 2009. Life history and management of a bullet gall wasp, *Disholcaspis quercusvirens* (Hymenoptera: Cynipidae) on Cathedral® live oak (*Quercus virginiana*) in northern Florida. Master's Thesis, University of Florida, Gainesville, Florida.

Walsh B.D., 1869. Galls and their architects. The American Entomologist 1(6): 101-110.

CHAPTER FIVE

IDENTIFICATION OF THE SEXUAL GENERATION OF *DISHOLCASPIS QUERCUSMAMMA* (WALSH) AND ITS BIOLOGY

Introduction

The rough bulletgall wasp, *Disholcaspis quercusmamma* (Walsh), is a gall making wasp (Hymenoptera: Cynipidae:Cynipini) that is a parasite of bur oak, *Quercus macrocarpa* Michx, and swamp white oak, *Q. bicolor* Willd (Basset 1890, Beutenmüller 1909, Eckberg 1993, Krombein et al. 1979). The agamic females are known to produce conspicuous twig galls in late summer and autumn. These galls contain a single larval cell, are rounded, have a pointed apex, and mature to have a woody texture. The galls often occur in clusters and in dense infestations can completely encompass newly maturing twigs (Basset 1890, Beutenmüller 1909, Eckberg 1993).

Quercus macrocarpa and *Q. bicolor* are popular horticultural plantings in northern Colorado due to their ability to endure Colorado growing conditions. Heavy infestations by *D. quercusmamma* can inhibit growth (Basset 1890, Eckberg 1993). An added concern to homeowners is that the honeydew exuded from the galls attracts various stinging insects and the galls can make trees look disfigured after leaf fall. Gall wasp populations are often hard to control using chemicals due to the protection of the gall during a large portion of the gall wasp life cycle. If chemical controls are to be most effective, there needs to be a clear understanding of the life cycle of the gall wasp and its natural enemies.

Current knowledge about the biology of *D. quercusmamma* is based only on the asexual generation (Eckberg 1993). Cynipids are known to alternate between asexual and sexual

generations but this discovery came after *D. quercusmamma* was described (Melika & Abrahamson 2000). Until now, the biology and associated natural enemies of the sexual generation of this species have not been researched. The purpose of this study is to contribute to the life history of *D. quercusmamma* and to document the parasitoids attacking the sexual generation in northern Colorado.

Methods and Materials

Collection of Sexual Generation Specimens

Sexual generation galls were collected during in spring 2009 and 2010. These galls are small, buff colored bud galls that are similar in shape to a kernel of rice or grain. *Quercus macrocarpa* trees at the Colorado State University (CSU) Forest Service Nursery (FS site) and the CSU Horticultural Research Center (HF site) were checked weekly for spring gall formation starting in March 2010. Once gall formation was noted galls were collected from *Q. macrocarpa* at six sites in the Fort Collins area: the FS site; the HF site; street-side trees from Shields Street and Stuart Street (S&S); Prospect Road and Yount Street (P&Y); Harmony Road and Starflower Drive (FRCC); and west side of the Fossil Ridge High School campus (FRHS). Gall collections were also made from a *Q. bicolor* located near the corner of White Willow Drive and MacKenzie Court, Fort Collins, Colorado.

At collection a small portion of the shoot where the gall was attached was collected with the gall to prevent gall damage and reduce desiccation. Galls were placed in 100x15 mm polystyrene petri dishes until emergence. Emerged wasps were either killed for preservation or were used for the subsequent rearing trial. Preserved wasps were identified using a Nikon SMZ800 with a mounted InfinityX-21C camera.

Species Confirmation

Rearing Trial (2010)

Rearing trials were performed on *Q. macrocarpa* at the HF and FS sites. The FS site consists of two closely planted rows of mature trees while the HF site is a single row of younger trees.

Polypropylene pollination bags, with the seams reinforced with hot glue, were placed over easily accessible branch tips on randomly chosen trees on 21 May and closed with twist ties. The branches were cleared of all galls and any visible insects. There were 14 branches bagged at the HF site; 15 branches at the FS site. Four of the bags at the HF site were on trees that appeared to be resistant to the asexual generation of *D. quercusmamma* based on the absence of rough bullet galls. A range of resistance to galling in *Q. macrocarpa* was noted by Eckberg (1993). The bags were rechecked for insect contaminants and newly forming bud galls every couple of days and other insects were removed when observed.

Wasps that emerged from spring galls collected from the HF, FS, S&S, P&Y, FRCC, and FRHS sites were sexed and confirmed to not be inquiline synergine wasps then grouped into vials. These vials contained 5 wasps made up of at least two of each sex. If the wasps were to be held overnight a small piece of cellulose paper soaked with sugar water was offered.

Wasps were released inside of the bags starting 26 May and the last bag was filled on 2 June. Each bag received the wasps from one vial within one day of the wasp emergence. Damaged bags were changed out as needed through 25 October. The fall-form galls that developed on the isolated, bagged branches were harvested on 25 October and the specimens reared out for identification.

DNA Testing

Five specimens were used for the comparative DNA analysis. Two sexual generation females, one sexual generation male, and 2 asexual generation females were used to determine if the wasps were the same species. The specimens used for the analysis were stored in 95% ethanol.

Specimens were homogenized using liquid nitrogen and a disposable microtube pestle inside an Eppendorf tube. Total DNA was extracted using the standard DNeasy® spin column protocol (QIAGEN 2006). DNA amplification was done by polymerase chain reaction (PCR). The PCR mixtures, conditions, and relevant genes for comparing were recommended by the Dr. Graham Stone and Dr. James Nicholls, University of Edinburgh. The cytochrome b (CB) region and the internal transcribed spacer (ITS) region were sequenced using the primers in Table 5.1.

Table 5.1. Oligonucleotide primers for amplification and sequencing of the cytochrom b (CB) and internal transcribed spacer (ITS) regions courtesy of Dr. Nicholls and Dr. Stone, University of Edinburgh.

Primer	Sequence	Fragment Length
CB1[1]	5' TAT GTA CTA CCA TGA GGA CAA ATA TC 3'	433 bp
CB2[1]	5' ATT ACA CCT CCT AAT TTA TTA GGA AT 3'	
ITS2f[2]	5' TGT GAA CTG CAG GAC ACA TG 3'	510-520 bp
ITS2r[2]	5' AAT GCT TAA ATT TAG GGG GTA 3'	

[1] Original reference is Jermiin & Crozier 1994
[2] Original reference is Campbell et al. 1993

PCR amplifications of the CB samples was performed in 20 µl volumes of 15.34µl water, 2 µl 10x PCR buffe, 0.8µl $MgCl_2$ (50 mM), 0.3µl CB1 (20µM), 0.3µl CB2 (20µM), 0.16µl dNTPs (25mM), 0.1µl Taq, and 1.0µl DNA extract. Cycle conditions for the CB samples were: 94°C for 2 minutes; denaturing 94°C for 30 seconds; annealing at 46-50°C for 30 seconds; extension at72°C for 40 seconds; denaturing, annealing, and extension steps were repeated thirty-five times; 72°C for five minutes; and 10°C until amplificaiton. The PCR amplification of the ITS samples was performed in 20µl volumes of 15.94µl water, 2µl 10x PCR buffer, 0.6µl $MgCl_2$ (50mM), 0.3µl ITSf (20µM), 0.3µl ITSr (20µM), 0.16µl dNTPs (25mM), 0.1µl Taq, and 0.6µl DNA extract. The CB samples were separated on agarose gels and a Zymo Research gel extraction kit was used to isolate the desired material for sequencing.

Cycle conditions for the ITS samples were: 94°C for two minutes; denaturing at 94°C for 30 seconds; annealing at 50°C for 40 seconds; extension at72°C for one minute; denaturing, annealing, and extension steps were repeated thirty-four times; 72°C for five minutes; and 10°C until purification. The ITS samples were then treated with a QIAquick PCR purification kit using the microcentrifuge protocol (QIAGEN 2008).

Sequencing was performed by the Colorado State University Proteomics and Metabolics Facility (Fort Collins, Colorado). The sequences were analyzed and compared by Dr. Nicholls at the University of Edinburgh.

Seasonal Activity of the Sexual Generation and Associated Parasitoids

Pherocon AM No Bait® yellow sticky traps were used at four random trees at both the HF and FS sites to determine seasonal activity of *D. quercusmamma* and associated parasitoids. The traps were replaced at biweekly intervals starting 5 April and switched to weekly intervals from 25 May until 15 December. Samples taken by sweep netting and beating the branches over the sweep nets were also taken from four random trees at each site at the same time

intervals as the sticky traps. The traps and sweep net samples were placed in the freezer then transferred to the refrigerator. The trap and sweep net samples were later examined so that the presence or absence of the species reared from the galls could be recorded. Traps were viewed with a Nikon SMZ800.

Parasitoids reared from the collected sexual generation galls were used to identify the parasitoids associated with this generation. The specimens from the family Pteromalidae were identified by Dr. Steve Heydon, Curator and Collections Manager, Bohart Museum of Entomology, University of California, Davis, CA. All other parasitoids were identified by Dr. Michael Gates (Eurytomidae, Torymidae), Systematic Entomology Laboratory, Agricultural Research Service, U.S. Department of Agriculture.

Parasitoids Reared from the Asexual Generation Galls

Asexual generation galls were collected from the HF and FS sites on 9 October and 21 October, 2009. The galls were stored in 100x15 mm polystyrene petri dishes until emergence. The dishes were checked periodically over the winter and stored at room temperature. Parasitoids were identified by Dr. Michael Gates (Eurytomidae, Torymidae, Eulophidae), Systematic Entomology Laboratory, Agricultural Research Service, U.S. Department of Agriculture.

Results

Collection of Sexual Generation Specimens

The spring bud galls of the sexual generation were observed shortly after bud break after the leaves start to expand. The galls are usually toward the tips of new growth, surrounded by a whorl of leaves. The tips where the galls usually appear tend to swell slightly around the gall. At the FS site was noted that the sexual generation galls were easier to find on lower branches when the branches faced west. The branches on the west side were less shaded

and were facing an open field. In this study the galls start to appear toward the end of May and by mid-June most of the wasps had emerged.

The number of sexual generation specimens reared from galls collected in 2009 was not recorded. During 2010, a total of 271 sexual generation specimens were reared (Table 5.2) and these were at approximately 1.15:1 ratio of females to males.

The sexual generation was confirmed to attack both host species, *Q. macrocarpa* and *Q. bicolor*. The sexual generation galls were also noted to be present on the trees that had appeared to be resistant to the asexual generation twig galls.

Table 5.2. Wasps reared from sexual generation *Disholcaspis quercusmamma* galls harvested from *Quercus macrocarpa* during spring 2010 in Fort Collins, Colorado.

Wasp Type	Total
D. quercusmamma Females	145
D. quercusmamma Males	126
Parasitoids	61

*Galls were collected from one of the following locations: the Colorado State University (CSU) Forest Service Nursery; the CSU Horticultural Research Center; street-side trees from Shields Street and Stuart Street; Prospect Road and Yount Street; Harmony Road and Starflower Drive; and the west side of the Fossil Ridge High School campus.
**Galls were collected 21 May through 27 May, 2010 and stored in petri dishes until the wasps emerged.
***Sexual generation specimens emerged in the lab 22 May through 7 June, 2010.
******Parasitoids emerged in the lab 30 May through 16 June, 2010.**

Species Confirmation

Rearing Trial (2010)

Sexual generation wasps reared from spring bud galls then isolated on newly developing *Q. macrocarpa* shoots were shown to result in the formation of the previously described twig galls in which agamic females develop. Of the ten bags that were placed on non-resistant trees at the HF site, eight of them had formed the typical asexual generation galls. The wasps reared from these galls were identified as *D. quercusmamma*. There were no galls formed in the bags that were on trees that appeared resistant to the asexual generation galls. The number of galls found inside the bags ranged from 0-154 galls per bag.

Ten of the fifteen bags at the FS site had asexual generation gall formation. The wasps reared from these galls were identified as *D. quercusmamma*. The number of galls found inside the bags ranged from 0-51 galls per bag.

DNA Testing

DNA sequences from wasps reared from spring bud galls of *D. quercusmamma* were similar to those of the asexual generation wasps (Dr. James Nichols, University of Edinburgh, personal communication 17 November, 2010). These results, when combined with the rearing experiment, strongly support the conclusion that the wasps reared from the spring bud galls are correctly identified as the sexual generation of *D. quercusmamma*.

Seasonal Activity of the Sexual Generation and Associated Parasitoids

Disholcaspis quercusmamma adults collected from spring bud galls were observed to emerge from 22 May through 7 June. Wasp collections on sticky traps from the HF site showed that the sexual generation wasps were first noted on traps 8-19 May and collection continued until 9-15 June trap interval. The sticky traps from the FS site showed that the sexual generation wasps were active from 20-25 May until 9-15 June.

Parasitoids were reared from approximately 23% of the galls collected in 2010 (Table 5.2). Parasitoids reared from spring bud galls emerged from 29 May until 19 June. The most common parasitoids reared were from the family Pteromalidae and were identified by Dr. Steve Heydon (UC Davis) as *Lyrcus* nr. *nigroaeneus* (Ashmead). There were two other pteromalid species but both were males so they could only be identified to the genus level, *Pteromalus* sp. and *Mesopolobus* sp.

The other parasitoids reared from the sexual generation galls were identified by Dr. Michael Gates (Systematic Entomology Laboratory, Agricultural Research Service, U.S. Department of Agriculture) as *Torymus denticulatus* (Breland) (Torymidae) and *Sycophila dubia* (Walsh) (Eurytomidae). The *T. denticulatus* and *S. dubia* specimens were donated to the U.S. National collection at their request.

Parasitoids Reared from the Asexual Generation Galls

The parasitoids reared from the asexual generation galls were identified by Dr. Michael Gates (Systematic Entomology Laboratory, Agricultural Research Service, U.S. Department of Agriculture) as *S. dubia* (Eurytomidae), *Eurytoma querciglobuli* (Fitch) (Eurytomidae), *T. denticulatus* (Torymidae), and *Baryscapus racemariae* (Ashmead) (Eulophidae). The *S. dubia* and *T. denticulatus* specimens were donated to the U.S. National collection at their request. *Baryscapus racemariae* are gregarious parasitoids and the reared broods ranged in size from 7-32 wasps from a single asexual generation gall.

Discussion

The identification and description of the sexual generation of *D. quercusmamma* helps complete the life history of this species (Figure 5.1). The sexual generation bud galls develop and become visible in spring shortly after bud break. In northern Colorado, the sexual generation wasps emerge from mid-May through beginning of June at which time sexual

generation females oviposit in newly developing stems. The typical twig gall of the asexual generation begins to visibly erupt from first year twigs in July and is fully formed in late summer. Asexual females emerge mid-October through November from the twig galls and oviposit in dormant buds. The latter observations are consistent with Eckberg (1993).

There is evidence that oak trees have varying degrees of resistance to the twig galls produced by *D. quercusmamma* (Eckberg 1993). It is not unusual to observe heavily infested trees and trees supporting very low infestations or even the complete absence of twig galls growing next to each other. While the resistant trees can be nearly devoid of any asexual generation galls, some of them did contain sexual generation galls, indicating that resistance may be against only one generation of *D. quercusmamma*.

The current study is the first record of parasitoids associated with the sexual generation of *D. quercusmamma*. Furthermore, no pteromalids were previously known to be associated with *D. quercusmamma*, so the three pteromalid species recorded in this study constitute new records for this species (Krombein et al. 1979; The National History Museum, London © 2011). Both *S. dubia* and *T. denticulatus* are parasitoids that are associated with both generations.

During 1992, Eckberg (1993) only recovered *S. dubia* attacking the asexual generation. The current study expands on the parasitoids associated with this generation in northern Colorado. The Universal Chalcidoidea Database (The National History Museum, London © 2011) lists 15 chalcidoid parasitoids known to be associated with the asexual generation of *D. quercusmamma* including *S. dubia*, *E. querciglobuli*, and *B. racemariae*. This appears to be the first record of T. *denticulatus* being reared from *D. quercusmamma* though it is known to attack other cynipids.

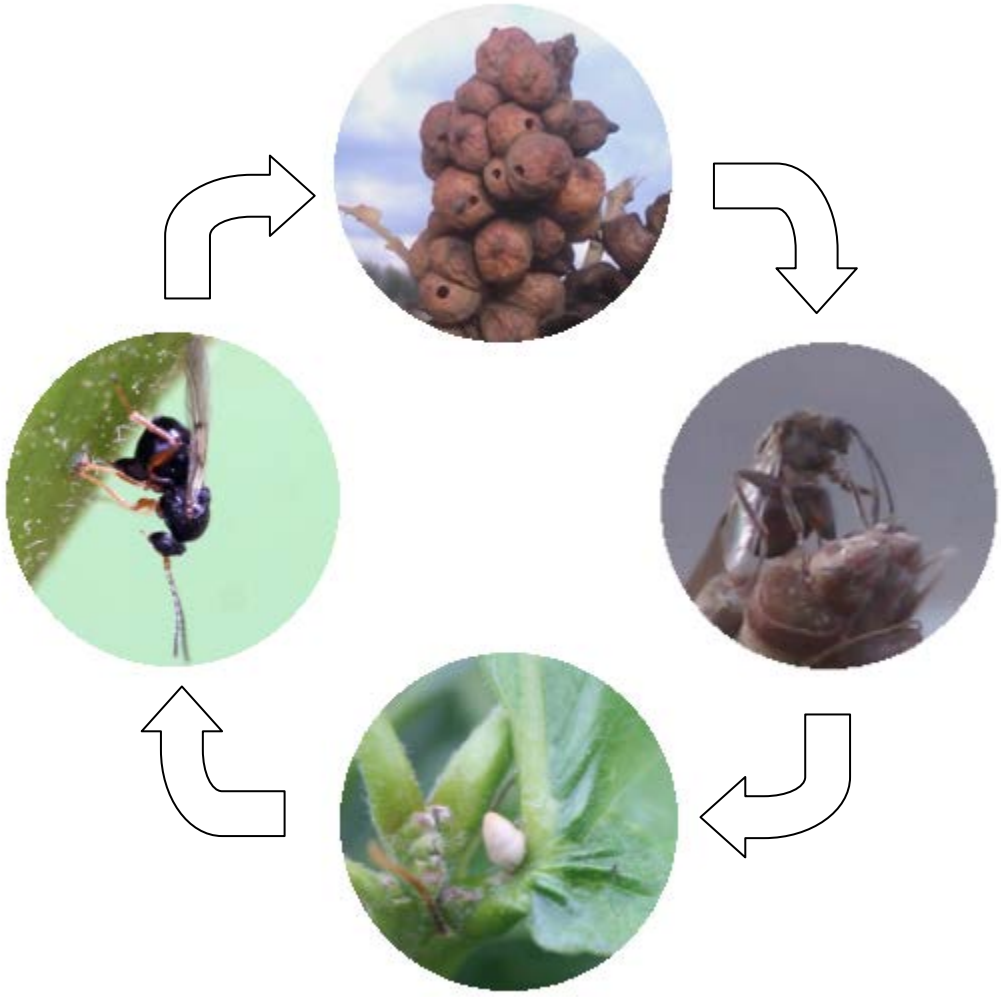


Figure 5.1. Life history of *Disholcaspis quercusmamma* (Walsh) on *Quercus macrocarpa* or *Q. bicolor*. Top: Rough bullet galls on twigs in late-summer or autumn, asexual generation emerges in autumn.
Right: Asexual female ovipositing into dormant bud. Bottom: Bud gall in spring, sexual generation emerges in spring or early summer. Left: Sexual generation female ovipositing in newly forming stem.

References Cited

Basset H.F., 1890. New Species of North American Cynipidae. Transactions of the American Entomological Society 17(1): 59-92.

Beutenmuller W., 1909. Article V. The Species of *Holcaspis* and their galls. Bulletin of the American Museum of Natural History 26: 29-45.

Campbell B.C., Steffen-Campbell J.D. 1993. Insect molecular biology: Phylogeny of the *Nasonia* species complex (Hymenoptera: Pteromalidae) inferred from an internal transcribed spacer (ITS2) and 28s rDNA sequences. Insect Molecular Biology 2(4): 225-237.

Eckberg T.B., 1993. Observations on the biology and control of four landscape pests of the Colorado front range. Master's Thesis, Colorado State University, Fort Collins, Colorado.

Jermiin L.S. and Crozier R.H. 1994. The cytochrome b region in the mitochondrial DNA of the ant *Tetraponera rufoniger*: Sequence divergence in Hymenoptera may be associated with nucleotide content. Journal of Molecular Evolution 38: 282-294.

Krombein K.V., Hurd Jr. P.D., Smith D.R., and Burks, B.D., 1979. Catalog of Hymenoptera in America North of Mexico, Vol. 1, Symphyta and Apocrita (Parasitica). Smithsonian Institution Press., Washington, D.C., pp. 1091-1092.

Melika G. and Abrahamson W.G., 2000. Historical review and current state of the world generic classification of oak gall wasps (Hymenoptera: Cynipidae: Cynipini). *In* Austin A.D. and Dowton M. (Eds.), *Hymenoptera, Evolution, Biodiversity and Biological Control*. CSIRO Publishing, Melbourne, pp. 218-230.

The National History Museum, London © 2011. Universal Chalcidoidea Database. www.nhm.ac.uk/research-curation/research/projects/chalcidoids. Accessed multiple times 2010-2011.

QIAGEN, 2006. Protocol: Purification of total DNA from animal tissues (spin-column protocol). *In*: DNeasy® Blood & Tissue Handbook. Available at www.qiagen.com/literature/default.aspx. Accessed 28 October 2010. pp. 28-30.

QIAGEN, 2008. QIAquick PCR purification kit protocol using a microcentrifuge. *In*: QIAquick® Spin Handbook. Available at www.qiagen.com/literature/default.aspx. pp. 19-21.