Evaluating natural and induced resistance of broccoli (*Brassica oleracea* var. *italica*) against *Pieris rapae*

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Dedication

This thesis is dedicated to my family – Mom, Dad, Spring, Brian, Sage, Briar, Cassius, and Mike.

Abstract

Pieris rapae is an insect pest the feeds exclusively on species of the Brassicaceae. It is a serious economic pest in commercial horticulture as larvae chew large holes in leaves, can destroy developing heads of broccoli, cauliflower, and cabbage, and otherwise contaminate or stain produce, rendering it unmarketable. *P. rapae* is notoriously difficult to manage in organic production systems, and organic brassica growers traditionally rely upon floating row covers or biopesticides to prevent feeding damage. Natural pest resistance is important when making cultivar recommendations for production systems with limited pest management options, and our research evaluates natural and induced resistance in broccoli (*Brassica oleracea* var. *italica*) against *P. rapae* with a focus on the roles that glucosinolates (GSLs) play in resistance. GSLs are constitutive and inducible secondary metabolites in members of the Brassicaceae and are toxic to generalist insect pests. However, the relationship between GSL and *P. rapae*, a brassica specialist, is complex and not well understood. A controlled greenhouse study evaluated the effects of *P. rapae* herbivory on GSL induction and subsequent larval growth and performance on two broccoli cultivars ('Beneforte' and 'Green Magic') that varied in concentration of two GSLs, glucobrassicin and neoglucobrassicin. Our results show that neoglucobrassicin and glucobrassicin concentrations did not significantly increase as a result of herbivory, but the overall growth and performance of *P. rapae* was influenced by cultivar. Larvae consumed less total leaf area and weighed less as pupae when feeding on 'Beneforte', which also had a significantly higher glucobrassicin concentration, indicating that cultivar may be important in resistance against *P. rapae.* A field study performed at two certified organic farms in Minnesota in 2015 and 2016 evaluated six broccoli cultivars ('Belstar,' 'Fiesta,' 'Green Magic,' 'Marathon,' 'Packman,' and 'Thompson') for natural resistance to *P. rapae* and for yield performance. The cultivars in this study did not significantly differ in susceptibility to *P. rapae* infestation, and neoglucobrassicin and glucobrassicin concentrations did not explain *P. rapae* egg and larval abundance. We conclude that cultivar selection should not be based on natural resistance to pests in this case but rather based on cultivar performance. 'Belstar,' 'Fiesta', 'Green Magic,' and 'Packman' were top performers and produced a consistent and high quality product, and therefore we would recommended these cultivars as acceptable selections for organic systems in Minnesota.

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1.1 Summary

Pieris rapae Linneaus, or imported cabbageworm, (Lepidoptera: Pieridae) is a specialist herbivore that feeds exclusively on species in Brassicaceae (mustard or cruciferous). It is a serious economic pest in commercial horticulture because larvae chew large holes in leaves, can destroy developing heads of broccoli, cauliflower, and cabbage, and may otherwise contaminate or stain produce, rendering the produce unmarketable. In conventional systems, this pest is controlled with broad-spectrum insecticides on a calendar-based spray schedule, but *P. rapae* is difficult to manage in organic systems. Organic brassica growers are limited in pest management options and must rely on alternative strategies to avoid insect damage, including biopesticides and botanical products, cultural practices, biological control, diversified cropping systems, and natural host plant resistance.

1.2 *Pieris rapae*

History and Distribution

Pieris rapae was introduced to North America from Europe. It was first observed in Quebec City, Canada in 1860, and in 1865, it was observed in the Northeastern United States in Maine. From there, the cabbageworm spread south and westward, and by 1886, it had inhabited much of the U.S., including the entire east coast, south to Florida, and west to the Rocky Mountains. It is now found throughout the U.S., Canada, Europe, Australia, New Zealand, and other temperate regions of the world (Chittenden 1916).

Life Cycle

The complete *Pieris rapae* life cycle takes three to six weeks, depending on temperature. There are three to five overlapping generations in much of the U.S., including Midwestern states such as Wisconsin and Minnesota and Northwestern states such as Oregon (Maltais et al. 1998). Since *P. rapae* is one of the first butterflies to emerge in the spring and remains active into the fall in most regions, monitoring should start early in the season and continue into the fall. Adults are very active and the presence of the butterflies may be a sign that larvae are present on brassica plants. Number of larvae and eggs as well as larval size or instar should be noted.

When scouting plants for infestation, the underside of leaves should be turned over and scanned for eggs, as adult females oviposit eggs singly on the underside of leaves (Lasota and Kok 1989, Maltais et al. 1998b). Eggs are off-white to yellow or orange, ribbed longitudinally, oblong, and attached endwise. Eggs hatch in 3 to 7 days.

After hatching, complete larval development lasts between 2 to 3 weeks, and larvae undergo 4 molts and 5 instars. Larvae have soft bodies and 5 pairs of prolegs. First instars are pale green and turn to a brighter green thereafter. Older larvae are velvety with a yellow dorsal stripe and an intermittent yellow stripe down each side of the body. When prodded, they are sluggish to respond. Fourth and fifth instars are the most voracious and thus most damaging to crops.

Pupae attach to the plant or nearby debris by a silk thread at the tip of the abdomen. Pupation lasts 7 to 12 days. The pupa varies in color from yellow to green, turning a light speckled brown or gray when close to emergence. Prominent, sharp ridges run along the thorax dorsally and dorsolaterally. *P. rapae* overwinters at this stage, entering diapause in the fall. After emergence, adult butterflies live for 3 to 4 weeks. The

upper side of the wings is creamy white while the underside is faint yellow. Forewings have black tips. Females have two black dots on the central portion of each forewing while males have one black dot on each forewing. Adults are active during the day (diurnal) when the weather is warm and sunny and feed on nectar of flowering plants. A single female lays 300 to 400 eggs in her lifetime (Richards 1940).

Host Plants and Damage

Pieris rapae can be a serious economic pest in commercial horticulture. Larvae are commonly found feeding on high-value crops of the *Brassica oleracea* species, including broccoli (var. *italica*), cauliflower (var. *botrytis*), Brussels sprouts (var. *gemmifera*), kale (var. *acephala*), kohlrabi (var. *gangylodes*), cabbage (var. *capitata*), and other specialty crops, as well as other Brassicaceae species such as radish (*Raphanus sativus*), horseradish (*Armoracia rusticana*), and weedy species. The larvae feed on foliage, leaving large and irregular holes. On occasion, they may completely defoliate a plant, reducing it to the stem and large veins. They can also destroy developing broccoli, cauliflower, and cabbage heads by burrowing into the heads to feed or pupate or by stunting growth. Larvae may also contaminate or stain produce with copious amounts of frass, or fecal matter, rendering produce unmarketable.

1.3 Management Strategies

Tolerance for insect damage and contamination in these cole crops is near zero (Maxwell and Fadamiro 2006). In conventional systems, *P. rapae* is controlled with broad-spectrum synthetic pesticides that are routinely applied on a calendar-based spray schedule, often regardless of the presence or absence of the insect pest (Maxwell and Fadamiro 2006). However, the use of these pesticides can negatively affect beneficial

insects, cause secondary pest outbreaks, and contribute to pesticide resistance (Metcalf 1994), and they are not compliant with organic standards. Organic brassica growers are limited in pest management options and must rely on alternative strategies to avoid *P. rapae* damage. Organic pest management often focuses on minimizing damage and pest abundance first with preventative measures rather than treatment. Here we will review specific strategies for the management of *P. rapae,* including biopesticides and botanical products, cultural practices, biological control, diversified cropping systems, and host plant resistance.

Biopesticides and Botanical Products

Calendar-based applications of broad-spectrum pesticides, such as organophosphates, carabamates, and pyrethroids, are typically executed to manage lepidopteran damage on brassica crops in conventional systems (Maxwell and Fadamiro 2006), and the negative consequences of these pesticides, such as pesticide resistance and adverse effects on beneficial insects, are well known (Metcalf 1994). "Reduced-risk" pesticides provide an alternative to synthetic broad-spectrum pesticides, and they are generally less toxic to humans and non-target organisms, including natural enemies (Maxwell and Fadamiro 2006). Many are referred to as "biopesticides", which the EPA defines as "certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals" (EPA 2016). They can be biochemically based, microbially based, or based on plants containing added genetic material (plant-incorporated protectants). Biopesticides also generally have shorter pre-harvest intervals and re-entry intervals, making them appealing to organic growers (Caldwell et al. 2013).

Organic-approved biopesticides formulated for *P. rapae* caterpillars include *Bacillus thuringiensis,* commonly referred to as *Bt,* and spinosad products (Caldwell et al. 2013). *Bt* is a naturally occurring soil bacterium that is a highly toxic stomach poison to caterpillars through ingestion (Mader and Adamson 2013). Younger larvae (first and second instars) are more susceptible than older larvae (Mahr et al. 1993). Many strains have been formulated in commercial products such as Agree® and XenoTari® (*B. thuringiensis* subspecies *aizawai*) and Dipel® and Javelin® (*B. thuringiensis* subspecies *kurstaki*) (Guerena 2006, Maxwell and Fadamiro 2006). *Bt* is widely used (Maxwell and Fadamiro 2006) and has been shown to be reliable and effective in reducing *P. rapae* abundance while also yielding marketable crops (Endersby et al. 1992, Furlong et al. 2008, Jacques 1988, Lundgren et al. 2002, Maxwell and Fadamiro 2006, Sears et al. 1983). Since *Bt* must be ingested to be effective, proper application and full coverage of the plant, especially on the underside of the leaves where the caterpillar feed, is crucial (Caldwell et al. 2013). Furthermore, *Bt* is photo sensitive and degrades in the sunlight within 2 to 3 days of application, so frequent re-application may be necessary (Caldwell et al. 2013).

Spinosad is a fermentation by-product from a soil bacterium, *Saccharopolyspora spinose,* that has ingestion and contact activity, and it is the active ingredient in products such as Entrust® and SpinTor® (Hines and Hutchison 2001, Maxwell and Fadamiro 2006). Studies have shown that spinosad is effective in reducing *P. rapae* and other lepidopteran abundance (Burkness and Hutchison 2008, Caldwell et al. 2013, Hines and Hutchison 2001, Maxwell and Fadamiro 2006). However, care must be taken in application of spinosad, as it is highly toxic to bees and has been shown to adversely

impact natural enemies such as *Trichogramma* wasps (Cisneros et al. 2002, Mader and Adamson 2013, Maxwell and Fadamiro 2006). One goal of organic agriculture is to make decisions that cause the least amount of harm to people and the environment, and therefore, *Bt* may be a more suitable pesticide alternative than spinosad.

Additionally, botanical products such as neem have been reviewed for the management of *P. rapae* (Endersby and Morgan 1991), and may often be recommended in organic pest management (Guerena 2006). Neem, a botanical extract from the seeds and leaves of the tropical tree *Azadirachta indica*, has been shown to be an oviposition deterrent and antifeedant for several lepidopterans (Hasan and Shafiq Ansari 2011, Leskovar and Boales 1996). In a field study, Peric et al. (2009) found an 86% efficacy of the active ingredient in neem, azadirachtin, against *P. rapae* larvae, which was comparable to conventional pesticides. Neem may also prolong the development of *P. rapae* larvae, thus exposing the larva to natural enemies for a longer period of time, with little adverse effects on the *H. ebeninus* parasitoid if applied at low rates (Matter et al. 2002). Neem may need to be applied frequently because it degrades in the sun and does not persist on plant surfaces (Caldwell et al. 2013).

Cultural Practices

Floating row covers and early planting dates may provide additional prevention of *P. rapae* colonization. Floating row cover is a spun bond, lightweight fabric that is draped over the plants to act as a physical barrier to oviposition and thus prevent herbivore colonization (Endersby et al. 1992, Mahr et al. 1993). It is readily available from many suppliers and has been shown to provide adequate pest control (Endersby et

al. 1992). Though floating row covers may be difficult to implement on large scale operations, they can be an effective tool in smaller scale operations.

Planting brassicas early in the season is often a recommendation to prevent *P. rapae* colonization since the population has not yet had time to peak (Mahr et al. 1993). Maltais et al. (1998b) reported that *P. rapae* population peaked and the economic threshold was surpassed later into the season (late July) in New Brunswick where there are 3 to 4 overlapping generations of *P. rapae.* In Virginia, Gaines and Kok (1995) reported consistently high egg and larval counts in second and third generations occurring in mid-June and mid-July. Based on these studies, it appears that *P. rapae* abundance generally peaks in July and that early plantings may be effective in reducing feeding damage. However, location should be taken into consideration, as populations may vary by location (Lasota and Kok 1989).

Biological Control

Biological control is the use of natural enemies to reduce or mitigate insect pests through use of beneficial insects such as predators and parasitoids (Dreistadt 2014). Parasitoids of *P. rapae* include *Pteromalus puparum* (Hymenoptera: Eulophidae), *Phryxe pecosensis* (Townsend, Diptera: Tachinidae), *Trichogramma* sp. (Hymentopera: Trichogrammatidae), *Telenomus* sp. (Hymentopera: Scelionidae), *Hyposter ebeninus* (Gravenhorst, Hymenoptera: Ichneumonidae), *Compsilura concinnata* (Meigen, Diptera: Tachinidae), and *Cotesia rubecula,* which has widely replaced *C. glomerata* (Marshall) (Hymentopera: Braconidae) (Bryant et al. 2014, Harvey et al. 2010, Hines 1998, Mahr et al. 1993, Pfiffner et al. 2009). Predators that feed on *P. rapae* eggs and larvae include soldier bugs (Hemiptera: Pentatomidae), lady beetles (Coleoptera: Coccinellidae), hover

flies (Diptera: Syrphidae), lacewings (Neuroptera*:* Chrysopidae and Hemerobiidae), tarnished plant bugs (*Lygus lineolaris,* Hemiptera: Miridae), spiders, ants, chrysopids, staphylinids, and carabids (Bryant et al. 2014, Mahr et al. 1993, Pfiffner et al. 2009, White et al. 1995).

Trichogramma sp. and *Cotesia rubecula,* have been reported as the most effective and abundant parasitoids in natural field settings (Mahr et al. 1993). However, generalist predators may be more important than parasitoids in the mortality of *P. rapae* (Mahr et al. 1993). In addition to supporting and increasing natural enemy abundance through conservation biocontrol, growers may buy and release some of these insects to augment natural populations. *Trichogramma* species and green lacewings are available commercially (Mahr et al. 1993).

Diversified Cropping Systems

Diversification of cropping systems was originally researched as a way to develop more ecologically-based strategies to insect pest management (Hooks and Johnson 2002). These techniques have been used and anecdotally verified by growers for a long time, though their effectiveness remains controversial (Parker et al. 2013, Theunissan and Ouden 1980). Root (1973) coined the "resource competition" hypothesis, which hypothesizes that herbivores are more likely to concentrate and remain in monoculture plant stands versus diversified plant stands, because food and other resources are concentrated in monocultures. Diversification of cropping systems can aid in insect pest management through two avenues: 1) direct interference of the insect pest by disrupting biological parameters such as host location, oviposition, movement and searching, or otherwise deterring the insect pest through visual and chemical cues, masking the target

crop, or providing alternative hosts; and 2) indirect interference by providing habitat and food sources for natural enemies (predators and parasitoids) of the insect pest (Broad et al. 2008, Hokkanen 1991, Hooks and Johnson 2003, Parker et al. 2013). Ideally diversified cropping systems should support beneficial insects while also reducing herbivore colonization.

A strategy often employed in organic production to create a diversified cropping system or polyculture is companion planting (Parker et al. 2013). Companion planting is a polyculture in which a companion plant and a target crop are grown together for the benefit of the target crop. Intercropping, trap cropping, or otherwise planting cash crops near non-host plants are examples of companion planting (Parker et al. 2013). However, little scientific research validates their efficacy in suppression of *P. rapae* abundance, as most research that has been conducted shows mixed or conflicting results. Furthermore, mixed cropping systems may compete with the cash crop, complicate crop management, and increase costs and labor, so it is important to evaluate whether these strategies contribute to a reduction in herbivore pest populations.

The response of *P. rapae* to diversified cropping has been variable and inconsistent across techniques and intercrop species. Mixed cropping systems that have been evaluated in their effectiveness to directly reduce *P. rapae* populations include cover crop mulches, herbs, tomatoes (*Lycopersicon esculentum* Miller), yellow sweetclover (*Melilotus officinalis* L.), potato (*Solanum tuberosum*), chili peppers (*Capsicum annuum* L.), other brassicas, and weeds, which are summarized in the following paragraphs.

In addition to improving soil fertility, cover crop mulches have been shown to reduce insect attack (Andow et al. 1986), and several studies have investigated their efficacy in suppressing *P. rapae* when interplanted with a brassica cash crop but show conflicting results. Bryant et al. (2014) found that *P. rapae* abundance was higher on broccoli intercropped with a winter rye (*Secale cereale*) cover crop compared to hairy vetch (*Vicia villosa*) plots and bare soil plots, contradicting Bottenberg et al. (1997) who reported lower *P. rapae* abundance in the same rye mulch system. In a study evaluating cabbage interplanted with nine living mulches, Andow et al. (1986) found that second generation *P. rapae* populations were lower on cabbage interplanted with a clover (*Festuca* spp.) living mulch compared to cabbage monocultures, but first generation larvae were more abundant in the mixed cropping system. The reason for these variations in populations is not clear, as *P. rapae*'s response to nonhost plants and oviposition is complex and not well understood. Oviposition preferences may change over the generations, and/or oviposition rate may change due to establishment of undersown crops (Finch and Kienegger 1997, Hooks 2000, Radcliff and Chapman 1965).

Furthermore, some non-host plants contain chemicals that may repel or deter oviposition by herbivores (Hooks and Johnson 2003). Several studies have suggested that non-host plants such as tomato and yellow sweet clover contain chemicals that deter *P. rapae* oviposition (Renwick and Radke 1985, Tabashnik 1987). However, other studies report that odorous non-host plants have little to no negative effect on *P. rapae* populations (Hooks and Johnson 2002, Latheef and Irwin 1979, Latheef and Irwin 1983a, b, Maguire 1984). In response to organic gardening publications recommending planting herbs to repel *P. rapae,* Latheef et al. conducted three separate experiments to evaluate

these claims (Latheef and Irwin 1979, Latheef and Ortiz 1983a, b). In the first study (Latheef and Irwin 1979), cabbage was interplanted with six herbs: French marigold (*Tagetes patula* L.), garden nasturtium (*Tropacolum minus* L.), pennyroyal (*Mentha pulegium* L.), peppermint (*Mentha piperita* L.), garden sage (*Salvia officinalis* L.), and thyme (*Thymus vulgaris* L.). The results suggest that the companion plants have no effect on *P. rapae* larvae and even trend towards attracting higher *P. rapae* populations, resulting in more damage than cabbage grown in a monoculture. Latheef and Ortiz (1983a, b) found similar results in two additional studies in which they evaluated intercropped species such as hyssop (*Hyssopus officinalis* L.), southernwood (*Artemesia abrotanum* L.), wormwood (*A. absinthium* L.), tansy (*Tanacetum vulgare* L.), catnip (*Nepeta cataria* L.), santolina (*Santolina chamaecyparissus* L.), garlic (*Allium sativum*), lavender (*Lavandula angustifolia* L.), and other species, suggesting that these companion plants are also not effective. They may have even promoted higher populations, as the butterfly may have exploited the nectar of the flowering herbs as a food source, similar to what Maguire (1984) found when collards were interplanted with tomatoes.

Additional studies suggest that *P. rapae* abundance is not negatively affected by other non-host interplants, including potato (Broad et al. 2008) and spurry (*Spergula arvensis*) (Theunissan and den Ouden 1980). Broad et al. (2008) evaluated a potato/broccoli cropping system in its effectiveness to disrupt host plant location by the cabbage white butterfly and found no significant reduction in *P. rapae* egg or larval abundance. Theunissan and den Ouden (1980) hypothesized that spurry, a low growing plant with good ground coverage, grown around Brussels sprouts would provide habitat for predators and parasitoids of *P. rapae* as well as mask the Brussels sprouts from the

cabbage white butterfly. They reported no significant differences in *P. rapae* abundance on Brussels sprouts grown with spurry compared to those grown in bare-soil monocultures. The results of both of these studies are likey due to the cabbage white butterfly's superior host finding ability and active egg laying activity.

Moreover, several studies have evaluated the effects of mixed cropping to support natural enemies that serve as biological control agents of *P. rapae,* thus potentially enhancing control of *P. rapae* populations indirectly. Diversification of cropping systems may provide habitat, nectar, pollen, and alternative prey for natural enemies, enhancing their longevity, fecundity, and population (Harvey and Wagenaar 2006, Parker et al. 2013).

Many adult predators and parasitoids rely on floral resources to meet nutritional needs (Parker et al. 2013, Philips et al. 2014b), so flowering plants grown near target crops may elicit biological control of *P. rapae* indirectly through support of natural enemies. Zhao et al. (1992) reported a 39.9% parasitism rate of *P. rapae* by the parasitoid wasp, *Cotesia rubecula*, in broccoli interplanted with nectar-producing plants compared to a 26.2% parasitism rate in a broccoli monoculture and a 22.2% rate in broccoli grown adjacent to nectar-producing plants. Philips et al. (2014a) found an overall parasitism rate by *C. glomerata* of 68% when collards were grown near buckwheat (*Fagopyrum esculentum* Moench) with no significant difference to distance between collards and buckwheat. Lee and Heimpel (2005) concur, finding an increased parasitism rate of *P. rapae* on cabbage in fields close to buckwheat.

However, the extent of biological control is unclear and may differ across location. Pfiffner et al. (2009) found that *P. rapae* parasitism rates were significantly

higher in plots next to a wildflower strip at one site location but not the second location, suggesting site-specific factors more strongly influence parasitism than the presence or absence of wildflower strips alone. Across seven locations in northwestern Spain, parasitism rate and parasitoid species richness varied significantly with locality (Santolamazza-Carbone et al. 2013).

Moreover, since lepidopteran butterflies feed on nectar, these same flowering plants may increase abundance of *P. rapae*, and several of the aforementioned studies also found that *P. rapae* populations were increased when the cash crop was grown in close proximity to flowering plants (Hooks and Johnson 2002, Lee and Heimpel 2005). For example, Zhao et al. (1992) found that *P. rapae* eggs and larvae were more abundant on broccoli interplanted with nectar-producing plants than on broccoli grown in a monoculture.

Additionally, non-flowering vegetation, such as weeds – often present on organic farms – and other cruciferous crops, may also influence herbivore and natural enemy abundance. Weeds can provide cover and a favorable microhabitat for arthropod predators such as coccinellids, carabids, and staphylinids. Schellhorn and Sork (1997) found a higher abundance of these predators in collard-weed polycultures compared to collard monoculture, which may have been responsible for *P. rapae* mortality observed in these plots. Brassica cultivar may also influence host-parasitoid interactions. Results from Santolamazza-Carbone et al. (2013) show that parasitoid species richness was higher while herbivore abundance was lower in cabbage than kale. They also showed that the abundance of *P. rapae*-specific parasitoids (*Telenomus* sp. and *C. glomerata*) was higher in kale, which may have accounted for the higher parasitism rate of *P. rapae* in kale.

In many cases, yield is reduced in intercropped plantings due to competition from the intercrop (Andow et al. 1986, Bryant et al. 2014, Dempster 1969, Hooks and Johnson 2001, 2002, Latheef and Ortiz 1983b), and thus reduction in herbivore abundance may not negate the reduction in yield. This is a large constraint when incorporating diversified plantings in an organic pest management program. More research must be done to evaluate the economic trade-offs.

Diversification alone may not be enough to elicit control of *P. rapae*, and there are numerous challenges associated with mixed cropping systems. The optimum intercrop-cash crop mixture is not clear, and there is large variation and inconsistency in effectiveness of intercrop species in direct and indirect suppression of *P. rapae*. Ideal proximities and locations of the companion plants are also not entirely known. Host location and oviposition behavior of *P. rapae* and tri-trophic interactions are complex and not fully understood, complicating pest management decisions. Furthermore, yield reduction, economic feasibility and pay-off, and logistics of growing multiple crops are additional challenges to implementing diversified cropping systems in an insect pest management program.

Host Plant Resistance

As sessile organisms, plants have evolved a multitude of defense strategies against insect and pathogen attack (Ratzak et al. 2002), and growers may use this to their advantage by cultivating plants that are more naturally resistance to insect colonization. Inherent traits found in brassicas that may play a role in resistance against *P. rapae* include chemical compounds such as glucosinolates and tactical or visual traits such as color and waxy or glossy surfaces, and these can vary by species and even cultivar.

Several studies have evaluated brassica species and cultivars and attempted to explain these differences by plant characteristics such as genotype, glucosinolate content, morphology, and nutritional content. For example, Broekgaarden et al. (2010) found lower *P. rapae* abundance on 'Rivera' cabbage cultivar compared to 'Christmas Drumhead' in a natural field setting. Previous studies have shown *P. rapae* oviposition preference for 'Christmas Drumhead' (Poelman et al. 2009) as well as improved larval performance on this cultivar (Broekgaarden et al. 2007, Poelman et al. 2009). Broekgaarden et al. (2007, 2010) attribute these differences to expression of defense genes in the two cultivars while Poelman et al. (2009) attribute differences to glucosinolate diversity, chiefly glucoiberin content. Though glucosinolates (GSLs) are toxic to a wide range of generalist insect pests, the interactions between GSLs and *P. rapae* are complex, as *P. rapae* is a specialist that has co-evolved with brassicas. As such, it is not entirely clear how and to what extent GSLs and their breakdown products affect *P. rapae* oviposition and larval fitness, and this plant-insect interaction has been the focus of many studies and several reviews (Ahuja et al. 2010, Hopkins et al. 2009). Additionally, in a study in Spain, *P. rapae* abundance did not significantly differ between kale and cabbage, but significantly more larvae and pupae were parasitized on kale than cabbage, and the authors suggest the differences may be due to the morphological and/or foliar nutritional content (Santolamazza-Carbone et al. 2013).

Moreover, constitutive traits such as glossiness and color may affect *P. rapae.* The glossy cauliflower PI 234599 has been shown to be more resistant to *P. rapae* than other varieties and has been used in breeding programs (Dickson and Eckenrode 1975). Stoner (1990) evaluated several genetic lines of broccoli, cauliflower, Brussels sprouts,

collards, and kale that differed in their glossiness and found that the glossy lines consistently had lower *P. rapae* egg and larvae abundance (Stoner 1990), which Shelton et al.'s (1988) study also concluded on several genetic varieties of green and red cabbage. In a follow up study, Stoner (1992) further concluded that glossy lines of broccoli and cauliflower were more resistant to *P. rapae* colonization. Moreover, Shelton et al. (1988) found that green cabbage varieties were more susceptible to *P. rapae* damage than red varieties. Red varieties have also been less preferred in the greenhouse and field in oviposition studies (Dickson and Eckenrode 1975).

It appears that brassica cultivars and species differ in their natural resistance against *P. rapae* to some extent, but *P. rapae* co-evolved with hosts and may have adapted to some defensive characteristics of brassicas. The mechanisms of resistance in brassicas are yet unclear, and more research must be done to investigate the mechanisms, especially on cultivated brassicas in agricultural settings.

1.4 Conclusion

There are numerous novel management strategies that are compatible with organic production systems, and several strategies may need to be used simultaneously for successful management of *P. rapae*. The reduced-risk biopesticides *Bt*, spinosad, and neem are effective in reducing larval abundance. However, though considered reduced-risk, care should still be taken in application, especially with spinosad as spinosad can adversely affect beneficial predators and parasitoids. Pesticide resistance is also a concern with biopesticides, and rotation of biopesticide class should be implemented.

There are numerous natural predators and parasitoids of *P. rapae. C. glomerata* and *Trichogramma* sp. appear to be the most effective parasitoids, but generalist

predators such as lady beetles and green lacewings may be more important in managing *P. rapae* abundance. Organic pest management is often a long-term approach to insect management, and efforts should focus on conservation biocontrol which emphasizes augmenting the abundance and diversity of naturally occurring beneficial insects by providing food (nectar, pollen, honeydew) and shelter.

Crop diversification in a brassica agroecosystem shows mixed results in *P. rapae* management. Most studies suggest that diversification alone is not enough to elicit control of *P. rapae.* Diversification may even increase larval populations in some cases, as the adults exploit floral resources of the diversified crop. The primary benefit of a diversified cropping system appears to be the support of beneficial predators and parasitoids of *P. rapae,* which can contribute to control of larvae. More research needs to be done to evaluate the efficacy of companion planting as well as the economic trade-offs.

The relationship between brassicas and *P. rapae* is complex and not yet well-understood. *P. rapae* co-evolved with brassicas and seem to have adapted to the brassica defense system to some extent. Understanding the mechanisms of oviposition preference and selection of host-plant would improve crop protection, and more research needs to be done to elucidate these interactions. More applied research should also be done in field settings to determine susceptible and resistant brassica cultivars and the mechanisms for host plant resistance.

2 Effects of *Pieris rapae* herbivory on glucosinolate concentration and larval growth and performance on broccoli (*Brassica oleracae* var. *italica*)

2.1 Summary

A controlled greenhouse study evaluated the effects of *Pieris rapae* herbivory on glucosinolate (GSL) induction and subsequent larval growth and performance on two broccoli (*Brassica oleracea* var. *italica*) cultivars ('Beneforte' and 'Green Magic'). 'Beneforte' was chosen for its higher than average levels of GSL while 'Green Magic' was chosen for its lower than average GSL concentrations. Fourth instar *P. rapae* fed on broccoli plants, and plant response was measured by changes in GSL concentrations, specifically neoglucobrassicin and glucobrassicin. Second instar *P. rapae* then fed on plants, and days to pupation, pupal weight, and leaf area consumed were measured. Neoglucobrassicin and glucobrassicin concentrations did not significantly increase as a result of herbivory, but the overall growth and performance of *P. rapae* was influenced by cultivar. Larvae consumed less total leaf area and weighed less as pupae when feeding on 'Beneforte', which also had a significantly higher glucobrassicin concentration, indicating that cultivar may be important in resistance against *P. rapae.* It appears that cultivar is more important in larval growth and performance than the presence or absence of previous *P. rapae* damage.

2.2 Introduction

As sessile organisms, plants have evolved a multitude of defense strategies against insect and pathogen attack, and these defenses can be innate and/or induced. Innate defenses are constitutively present in a plant, regardless of the presence or absence

of attack, and are often the first line of defense. Examples of innate defense traits include morphological characteristics, such as trichomes and thorns, that act as physical barriers and chemical defenses, such as secondary metabolites and allelochemicals, that are antixenotic or antibiotic (Walling 2000). An induced response is when a plant responds to insect or pathogen attack by activating or increasing certain defensive traits. Induced responses are important to resistance against insect attack because the plant is able to selectively allocate resources to otherwise metabolically costly defenses, using damage as an induction cue, to resist future attack, ideally negatively affecting subsequent insects (Poelman et al. 2008b). Induced responses also often involve a systemic response. For example, a plant may be attacked on one part, and the plant then responds by increasing defensive traits in undamaged parts, thereby increasing the defensive capacity of the entire plant (Kaplan et al. 2008).

The glucosinolate-myrosinase system is a well-studied, classic example of a plant defensive system that is both innate and inducible. Glucosinolates (GSL) are characteristic secondary metabolites constitutively present in members of the Brassicaceae (mustard) family. There are more than 120 known GSL compounds divided into three classes: indolic (derived from tryptophan); aliphatic (derived from methionine, alanine, valine, leucine, and isoleucine); and aromatic (derived from phenylalanine or tyrosine). GSLs have long been known for their antifungal, antibacterial, and other antibiosis properties as well as their role in herbivory and plant defense (Fahey et al. 2001). Many studies have shown that the concentration of GSLs in brassica foliage changes in response to insect herbivory, indicating that GSLs are also an inducible

defense trait (Gols et al. 2008b, Kos et al. 2012, Poelman et al. 2008b, Traw and Dawson 2002, van Dam et al. 2005).

Upon cell rupture, myrosinase enzymes hydrolyze GSLs, and the resulting breakdown products, including isothiocyanates, thiocyanates, and nitriles, are noxious products known as "mustard oil bombs" (Ratzka et al. 2002). These products are toxic to a wide range of generalist insects and decrease generalist performance and growth (Poelman et al. 2008b, Textor and Gershenzon 2009). However, the interactions between GSLs and specialist insects are complex. Some specialists have developed strategies to circumvent the toxicity of these breakdown products, including detoxification, sequestration, and excretion (Hopkins et al. 2009).

One such specialist insect is *Pieris rapae* (Linneaus, Lepidoptera: Pieridae), known commonly as the imported cabbageworm. *P. rapae* is a serious pest in horticultural crop production, as the larva chews large holes and can contaminate the high-value crops of the *Brassica oleracea* species, including broccoli (var. *italica*), cauliflower (var. *botrytis*), and cabbage (var. *capitata*), rendering the produce unmarketable. *P. rapae* has co-evolved with these and other brassica species, and as such, it is not entirely clear how and to what extent innate and induced responses in brassicas, particularly GSLs and their breakdown products, affect *P. rapae* fitness and performance.

There does not appear to be a general pattern that indicates a positive or negative relationship between herbivore damage and subsequent larval fitness. Some studies indicate that initial herbivore damage may deter further feeding or reduce subsequent herbivore health on the damaged plant, mediated by an induced plant response. Parameters that measure larval health includes time spent on the host plant, weight gain,

fecundity, mortality, and leaf consumption, and these can affect subsequent generations of *P. rapae*. For example, damage on black mustard (*Brassica nigra*) from flea beetles (*Phyllotreta cruciferae*) and *P. rapae* reduced subsequent damage from these pests as the plant matured and reduced growth rate and survival of herbivores that were fed new leaves from plants that were previously damaged (Traw and Dawson 2002). Agrawal (2000b) concurs, finding that *P. rapae* weight was significantly reduced on wild radish (*Raphanus sativus*) plants that were previously damaged by conspecifics or *Trichoplusia ni.* Additionally, Zhang et al. (2013) show that *P. rapae* development was slower and larval weights were lower when feeding on plants previously infested by *Bemisia tabaci*. However, in two separate studies, Agrawal (1999, 2000a) did not find a significant difference in the growth of *P. rapae* feeding on wild radish and *Lepidium virginicum* previously damaged by *P. rapae.*

Moreover, studies also report conflicting results about the effects of GSLs on *P. rapae* health. Some studies have reported that larvae are adversely affected by GSLs and breakdown products, exhibited by reduced survival or prolonged development time (Agrawal and Kurashige 2003, Gols et al. 2008b, Kos et al. 2012). Other research has shown that GSLs and their breakdown products have little to no effect on larvae fitness (Mumm et al. 2008, Poelman et al. 2008b, Santolamazza-Carbone et al. 2014, Wittstock et al. 2004). One hypothesis to explain why GSLs do not negatively affect *P. rapae* larvae is because, being specialist insects, the larva may be able to circumvent the toxicity of isothiocyanates by redirecting breakdown products into less-toxic nitriles through a nitrile-specifier protein in the gut (Wittstock et al. 2004), which allows the larvae to feed without adverse effects. Additionally, some research has even suggested

that specific GSLs, such as gluconasturtiin, may be feeding stimulants for this specialist (Miles et al. 2005, Muller et al. 2010).

Although *P. rapae* feeding on wild brassicas is a well-studied plant-herbivore interaction, the relationship is still poorly understood, and little research has been done to explore induced resistance against *P. rapae* on cultivated brassica species. This research addresses *P. rapae* feeding on the induction of GSLs and the effects on subsequent *P. rapae* larvae feeding on two broccoli (*Brassica oleracea* var. *italica*) cultivars in a controlled greenhouse setting. In our study, we tested i) if damage by *P. rapae* influences broccoli defense by inducing glucosinolates, specifically glucobrassicin and neoglucobrassicin; and ii) if previous herbivore damage affects subsequent *P. rapae* growth and performance due to induced glucosinolates.

2.3 Materials and Methods

Plant Material

Two broccoli (*Brassica oleracea* var. *italica*) cultivars that vary in GSL concentration were selected as model crops. 'Green Magic' (Johnny's Seeds, Winslow, ME) was chosen due to its lower-than-average GSL concentration, specifically glucoraphanin. It is also a common cultivar in commercial broccoli production. 'Beneforte' (Seminis, St. Louis, MO) was chosen due to its higher-than-average GSL concentration, specifically glucoraphanin, as it was developed to assimilate more sulfate for GSL production (Armah et al. 2013).

Untreated broccoli seeds were sown in 50 cell plug trays with compost-based potting mix (Purple Cow Organics, LLC, Middleton, WI). Plants were grown in a greenhouse with 21ºC daytime temps and 15.5ºC nighttime temps and 12 hours of

supplemental daylight (6:00h to 18:00h). Plants received water daily as needed, and beginning two weeks after sowing, plants were fertilized with 26 mL/gal of 2N-3P-1K fish emulsion (Neptune's Harvest, Gloucester, MA) to provide 150 ppm nitrogen weekly until the end of the experiment. At approximately one month old, seedlings were transplanted into 6" standard pots with the same potting mix.

Insect Material

Adult *Pieris rapae* were acquired from a lab colony containing offspring from wild-caught cabbagewhite butterflies in Minnesota, but some genes were mixed from butterflies in surrounding states. Two-month old broccoli ('Green Magic') plants used exclusively for *P. rapae* rearing were exposed to multiple gravid females until desired number of eggs were oviposited (~4 hours). Eggs hatched and larvae developed on the broccoli in a greenhouse with 21°C daytime temps and 15.5°C nighttime temps and 12 hours of supplemental daylight (6:00h to 18:00h). In the third replication, larval supply was supplemented with 4th instars from Carolina Biological (Burlington, NC) for the feeding damage phase of the experiment.

Objective One – Pieris rapae *Feeding Damage*

Three replicate experiments were performed in one year (Table 2.1) at the University of Minnesota – Twin Cities Plant Growth Facilities in St. Paul, MN.
The experiment was a Completely Randomized Design with pots randomly arranged on a greenhouse bench. There were eight replicates of three damage treatments x two broccoli cultivars. When plants were between 49 - 55 days old, they were infested with 4th instar *P. rapae* in order to cause feeding damage to plants. Damage treatments consisted of varying levels of infestation to inflict: no damage (zero larvae as a control), light damage

(one larva), or heavy damage (4 larvae). Larvae were added to the top of one young broccoli leaf per plant and contained to the leaf by enclosing leaf and larvae in a 10 x 30 cm mesh drawstring bag (BugDorm, Taichung, Taiwan). Control plants received bags without larvae. Larvae were allowed to feed for 72 hours and then removed.

Due to the need to remove leaf material for GSL analysis, GSL could not be measured in the same plants used for subsequent herbivore growth and performance. Therefore, after larvae were removed, four of the replicates were analyzed for GSL content (objective 1) and the other four were used to measure *P. rapae* growth and performance (objective 2).

Glucosinolate Extraction and Analysis

To test our first objective, GSLs were extracted from control (n = 12), lightly-damaged (n = 12), and heavily-damaged (n = 12) plants from each cultivar. GSL quantification and extraction methods were adapted from Rosen et al. (2005). The four youngest, fully expanded leaves on plants reserved for GSL analysis were collected on the same day that 4th instars were removed. Samples were stored at 4℃, prepared for analysis, and frozen at -80℃ within 8 hours of collection. Samples were boiled in six times the weight per volume of deionized water for five minutes to deactivate myrosinase and then macerated for two minutes in a blender. A 40-mL aliquot of the macerated sample was stored at -80℃ until GSL analysis could be performed.

Desulfonated glucosinolates (df-GSL) were extracted on solid phase strong-anion exchange (SAX) columns (Sigma-Aldrich, St. Louis, MO) before identification and quantification. Samples were thawed and then homogenized for two minutes at 12,000

rpm with a Polytron PT 1300 D homogenizer (Kinematica AG, Lucerne, Switzerland). A 2-mL sample of the aliquot was centrifuged for 4 minutes at 4°C and 8000 rcf. SAX columns were prepared on a vacuum manifold by washing with 2-mL of 0.5 M sodium acetate (pH 4.6) buffer followed by 2-mL of deionized water. A 500-µL sample of centrifuged broccoli supernatant was then filtered through the SAX column, followed by 1-mL of 0.02 M sodium acetate (pH 4.0) buffer. To desulfate glucosinolates, 1-mL of a sulfatase solution (aryl-sulfate sulfohydrolase from *Helix pomatia* - Type H-1; Sigma-Aldrich, St. Louis, MO) was added to the column, and columns sat at room temperature (~21℃) for ~15 hours. Samples were then eluted with 3-mL of deionized water, and eluent was weighed to determine volume. Finally, eluents were filtered through 0.2 micron PTFE filters (Chrom Tech, Apple Valley, MN).

HPLC analysis was performed on an Agilent 1200 Series Quaternary system (Agilent Technologies, Inc., Santa Clara, CA). GSL were separated on a Luna C18 column (5 µm, 250 x 4.6 mm; Phenomenex, Torrence, CA) set at 30℃ and detected with a diode array detector set at λ=229 nm. A 50-µL aliquot of the eluent was separated with the following flow rates and gradients: solvent A = water and solvent B = acetonitrile; 0 minutes: 95% A + 5% B, 1.0 mL·min^{-1}; 0-2 minutes: 85% A + 15% B, 1.0 mL·min^{-1}; 2-20 minutes: 53% A + 47% B, 1.0 mL·min^{-1}; 20-22 minutes: 0% A + 100% B, 1.15 mL·min^{-1}; 22-26 minutes: 0% A + 100% B, 1.3 mL·min^{-1}; 26-28 minutes: 0% A + 100% B, 1.0 mL·min^{-1}; 28-34 minutes: 95% A + 5% B, 1.0 mL·min^{-1}. GSL peaks were presented using OpenLAB Chromatography Data System with rev. C.01.06 software. Peaks were identified using relative retention times, and ds-GSL concentrations were

calculated using relative quantification with an internal standard (sinigrin) and previously published response factors (EU 1990). Concentrations are reported on a $\mu mol \cdot g^{-1}$ fresh weight (fw) basis.

Objective Two - Pieris rapae *Growth and Performance*

To test our second objective, growth and performance of *P. rapae* was assessed on control (undamaged), lightly-damaged, and heavily-damaged plants. Growth was measured by pupal mass and days to pupation while performance was measured as leaf area consumed. Three days after the removal of initial 4^{th} instar *P. rapae*, one 2nd instar was added to the youngest fully expanded leaf of each plant and enclosed in the same way as initial larvae. Larvae were allowed to feed freely on the one leaf until pupation. To determine feeding performance, leaves were scanned with a LC4800P color optical scanner (Regent Instruments Inc., Ville de Quebec, Canada) and traced using WinFOLIA Basic software (Regent Instruments Inc., Ville de Quebec, Canada) to quantify total leaf area removed.

To account for reduction of the number of surviving larvae on plants and correct unbalanced samples sizes, each *P. rapae* fitness parameter was censored by randomly discarding some observations so that $n = 8$ for each parameter.

Statistical Analysis

Data were analyzed using R statistical software version 3.1.2 (The R Foundation for Statistical Computing, Vienna, Austria). Days to pupation data were log transformed to obtain normality; analysis was done on transformed data but untransformed means are reported. The significance of differences between treatments for GSL concentrations and *P. rapae* fitness parameters was determined using a one-way ANOVA. Significance of

treatment by variety was done using a two-way ANOVA. Differences in mean values were identified using Tukey's Honest Significant Difference (HSD) and were considered significant at the $P < 0.05$ level.

2.4 Results and Discussion

Glucosinolate Concentrations

Concentrations of GSLs were significantly affected by cultivar (Tables 2.2 and 2.3), which is what we expected because we selected 'Beneforte' and 'Green Magic' specifically for their differences in GSL concentration. Glucobrassicin was significantly higher in 'Beneforte' (Tables 2.4a and 2.2, P=5.21e-10) overall while, unexpectedly, neoglucobrassicin was slightly higher in 'Green Magic' (Tables 2.4b and 2.3, P=0.0437). The primary GSLs found in broccoli include glucoraphanin (4-methylsulfinylbutyl-glucosinolate), glucobrassicin (3-indolemethyl-glucosinolate), gluconapin (3-butenyl-glucosinolate), and neo-glucobrassicin (1-methoxy-3-indolylmethyl-glucosinolate) (Kushad et al. 1999, Rosa and Rodrigues 2001). Our results show that neoglucobrassicin and glucobrassicin were the most abundant indolic glucosinolates in broccoli foliage.

Interestingly, feeding by 4th instar *P. rapae* did not significantly affect the concentrations of neoglucobrassicin or glucobrassicin in any of the damage treatments (P=0.677 for glucobrassicin and P=0.4456 for neoglucobrassicin). This is contradictory to many of the studies that have evaluated induced responses to *P. rapae* herbivory in brassicas. Many studies have report that GSL concentration in brassica foliage increases as a result of herbivory (Gols et al. 2008b, Kos et al. 2012, Poelman et al. 2008b, Traw and Dawson 2002, van Dam et al. 2005). Perhaps we did not observe an increase in GSL because domesticated brassicas have lower GSL concentrations than wild brassicas (Gols

et al. 2008b). Gols et al. (2008b) found that *P. rapae* herbivory significantly increased the concentrations of neoglucobrassicin and glucobrassicin in wild brassicas but saw no significant differences in domesticated brassicas, and they suggest that this difference may be due to the reduction of direct defenses in domesticated brassicas from artificial selection.

Objective two - Pieris rapae *Growth and Performance*

Our results show that the overall growth and performance of *P. rapae* is differentially influenced by cultivar. Overall, larvae fed significantly less (Figure 2.2a; P=0.00733), and pupae weighed significantly less on 'Beneforte' than on 'Green Magic' (Figure 2.2b; P=0.0158). This is similar to Poelman et al.'s (2008a) results, in which they found that *P. rapae* feeding on the 'Rivera' cabbage cultivar had significantly lower mass compared to those feeding on 'Badger Shipper.' Another study has also shown that *P. rapae* fed significantly less and took longer to develop on a kale (*Brassica oleracea* var. *acephala*) variety with a higher concentration of glucoiberin. We propose that this study be done with a wider variety of cultivars, especially cultivars that are commonly used in agricultural systems in order to get a better understanding of natural resistance to *P. rapae* feeding in an agricultural setting.

However, we did not observe a strong positive relationship between previous herbivory and subsequent *P. rapae* growth and performance. In only once instance larval performance was significantly different between damage treatments. Leaf area consumed was significantly less on lightly-damaged 'Green Magic' plants than on heavily-damaged 'Green Magic' (P=0.01) but not significantly different from undamaged control plants; however, this trend was not observed when larvae fed on previously damaged

‘Beneforte’. None of the other treatment and variety combinations showed differential effects on *P. rapae* growth and performance. This is similar to a study done by Agrawal (1999), in which the author did not observe any significant effects of induced plant response on the growth of *P. rapae*.

Effects of Glucosinolates on Pieris rapae

Our research appears to provide evidence that the indolic GSL, glucobrassicin, has a differential effect on the fitness and performance of *P. rapae*. Our data points to a relationship between a higher concentration of glucobrassicin found in ‘Beneforte’ and the more negative fitness and performance of the *P. rapae* feeding on this cultivar. However, due to the need to remove leaf material to measure GSL concentration, we could not collect leaf samples from the same plants that received a subsequent infestation of *P. rapae*. Therefore, we could not make direct correlations between GSL concentrations and *P. rapae* fitness and performance parameters on the same plants.

3.5 Conclusion

Our data reaffirms that glucosinolate concentration varies by cultivar, and the overall growth and performance of *P. rapae* is more influenced by these cultivar differences than by previous herbivory. ‘Beneforte’ had a significantly higher glucobrassicin concentration while also more negatively affecting larval growth and performance, as larvae feeding on ‘Beneforte’ both consumed less total leaf area and weighed less as pupae. We did not, however, observe a positive relationship between previous herbivory and current GSL concentrations. Neoglucobrassicin and glucobrassicin concentrations did not significantly increase as a result of herbivory. It

appears that cultivar is more important in larval growth and performance than the presence or absence of previous *P. rapae* damage.

Table 2.1 Schedule of activities in 2016

Replication	Seeds sown	Seedlings potted	4th instars introduced (age of plants)	2nd instars introduced (age of plants)
1	16-Feb	16-Mar	11 April (55 days)	17 April (61 days)
2	16-Mar	12-Apr	6 May (51 days)	12 May (57 Days)
3	28-Jun	27-Jul	19 August (52 days)	25 August (58 days)

Table 2.2 Analysis of variance for glucobrassicin in broccoli foliage after *P. rapae* feeding; * is significant at P<0.001**

Reponse:	Glucobrassicin				
	d.f.	Sum Sq	Mean Sq	F value	P(>F)
Cultivar	1	0.8471	0.8471	54.097	5.21e-10***
Treatment	2	0.0128	0.0065	0.407	0.677
Cultivar:Treatment	2	0.0012	0.0006	0.037	0.964
Residuals	62	0.9709	0.0157		

Table 2.3 Analysis of variance for neoglucobrassicin in broccoli foliage after *P. rapae* feeding; * is significant at P<0.05

Reponse:	Neoglucobrassicin				
	d.f.	Sum Sq	Mean Sq	F value	P(>F)
Cultivar	1	0.373	0.373	4.239	0.0437*
Treatment	2	0.144	0.0721	0.819	0.4456
Cultivar:Treatment	2	0.023	0.0115	0.131	0.8775
Residuals	62	5.456	0.088		

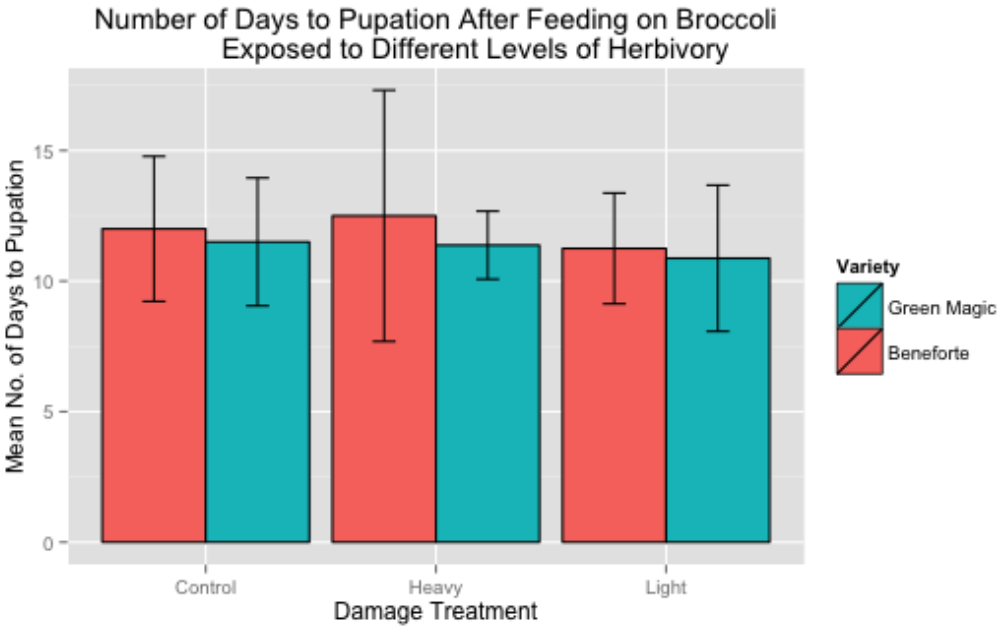
Table 2.4 Comparison of the mean values of a) glucobrassicin and b) neoglucobrassicin in foliage of two broccoli cultivars after damage by *P. rapae* feeding; ** is significant at P<0.01; * significant at P<0.001**

a	Glucobrassicin (µmol·g-1 fresh weight)		
Cultivar	No damage	Light damage	Heavy damage
Beneforte	0.37 ± 0.16	0.40 ± 0.14	0.41 ± 0.21
Green Magic	0.16 ± 0.02	0.18 ± 0.06	0.18 ± 0.03
ANOVA p values	0.0004421***	1.843e-05***	0.001398**

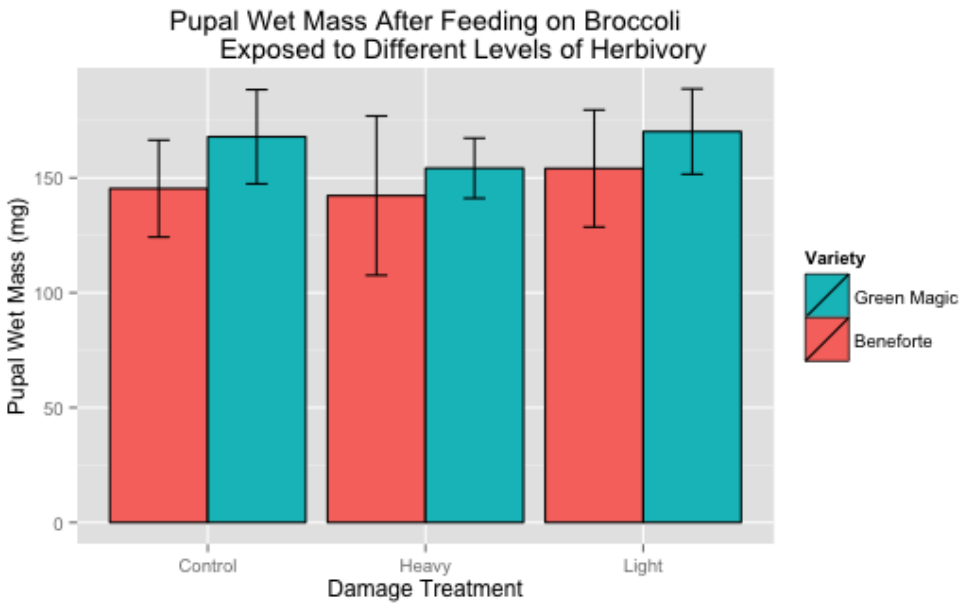
b	Neoglucobrassicin (µmol·g-1 fresh weight)		
Cultivar	No damage	Light damage	Heavy damage
Beneforte	0.32 ± 0.16	0.45 ± 0.22	0.43 ± 0.25
Green Magic	0.50 ± 0.31	0.56 ± 0.36	0.60 ± 0.38
ANOVA p values	0.11567	0.4433	0.2161

Figure 2.1 *P. rapae* growth and performance parameters after feeding on undamaged and damaged broccoli plants: a) number of days to pupation, b) pupal fresh mass, and c) leaf area consumed.

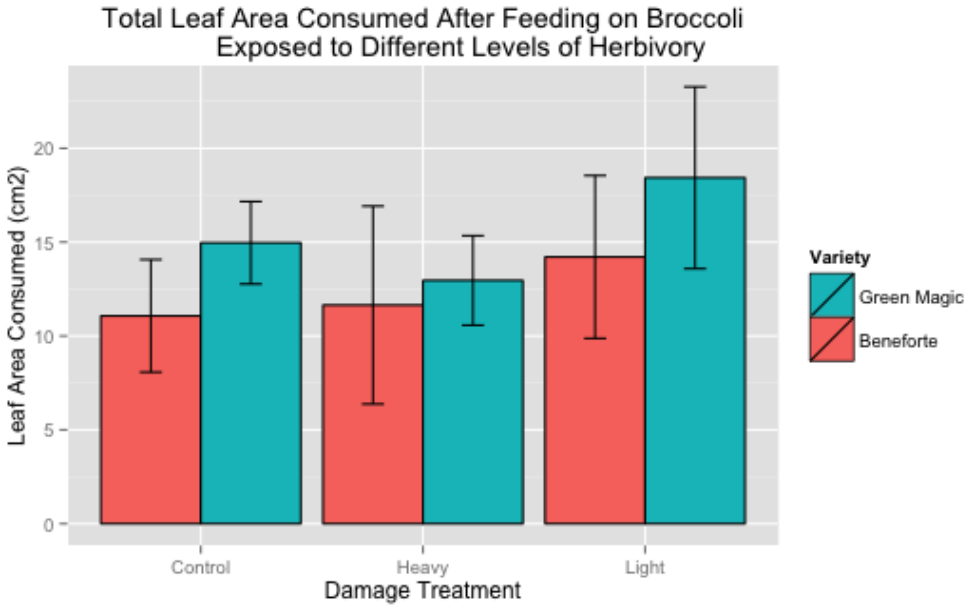
a



b



c


Total Leaf Area Consumed After Feeding on Broccoli
Exposed to Different Levels of Herbivory
Leaf Area Consumed (cm2)
20
15
10
5
0
Control
Heavy
Light
Damage Treatment
Variety
Green Magic
Beneforte

3 Evaluation of six broccoli (*Brassica oleracae* var. *italica*) cultivars for natural pest resistance and performance in organic production systems in Minnesota

3.1 Summary

A field study was performed in 2015 and 2016 at two sites to evaluate six broccoli cultivars (‘Belstar,’ ‘Fiesta,’ ‘Green Magic,’ ‘Marathon,’ ‘Packman,’ and ‘Thompson’) for natural resistance to *Pieris rapae* and for performance in yield and crown quality. The sites were certified organic farms: Garden Farme in Ramsey, MN and Cornercopia in St. Paul, MN. Seeds were started in a greenhouse and transplanted at one month old in early July. *P. rapae* eggs and larvae were counted weekly, and glucosinolate concentrations were measured in foliage collected from plants at early-heading stage in mid-August. GSL concentration and egg and larval counts were correlated. Crowns were weighed and sorted into marketable and unmarketable product. *P. rapae* egg and larval counts were not significantly different on any of the cultivars, and neoglucobrassicin and glucobrassicin concentrations did not explain *P. rapae* egg and larval abundance. We conclude that cultivar selection should not be based on natural resistance to *P. rapae* in this case but rather based on cultivar performance. ‘Belstar,’ ‘Fiesta’, ‘Green Magic,’ and ‘Packman’ were top performers and produced a consistent and high quality product over year and locations.

3.2 Introduction

Broccoli (*Brassica oleraceae* L. var. *italica*) belongs to the Brassicaceae (mustard or cruciferous), a large and economically important family with 375 genera and 3200 species. Broccoli is a *Brassica oleracea* species, which also includes other important

horticultural crops such as cauliflower (var. *botrytis*), Brussels sprouts (var. *gemmifera*), kale (var. *acephala*), kohlrabi (var. *gangylodes*), cabbage (var. *capitata*), and other specialty crops. Broccoli continuously ranks among the top most nutritious vegetables, providing vitamin C, iron, calcium, vitamin A, and fiber as well as chemopreventative compounds and antioxidants (Ahuja et al. 2010, Jahangir et al. 2009). Broccoli is also the most valuable cruciferous crop, estimated at $900M annually nationwide (NASS 2014). Furthermore, organic broccoli is a top-selling organic produce item and fetches a price premium of more than 120% over conventional broccoli (Oberholtzer et al. 2005).

Broccoli is produced in nearly every U.S. state with the majority of production in California, Arizona, Texas, and Oregon (Orzolek et al. 2012). However, consumer demand in Minnesota is shifting towards regionalized produce. There is a thriving and growing local foods community in Minnesota, including over 150 farmers markets, more than 40 food cooperative retail stores, and efforts such as farm-to-school programs and farm-to-table restaurants, that demand high-quality, nutritious, locally-grown produce. Minnesota is also in a unique position to produce broccoli and other brassicas due to its diversity of soil conditions and cool temperatures.

However while demand for local produce has increased at the consumer level, there has not been a concurrent increase in research to meet those demands. In surveys done in 2009, 2011, and 2014 by the Minnesota Department of Agriculture, organic fruit and vegetable growers ranked “organic variety trials”, “insect pests and management”, and “variety development” as a several of the top five research priorities (Moynihan 2010, 2016). Though there are recent broccoli cultivar recommendations for the Upper Midwest, these recommendations assume conventional management (Midwest Vegetable

Production Guide for Commercial Growers 2016). In organic systems, crops need to yield a consistent and high-quality product over diverse, sometimes sub-optimal, climatic conditions and endure various biotic and abiotic stressors, and therefore cultivar trials in organic systems should be performed to determine adaptability to local growing conditions and performance in systems with restricted agronomical practices.

Because pest management options are limited in organic systems, natural host resistance against insect pests can be an important management strategy. The insect pest *Pieris rapae* Linneaus (Lepidoptera: Pieridae), commonly referred as the imported cabbageworm, is a major brassica pest in Minnesota that feeds exclusively on members of the Brassicaceae. Larvae can damage developing heads of broccoli, cauliflower, and cabbage and contaminate heads with frass, and tolerance for insect damage and contamination is a near zero in these crops (Maxwell and Fadamiro 2006). Organic brassica growers traditionally rely on exclusion techniques, such as floating row covers, or organic approved pesticides, such as *Bacillus thuringiensis* (Bt) and spinosad, to avoid *P. rapae* damage. However, though effective, these strategies are expensive and increase labor, and organic compliant pesticides may negatively impact beneficial insects and pollinators. Understanding the roles that glucosinolates (GSL), naturally occurring defensive compounds, play in mediating plant-insect interactions may provide insight into natural host plant resistance against insect *P. rapae*. However, though brassicas may exhibit natural antibiosis against generalist insect pests due to GSLs, the relationship between GSL and *P. rapae* is complex and still poorly understood on cultivated crops. More research must be done linking this knowledge to practical pest management.

Our research aims to evaluate six broccoli cultivars for their natural pest resistance and performance in organic systems, and our objectives are: 1) to determine natural *P. rapae* egg and larval populations; 2) to determine glucosinolate content and correlate to insect pressure; and 3) to determine yield and crown grading. The results of this study will inform growers about cultivars that may be naturally pest resistant and perform well in organic systems, thereby increasing Minnesota's ability to produce high-quality, locally-grown organic broccoli.

3.3 Materials and Methods

Plant Material

This experiment was replicated over two years, in 2015 and 2016. On 1 June 2015 and 7 June 2016, seeds of six broccoli cultivars (*Brassica oleracea* var. *italica*), 'Belstar' (Johnny's Seeds, Winslow, ME), 'Fiesta'(Territorial Seed, Cottage Grove, OR), 'Green Magic' (Johnny's Seeds, Winslow, ME), 'Marathon' (Johnny's Seeds, Winslow, ME), 'Packman' (Park Seed, Hodges, SC and Best Cool Seeds, Anchorage, AK), and 'Thompson' (Territorial Seed, Cottage Grove, OR and Fedco Seeds, Clinton, ME), were sown in 50 cell plug trays with OMRI-approved potting mix (Purple Cow Organics, LLC, Middleton, WI) at the University of Minnesota - Twin Cities, St. Paul campus. Cultivars were chosen based on tolerance to heat and availability as certified organic or untreated seed. Seedlings from 2015 were kept under misters until relocation to a greenhouse with 21^0C daytime and 16^0C nighttime temperatures and 12 hour supplemental light (6:00h to 18:00h) on 16 June 2015. Seedlings from 2016 were immediately placed in the greenhouse.

Site Establishment and Plot Management

A field experiment was conducted in 2015 and 2016 at two certified organic farms: Cornercopia in St. Paul, MN (44°96' N, 93°17' W) and Garden Farme in Ramsey, MN (45°92' N, 93°45' W). Broccoli seedlings were hand-transplanted on 7 July 2015 and 12 July 2016 at each site location. Treatment (cultivars) plots were arranged in a randomized block design with 4 replicates. Broccoli plants were planted 12" apart within double rows spaced 18" apart. There were ten broccoli plants in each row, totaling 20 plants per plot. Plants experienced rabbit herbivory in 2015 and were hence covered with floating row cover (Johnny's Seeds, Winslow, ME) from 20 July to 29 July. In 2016, plants were immediately covered with floating row cover (Jordan's Seed, Woodbury, MN) to prevent rabbit herbivory and to encourage hardening off until plant establishment; row covers were removed 19 July. Because of high seedling mortality in 2016, likely due to transplant shock and heat stress, some seedlings had to be replaced at Cornercopia and Garden Farme with seedlings that were three weeks younger (sown on 28 June 2016 and grown in the same greenhouse). These were marked and excluded from being used in data collection.

Broccoli at Cornercopia was irrigated as needed with drip irrigation. At Garden Farme, broccoli was hand-watered at planting and as needed throughout the growing season. Weeds at both locations were manually managed as needed by hand-pulling. Straw mulch covered broccoli beds at both locations to provide weed suppression and moisture retention. Plants were not fertilized due to high levels of macronutrients and organic matter present in the soil at both locations in both years (see Table 3.1 with soil test results). Soils at Garden Farme have been amended with alternating layers of composted manure and hay since original plot establishment. Soils at Cornercopia have

not received additional inputs. In order to support beneficial predators and parasitoids, two 75’ x 5’ strips of organic buckwheat were sown on both sides of the experimental plot at Cornercopia on 8 June 2015 and 8 June 2016. Volunteer milkweed and sunflowers and cultivated asparagus and raspberries surrounded the experimental plot at Garden Farme.

Insect Sampling

Five plants in the center of each plot were chosen at random and scouted once weekly, beginning three to four weeks after transplant. The entire plant was evaluated, and *P. rapae* eggs and larvae were counted and recorded. Scouting took place 5 times at each location each year, for a total of 20 scouts across years and locations. See Table 3.2 for scouting dates across locations and years. Means are reported as cumulative seasonal abundance.

Glucosinolate Analysis

Glucosinolate quantification and extraction methods were adapted from Rosen et al. (2005). Foliar samples were collected from broccoli in the early heading (button) stage such that one sample was obtained from each plot by harvesting the two youngest, fully expanded leaves from two plants and aggregating into one sample. Samples were collected at the early heading stage because tolerance to insects at this stage decreases in order to protect the developing head from feeding damage and frass and thus produce a marketable, high quality product. Samples from each cultivar were collected at Cornercopia on 17 August 2015 and 23 August 2016 and at Garden Farme on 21 August 2015 and 23 August 2016. Samples were stored at 4℃ and then blended and frozen within 8 hours of collection. Samples were boiled in six times the weight per volume of

deionized water for five minutes to deactivate myrosinase and then macerated for two minutes in a standard blender. A 40-mL aliquot of the macerated sample was stored at -80℃ until glucosinolate analysis could be performed.

Desulfonated glucosinolates (df-GSL) were extracted on solid phase strong-anion exchange (SAX) columns (Sigma-Aldrich, St. Louis, MO) before identification and quantification. Samples were thawed and then homogenized for two minutes at 12,000 rpm with a Polytron PT 1300 D homogenizer (Kinematica AG, Lucerne, Switzerland). A 2-mL sample of the aliquot was centrifuged for 4 minutes at 4^0C and 8000 rcf. SAX columns were prepared on a vacuum manifold by washing with 2-mL of 0.5 M sodium acetate (pH 4.6) buffer followed by 2-mL of deionized water. A 500-µL sample of centrifuged broccoli supernatant was then filtered through the SAX column, followed by 1-mL of 0.02 M sodium acetate (pH 4.0) buffer. To desulfate glucosinolates, 1-mL of a sulfatase solution (aryl-sulfate sulfohydrolase from *Helix pomatia* - Type H-1; Sigma-Aldrich, St. Louis, MO) was added to the column, and columns sat for ~15 hours at room temperature (~21℃). Samples were then eluted with 3-mL of deionized water, and eluent was weighed to determine volume. Finally, eluents were filtered through 0.2 micron PTFE filters (Chrom Tech, Apple Valley, MN).

HPLC analysis was performed on an Agilent 1200 Series Quaternary system (Agilent Technologies, Inc., Santa Clara, CA). Glucosinolates were separated on a Luna C18 column (5 µm, 250 x 4.6 mm; Phenomenex, Torrence, CA) set at 30℃ and detected with a diode array detector set at λ=229 nm. A 50-µL aliquot of the eluent was separated with the following flow rates and gradients: solvent A = water and solvent B =

acetonitrile; 0 minutes: 95% A + 5% B, 1.0 mL·min^{-1}; 0-2 minutes: 85% A + 15% B, 1.0 mL·min^{-1}; 2-20 minutes: 53% A + 47% B, 1.0 mL·min^{-1}; 20-22 minutes: 0% A + 100% B, 1.15 mL·min^{-1}; 22-26 minutes: 0% A + 100% B, 1.3 mL·min^{-1}; 26-28 minutes: 0% A + 100% B, 1.0 mL·min^{-1}; 28-34 minutes: 95% A + 5% B, 1.0 mL·min^{-1}. GSL peaks were presented using OpenLAB Chromatography Data System with rev. C.01.06 software. Peaks were identified using relative retention times, and ds-GSL concentrations were calculated using relative quantification with an internal standard (sinigrin) and previously published response factors (EU 1990). Concentrations are reported on a μmol·g^{-1} fresh weight (gfw) basis.

Yield and Crown Grading

Broccoli crowns were harvested when they reached maturity. Due to variability in cultivar maturity dates, multiple harvests occurred. See Table 3.2 for harvest dates across locations and years and Table 3.3 for maturity classification for cultivars. In 2015, all crowns in each plot were harvested, and in 2016, five crowns were randomly selected from plants in the center of treatment plots. See Table 3.4 for number of crowns harvested of each variety in 2015 and 2016. Crowns were determined to be marketable or unmarketable. Marketable crowns were graded as 'Fancy', 'No. 1', or 'No. 2' based on the United States Standards for Grades of Italian Sprouting Broccoli, and individual crown weight was recorded. Those crowns that did not meet the standards for any grading category were considered 'unmarketable' and not included in average marketable crown weight. Qualities that deemed a crown unmarketable included severe discoloration, loose or flowering florets, crown size that was too small (less than 6.35 cm in diameter), or contamination by insects and/or frass. Yield is reported as average

marketable head weight determined by dividing total marketable weight in a plot by the total number of marketable heads in that plot. Number of marketable heads, unmarketable heads, 'Fancy', 'No. 1', and 'No. 2' were transformed into percentages.

Statistical Analysis

Data were analyzed using R statistical software version 3.1.2 (The R Foundation for Statistical Computing, Vienna, Austria). Analyses of egg and larval counts and crown grading percentages were performed with the Kruskal-Wallis rank sum test at the $P < 0.05$ level. If significant results were detected, additional posthoc tests were performed. The significance of differences in GSL concentrations as well as average marketable crown weights were determined using a one-way ANOVA. Significance of year and location was determined using a two-way ANOVA. Differences in mean values were identified using Tukey's Honest Significant Difference (HSD) and were considered significant at the $P < 0.05$ level.

3.4 Results and Discussion

P. rapae *Abundance*

Our data show no significant differences in mean number of seasonal *P. rapae* eggs and larval populations on cultivars across years and locations (Tables 3.5 and 3.6), suggesting no differences in natural resistance against *P. rapae* amongst the six cultivars tested. However, egg and larval abundance was lower in 2016 than 2015 at both locations, indicating a seasonal effect.

The effects of genotype on specialist insects such as *P. rapae* is unclear as specialists have co-evolved with host plants and adapted to host plant defensive tactics. It has been suggested that plant genotype may be able to influence herbivore abundance as

much as environmental conditions (Newton et al. 2010), but our data shows that mean seasonal egg and larval abundance was not statistically different on any of the broccoli cultivars, suggesting that genotype did not strongly influence oviposition preference of the adult female nor preference of the larvae. Our results are similar to findings from several other studies. Shelton et al. (1988) reported that *P. rapae* did not distinguish between green cabbage cultivars in a field study and thus these cultivars did not differ in susceptibility. A study evaluating six kale genotypes in the field also reported that *P. rapae* did not distinguish between kale genotype (Santolamazza-Carbone et al. 2014). However, this is in contrast with several studies that evaluated two cabbage cultivars ('Rivera' and 'Christmas Drumhead') and found intraspecific differences between the cultivars. Broekgaarden et al. (2010) found lower *P. rapae* abundance on 'Rivera' cabbage cultivar compared to 'Christmas Drumhead' in a natural field setting while other studies have shown *P. rapae* oviposition preference for 'Christmas Drumhead' (Poelman et al. 2009) as well as improved larval performance on this cultivar (Broekgaarden et al. 2007, Poelman et al. 2009). Broekgaarden et al. (2007, 2010) attribute these differences to expression of defense genes in the two cultivars while Poelman et al. (2009) attribute differences to GSL content, chiefly glucoiberin. The mechanisms for natural host resistance is unclear, but our results indicate that genotype did not strongly influence adult oviposition preference or larval performance.

Glucosinolate Concentrations

As expected, our results show that broccoli cultivars differ significantly in concentration of the two most abundant indolic GSLs, neoglucobrassicin and glucobrassicin, in foliage at early heading stage. Overall, 'Packman' had lowest mean

neoglucobrassicin concentration in both years and both locations while ‘Green Magic’ had the highest mean neoglucobrassicin concentration both years at Cornercopia (Table 3.7). ‘Green Magic’ also had the lowest mean concentration of glucobrassicin across seasons and locations, though the difference was not always significant (Table 3.8). Year and location interactions significantly affected concentrations of both glucosinolates (Tables 3.7 and 3.8). However, correlations between glucosinolate concentrations and *P. rapae* egg and larvae abundance did not indicate a strong relationship (Table 3.10).

Though GSL concentration varied between cultivars, the differences were not generally consistent across seasons and locations. Location and year:location interaction significantly affected neoglucobrassicin concentration (P=0.000493 and P=8.95e-05, respectively), while year and year:location interaction had significant effects on glucobrassicin (P=1.58e-18 and P=0.0122, respectively). This is likely due to the sensitivity of GSLs to biotic and abiotic stresses, which can significantly influence GSL concentration (Hopkins et al. 2009). We attempted to collect samples with consistency in regards to plant age, plant damage, and sample processing in order to control variability. Overall, neoglucobrassicin concentration was significantly lower in ‘Packman’ in most cases while neoglucobrassicin concentration was usually highest in ‘Green Magic’, though neoglucobrassicin concentration was highly variable depending on year and location. ‘Green Magic’ had the lowest glucobrassicin concentration overall, but there is not a strong trend in glucobrassicin concentration in the other five cultivars.

Though the glucosinolate-myrosinase defense system in brassicas is well studied, the relationship between *P. rapae*, and GSLs is complex and not well understood and is further compounded by biotic and abiotic factors that can influence GSL content. Our

data show no strong correlation between GSL concentration and egg abundance (Table 3.10), suggesting no natural resistance against *P. rapae* due to GSL content. Our results are in agreement with studies by Santolamazza-Carbone et al. (2014) and Newton et al. (2010), which showed that *P. rapae* did not differentially respond to GSL content in field plants even though GSL content and concentration varied in six kale genotypes and 12 wild cabbage types. However, many other studies have shown that cabbagewhite butterflies use GSLs as oviposition stimulants and that GSL class and concentrations show differential effects on oviposition (de Vos et al. 2008, Huang and Renwick 1994, Muller et al. 2010, Renwick et al. 1992).

Furthermore, there was no strong correlation between GSL concentration and larval populations. *P. rapae* larvae may redirect GSL breakdown from isothiocyanates to less toxic nitriles and thus the larvae may not have been adversely affected by different GSL concentrations found in the cultivars (Wittstock et al. 2004). Indeed much research has shown that *P. rapae* larvae are not negatively affected by GSLs (Mumm et al. 2008, Poelman et al. 2008, Santolamazza-Carbone et al. 2014, Wittstock et al. 2004), which may help to explain why we did not detect differences in larval populations amongst cultivars across locations and year.

Our study was limited in that we evaluated only glucobrassicin and neoglucobrassicin, and it is not entirely clear which GSLs most strongly influence oviposition and larval preference. We chose to measure the indolic GSLs glucobrassicin and neoglucobrassicin because they were two of the most abundant GSLs and some research has suggested that indolic GSL are the most important in providing antibiosis against insects (Gols et al. 2008b, Muller et al. 2010). Our study was also limited in that

we measured GSL content only once during the season in mid-August, while correlations were performed with seasonal egg and larval abundance. Future studies should investigate GSL content and egg and larval abundance during the same snapshot in time and at multiple sampling dates over the season.

Yield and Crown Grading

Overall, 'Green Magic' produced the highest average marketable crown weight (282 g) across locations and years, while it produced significantly heavier crowns in both locations in 2015 (Table 3.11). 'Green Magic' also had the lowest percentage of unmarketable crowns (20%) across locations and years (Figure 3.1). 'Marathon' produced the smallest marketable crowns (159 g) overall, although the results are not significant at the $P<0.05$ level (Table 3.11).'Belstar,' 'Fiesta,' 'Packman,' and 'Thompson' produced similar average marketable crown weights overall (220, 211, 220, and 180 g, respectively). Overall 'Belstar' produced the highest percentage of 'Fancy' crowns (69%) while 'Thompson' produced the lowest percentage of 'Fancy' crowns (32%) as well as the highest percentage of unmarketable crowns (53%) (Figure 3.1).

Similar to previous research, our results show that 'Green Magic' performed well and produced the largest crowns overall. In an extensive study by Renaud et al. (2014), 'Green Magic' consistently ranked in the top 3 cultivars for highest average crown weight when grown in organic systems in Maine and Oregon across two seasons and two years, suggesting yield stability across locations and seasons. The average marketable crown weight of 'Green Magic' (280 g) in our study, however, was much lower than in previous studies where average weight varied between 350 g and 685 g in conventional production systems (Lalla et al. 2007, Renaud et al. 2014, Toth et al. 2014). Additionally,

80% of all ‘Green Magic’ crowns we harvested were considered marketable, though due to relatively large crown size, fewer ‘Green Magic’ crowns (41%) were graded as ‘Fancy’, a category that is reserved for crowns between 6.35 cm and 12.7 cm. Despite lower grades, we conclude that ‘Green Magic’ is a suitable and adaptable cultivar for organic systems, as it produced a consistent and high yield across locations and years.

The poorest performer in our study was ‘Marathon,’ which is in contrast with previous research. Toth et al. (2014) performed a conventional broccoli cultivar trial in Croatia and found that ‘Marathon’ was a top performer out of 13 hybrid cultivars, and ‘Marathon’ was a mid-performer in a conventional cultivar study done in Brazil (Lalla et al. 2010). However, in our research, ‘Marathon’ was the poorest performer and had the least consistency in yield. Despite healthy plant growth, in 2016 at Garden Farme, ‘Marathon’ produced zero crowns, and in 2015 at Cornercopia, this cultivar was also the lowest performer with only ten total crowns harvested and a 159 g average marketable crown weight (Table 3.11). We observed some of the plants starting to turn purple near the end of the season when ‘Marathon’ was maturing, indicating a translocation of nitrogen in the plant. This may have contributed to lower performance, as nitrogen can affect yield (Vagen et al. 2007). However, lowered performance was not consistently observed across cultivars. Interestingly, though, ‘Marathon’ was a top performer at Garden Farme in 2015 with an average marketable crown weight of 353 g. Due to its inconsistency in crown production and relatively low crown weights, we would not recommend ‘Marathon’ as a reliable or top performing cultivar in organic systems in Minnesota.

‘Belstar,’ ‘Fiesta,’ and ‘Packman’ were overall average performers with mid-range average marketable crown weights (220, 211, and 220 g, respectively), and between 72 and 77% of total crowns were marketable. ‘Thompson’ produced the highest percentage of unmarketable crowns (53%) and had an average crown weight of 180 g. Overall we would recommend ‘Belstar,’ ‘Fiesta,’ ‘Green Magic,’ and ‘Packman’ as good candidates for organic systems in Minnesota, as they produced a consistent and quality product. ‘Thompson’ and ‘Marathon’ were the poorest performers, and more testing should be on these cultivars for plasticity in organic systems.

3.5 Conclusion

We conclude that though natural pest resistance is important when making cultivar recommendations for particular growing systems and goals, the broccoli cultivars evaluated in this study do not significantly differ in susceptibility to infestation by the insect specialist *P. rapae.* Concentrations of neoglucobrassicin and glucobrassicin did not explain *P. rapae* egg and larval abundance, and season appeared to play a larger role in *P. rapae* abundance. In this case, cultivar selection should not be based on natural resistance to pests but rather based on cultivar performance. ‘Belstar,’ ‘Fiesta’, ‘Green Magic,’ and ‘Packman’ were top performers and produced a consistent and high quality product, and therefore we would recommended these cultivars as acceptable selections for organic systems in Minnesota.

Table 3.1 Results from basic soil tests at both locations and year. Nitrate was not measured in 2015.

Location	Year	Soil Organic Matter (%)	pH	Phosporous (ppm)	Potassium (ppm)	NO_3 (ppm)
Cornercopia	2015	3.2	6.8	108	309	NA
	2016	2.8	7.4	85	166	6.55
Garden Farme	2015	8.1	6.6	199	496	NA
	2016	7.9	6.5	171	264	23.56

Table 3.2 Insect scouting and harvesting dates across locations and years.

Location	Year	Scouting Dates	Harvest Dates
Cornercopia	2015	10, 17, 24, 31 Aug; 8 Sept	4, 8, 15, 22 Sept
	2016	3, 10, 17, 24, 30 Aug	30 Aug; 7, 13, 20, 28 Sept
Garden Farme	2015	5, 12, 19, 16 Aug; 2 Sept	26 Aug; 2, 9, 16, 23 Sept
	2016	3, 9, 16, 23, 31 Aug	31 Aug; 7, 13, 20 Sept

Table 3.3 Maturity classification of cultivars evaluated

Cultivar	Maturity Classification
Belstar	Late
Fiesta	Late
Green Magic	Mid
Marathon	Late
Packman	Early
Thompson	Early

Table 3.4 Number of crowns harvested for each cultivar at each location and year. In 2015, all crowns were harvested while only 5 randomly selected crowns in the middle of plots were harvested in 2016 (for 2016, ideally n=20).

		Total number of crowns harvested					
		Cultivar					
Location	Year	Belstar	Fiesta	Green Magic	Marathon	Packman	Thompson
Cornercopia	2015	33	25	60	10*	56	48
	2016	20	20	20	20	20	20
Garden Farme	2015	38	62	24	28	43	21
	2016	15	10	15	0 *	15	15

* indicates that the cultivar did not produce many, if any, heads

Table 3.5 Mean number and standard error of seasonal *P. rapae* eggs per plant.

	Mean *P. rapae* eggs/plant ± SE			
	Cornercopia		Garden Farme	
Cultivar	2015	2016	2015	2016
Belstar	0.77 ± 0.18	0.35 ± 0.08	2.36 ± 0.56	0.33 ± 0.11
Fiesta	1.00 ± 0.11	0.40 ± 0.04	2.34 ± 0.24	0.28 ± 0.04
Green Magic	0.98 ± 0.15	0.43 ± 0.06	2.97 ± 0.39	0.32 ± 0.08
Marathon	0.85 ±0.13	0.44 ± 0.06	2.48 ± 0.19	0.38 ± 0.02
Packman	0.89 ± 0.18	0.34 ± 0.13	3.16 ± 0.06	0.43 ± 0.21
Thompson	0.91 ± 0.17	0.50 ± 0.09	3.41 ± 0.76	0.28 ± 0.16
P-value	0.9307	0.7206	0.2017	0.9306

Table 3.6 Mean number and standard error of seasonal *P. rapae* larvae per plant.

	Mean *P. rapae* larvae/plant ± SE			
	Cornercopia		Garden Farme	
Cultivar	2015	2016	2015	2016
Belstar	0.53 ± 0.10	0.13 ± 0.03	1.66 ± 0.38	0.31 ± 0.07
Fiesta	0.89 ± 0.34	0.23 ± 0.09	1.94 ± 0.19	0.40 ± 0.16
Green Magic	1.03 ± 0.24	0.29 ± 0.06	2.65 ± 0.13	0.43 ± 0.04
Marathon	0.97 ± 0.21	0.11 ± 0.03	1.86 ± 0.35	0.30 ± 0.10
Packman	0.74 ± 0.23	0.19 ± 0.02	2.91 ± 0.21	0.60 ± 0.08
Thompson	0.64 ± 0.03	0.16 ± 0.02	2.38 ± 0.18	0.37 ± 0.13
P-value	0.2639	0.1299	0.05092	0.4051

Table 3.7 Mean concentration and standard deviations of neoglucobrassicin in field grown broccoli, reported as µmol per gram fresh weight (gfw); * is significant at P<0.05; ** is significant at P<0.01; * significant at P<0.001.**

	Neoglucobrassicin µmol per gfw			
	Cornercopia		Garden Farme	
Cultivar	2015	2016	2015	2016
Belstar'	0.42 ± 0.10 ab	0.64 ± 0.22 abc	0.55 ± 0.13 b	0.22 ± 0.08
Fiesta	0.40 ± 0.13 ab	0.71 ± 0.19 bc	0.62 ± 0.27 b	0.18 ± 0.11
Green Magic	0.52 ± 0.29 a	0.86 ± 0.44 c	0.42 ± 0.06 ab	0.20 ± 0.08
Marathon	0.25 ± 0.11 ab	0.51 ± 0.14 abc	0.25 ± 0.04 a	0.18 ± 0.02
Packman	0.14 ± 0.01 b	0.14 ± 0.01 a	0.13 ± 0.04 a	0.06 ± 0.00
Thompson	0.29 ± 0.14 ab	0.35 ± 0.08 ab	0.31 ± 0.17 ab	0.26 ± 0.33
p-values	0.04339*	0.003461**	0.0009147***	0.5479

Factor	d.f.	F value	P value
Year	1	0.003	0.958742
Location	1	13.074	0.000493***
Year:Location	1	16.832	8.95e-05***

Table 3.8 Mean concentration and standard deviations of glucobrassicin in field grown broccoli, reported as µmol per gram fresh weight (gfw); * is significant at P<0.05; ** is significant at P<0.01; * significant at P<0.001.**

	Glucobrassicin µmol per gfw			
	Cornercopia		Garden Farme	
Cultivar	2015	2016	2015	2016
Belstar	1.12 ± 0.15	0.72 ± 0.21 ab	1.41 ± 0.13 c	0.51 ± 0.16
Fiesta	1.11 ± 0.28	0.69 ± 0.32 ab	1.38 ± 0.24 bc	0.43 ± 0.18
Green Magic	0.76 ± 0.15	0.24 ± 0.08 a	0.59 ± 0.07 a	0.25 ± 0.07
Marathon	0.88 ± 0.23	0.52 ± 0.33 ab	0.94 ± 0.16 ab	0.58 ± 0.16
Packman	0.97 ± 0.19	0.67 ± 0.19 ab	1.12 ± 0.15 bc	0.34 ± 0.06
Thompson	0.83 ± 0.35	0.91 ± 0.21 c	1.01 ± 0.28 bc	0.61 ± 0.39
p-values	0.2408	0.02078*	0.0001027***	0.1255

Factor	d.f.	F value	P value
Year	1	67.237	1.58e-18***
Location	1	0.362	0.5491
Year:Location	1	6.543	0.0122*

Table 3.10 R^2 values for correlations performed between means of glucobrassicin, neoglucobrassicin, egg and larval counts

		R^2 values for correlations			
		Glucobrassicin		Neoglucobrassicin	
Location	Year	Egg Count	Larval Count	Egg Count	Larval Count
Cornercopia	2015	0.0948	0.0045	0.0134	0.0247
	2016	0.0205	0.085	0.0076	0.0003
Garden Farme	2015	0.0902	0.0695	0.0182	0.1356
	2016	0.0568	0.0656	0.0052	0.134

Table 3.11 Average Marketable Crown Weights (g) (total marketable weight divided by number of marketable crowns)

	Average Marketable Crown Weight (g)				
	Cornercopia		Garden Farme		
Cultivar	2015	2016	2015	2016	overall
Belstar	206 ± 76 ab	121 ± 11	352 ± 36 ab	282 ± 39	220 ± 95
Fiesta	209 ± 53 ab	125 ± 18	312 ± 46 ab	233 ± 8	211 ± 81
Green Magic	295 ± 78 b	190 ± 67	393 ± 63 b	314 ± 89	282 ± 97
Marathon	51 ± 88 a	176 ± 35	352 ± 97 ab	0 ± 0	159 ± 146
Packman	196 ± 25 ab	165 ± 47	201 ± 16 a	343 ± 179	220 ± 102
Thompson	160 ± 50 ab	138 ± 55	171 ± 38 a	270 ± 289	180 ± 135
p-values	0.004929**	0.1532	0.01278*	0.3228	0.125

Factor	d.f.	F value	P value
Year	1	2.878	0.094
Location	1	18.962	4.28e-05***
Year:Location	1	0	0.999

Figure 3.1 Overall grading of crowns, divided into Fancy, No. 1, No. 2, and unmarketable categories, across locations and years.

4 Bibliography

Agrawal, A.A. 1999. Induced responses to herbivory in wild radish: Effects on several herbivores and plant fitness. Ecology. 80(5): 1713-1723.

Agrawal, A.A. 2000a. Benefits and costs of induced plant defense for *Lepidium virginicum* (Brassicaceae). Ecology. 81(7): 1804-1813.

Agrawal, A.A. 2000b. Specificity of induced resistance in wild radish: Causes and consequences for two specialists and two generalist caterpillars. OIKOS. 89: 493-500.

Agrawal, A.A. and N.S. Kurashige. 2003. A role for isothiocyanates in plant resistance against the specialist herbivore *Pieris rapae*. J. Chem. Ecol. 29(6): 1403-1415.

Ahuja, I., J. Rohloff, and A.M. Bones. 2010. Defense mechanisms of Brassicaceae: Implications for plant-insect interactions and potential for integrated pest management. Agron. Sust. Dev. 30: 311-348.

Andow, D.A., A.G. Nicholson, H.C. Wien, and H.R. Williams. 1986. Insect populations on cabbage grown with living mulches. Environ. Entomol. 15: 293-299.

Armah, C.N., M.H. Traka, J.R. Dainty, M. Defernez, A. Janssens, W. Leung, J.F. Doleman, J.F. Potter, and R.F. Mithen. 2013. A diet rich in high-glucoraphanin broccoli interacts with genotype to reduce discordance in plasma metabolite profiles by modulating mitochondrial function. Am. J. Clin. Nutr. 98(3): 712-722.

Bigger, D.S. and W.E. Chaney. 1998. Effects of *Iberis umbellata* (Brassicaceae) on insect pests of cabbage and on potential biological control agents. Environ. Entomol. 27: 161-167.

Bottenberg, H., J. Masiunas, C. Eastman, and D. Eastburn.1997. Yield and quality constraints of cabbage plants in rye mulch. Biol. Agric. Hortic. 14: 323-342.

Broad, S.T., N.A. Schellhorn, S.N. Lissont, and N.J. Mendham. 2008. Host location and oviposition of lepidopteran herbivores in diversified broccoli cropping systems. Agri. and Forest Entomol. 10: 157-165.

Broekgaarden, C., E.H. Poelman, G. Steenhuis, R.E. Voorrips, M. Dicke, and B. Vosman. 2007. Genetic variation in genome-wide transcription profiles induced by insect feeding: *Brassica oleracae-Pieris rapae* interactions. BMC Genomics. 8: 239.

Broekgaarden, C., E.H. Poelman, R.E. Voorrips, M. Dicke, and B. Vosman. 2010. Intraspecific variation in herbivore community composition and transcriptional profiles in field-grown *Brassica oleracea* cultivars. J. Exp. Botany. 61(3): 807-819.

Bryant, A., T. Coudron, D. Brainard, and Z. Szendrei. 2014. Cover crop mulches influence biological control of the imported cabbageworm (*Pieris rapae* L., Lepidoptera: Pieridae) in cabbage. Bio. Control. 73: 75-83.

Caldwell, B., E. Sideman, A. Seaman, A. Shelton, and C. Smart. 2013. Resource guide for organic insect and disease management. Cornell Univ.

Chalfant, R.B. W.H. Denton, D.J. Schuster, and W.B. Workman. 1979. Management of cabbage caterpillars in Florida and Georgia by using visual damage thresholds. J. Econ. Entomol. 72: 411-414.

Chittenden, F.H. 1916. The common cabbage worm. USDA Farmers' Bulletin 766. 16 pp.

Cisneros, J.D., D. Goulson, L.C. Derwent, D.I. Penagos, O. Hernandez, and T. Williams. 2002. Toxic effects of spinosad on predatory insects. Bio. Control. 23: 156-163.

Cromartie Jr., W.J. 1975. The effect of stand size and vegetational background on the colonization of cruciferous plants by herbivorous insects. J. Appl. Ecol. 12(2): 517-533.

Demptster, J.P. 1969. Some effects of weed control on the numbers of the small cabbage white (*Pieris rapae* L.) on Brussels sprouts. J. Appl. Ecol. 6: 339-346.

Dickson, M.H. and C.J. Eckenrode. 1975. Variation in *Brassica oleracea* resistance to cabbage looper and imported cabbageworm in the greenhouse and field. J. Econ. Entomol. 68: 757-760.

Dornan, A.P., J.G. Stewart, and M.K. Sears. 1994a. An action threshold for control of lepidopterous pests of cabbage in Prince Edward Island. Canadian Entomol. 126: 379-387.

Dornan, A.P., J.G. Stewart, and M.K. Sears. 1994b. Application of the cabbage looper equivalent model to lepidopterous pests of Brussels sprouts in Prince Edward Island. Canadian Entomol. 126: 961-969.

Dreistadt, S.H. 2014. Biological control and natural enemies of invertebrates. Univ. Calif. Agric. Nat. Res. Publ. 74140.

de Vos, M., K.L. Kriksunov, and G. Jander. 2008. Indole-3-acetonitrile production from indole glucosinolates deters oviposition by *Pieris rapae.* Plant Phys. 146: 916-926.

Endersby, N.M. and W.C. Morgan. 1991. Alternatives to synthetic chemical insecticides for use in crucifer crops. Bio. Agric. Hort. 8: 33-52.

Endersby, N.M., W.C. Morgan, B.C. Stevenson, and C.T. Waters. 1992. Alternatives to regular insecticide applications for control of lepidopterous pests of *Brassica oleracea* var. *capitata*. Bio. Agric. Hort. 8: 189-203.

Environmental Protection Agency. 2016. Biopesticides. US EPA. Accessed at: https://www.epa.gov/pesticides/biopesticides

EU. 1990. Off. J. Eur. Commun. L 170:03.07. 27-34.

Fahey, J.W., A.T. Zalcmann, and P. Talalay. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry. 56: 5-51.

Finch, S. and M. Kienegger. 1992. A behavioral study to help clarify how undersowing with clover affects host-plant selection by insect pests of *Brassica* crops. Entomol. Exp. Appl. 84: 165-172.

Furlong, M.J., K.H. Ju, P.W. Su, J.K. Chol, R. Chang II, M.P. Zalucki. 2008. Integration of endemic natural enemies and *Bacillus thuringiensis* to manage insect pests of *Brassica* crops in North Korea. Agriculture, Ecosystems and Environment. 125: 223-238.

Gaines, D.N. and L.T. Kok. 1995. Seasonal occurrence of *Pieris rapae* (Lepitopera, Pieridae) in southeast Virginia. J. Entomol. Sci. 30(2): 262-272.

Gols, R., T. Bukovinszky, N.M. van Dam, M. Dicke, J.M. Bullock, and J.A. Harvey. 2008a. Performance of generalist and specialist herbivores and their endoparasitoids differs on cultivated and wild *Brassica* populations. J. Chem. Ecol. 34: 132-143.

Gols, R., R. Wagenaar, T. Bukovinszky, N.M. van Dam, M. Dicke, J.M. Bullock, and J.A. Harvey. 2008b. Genetic variation in defense chemistry in wild cabbages affects herbivores and their endoparasitoids. Ecology. 89(6): 1616-1626.

Guerena, M. 2006. Cole crops and other brassicas: Organic production. ATTRA. 20 pp.

Hamilton, A.J., E.K. Waters, H.J. Kim, W.S. Pak, and M.J. Furlong. 2009. Validation of fixed sample size for monitoring lepidopteran pests of *Brassica oleracea* pests in North Korea. J. Econ. Entomol. 102(3): 1336-1343.

Harvey, J.A., E.H. Poelman, and R. Gols. 2010. Development and host utilization in *Hyposoter ebeninus* (Hymentopera: Ichneumonidae), a solitary endoparasitoid of *Pieris rapae* and *P. brassicae* caterpillars (Lepidoptera: Pieridae). Bio. Control. 53: 312-318.

Harvey, J.A. and R. Wagenaar. 2006. Development of the herbivore *Pieris rapae* and its endoparasitoid *Cotesia rubecula* on crucifers of field edges. J. Appl. Entomol. 130(9-10): 465-470.

Hasan, F. and M.S. Ansari. 2011. Toxic effects of neem-based insecticides on *Pieris brassicacae* (Linn.). Crop Protection. 30: 502-507.

Hines, R.L. 1998. Biologically intensive IPM of the lepidopteran pest complex in Minnesota cabbage. M.S. thesis, University of Minnesota, St. Paul, MN

Hines, R.L. and W.D. Hutchison. 2001. Evaluation of action thresholds and spinosad for lepidopteran pest management in Minnesota cabbage. J. Econ. Entomol. 94: 190-196.

Hokkanen, H.M.T. 1991. Trap cropping in pest management. Annu. Rev. Entomol. 36: 119-138.

Hooks, C.R.R. 2000. The impact of flora diversification on lepidopterous pests and associated natural enemies inhabiting a broccoli agroecosystem. Ph.D. Dissertation, Department of Entomology, University of Hawaii at Manoa, Honolulu, HI.

Hooks, C.R.R. and M.W. Johnson. 2001. Broccoli growth parameters and level of head infestations in simple and mixed plantings: Impact of increased flora diversification. Ann. Appl. Boil. 138: 269-380.

Hooks, C.R.R. and M.W. Johnson. 2002. Lepidoptera pest populations and crop yields in row intercropped broccoli. Agri. and Forest Entomol. 4: 117-125.

Hooks, C.R.R. and M.W. Johnson. 2003. Impact of agricultural diversification on the insect community of cruciferous crops. Crop Protection. 22: 223-238.

Hopkins, R.J., N.M. van Dam, and J.J.A van Loon. 2009. Role of glucosinolates in insect-plant relationships and multitrophic interactions. Annu. Rev. Entomol. 54: 57-83.

Huang, X. and J.A.A. Renwick. 1994. Relative activities of glucosinolates as oviposition stimulants for *Pieris rapae* and *P. napi oleracea.* J. Chem. Ecol. 20(5): 1025-1037.

Jacques, R.P. 1988. Field tests on control of the imported cabbageworm (Lepidoptera: Pieridae) and the cabbage looper (Lepidoptera: Noctuidae) by mixtures of microbial and chemical insecticides. Can. Ent. 120: 575-580.

Jahangir, M., I.B. Abdel-Farid, H.K. Kim, Y.H. Choi, and R. Verpoorte. 2009. Healthy and unhealthy plants: the effects of stress on the metabolism of Brassicaceae. Environ. Exp. Botany. 67: 23-33.

Kaplan, I., Halitschke, R., Kessler, A., Sardanelli, S., and Denno, R.F. 2008. Constitutive and induced defenses to herbivory in above- and belowground plant tissues. Ecology. 89(2): 392-406.

Kelsey, J.M. 1958. Control of *Pieris rapae* by granulosis virus. N.Z. J. Agri. Res. 1: 778-782.

Kogan, M. 1998. Integrated pest management: Historical perspectives and contemporary developments. Annu. Review of Entomol. 43: 243-270.

Kos, M., B. Houshyani, R.Wietsma, P. Kabouw, L.E.M. Vet, J.J.A. van Loon, and M. Dicke. 2012. Effects of glucosinolates on a generalist and specialist leaf-chewing herbivore and an associated parasitoid. Phytochemistry. 77: 162-170.

Kushad, M.M., A.F. Brown, A.C. Kurilich, J.A. Juvik, B.P. Klein, M.A. Wallig, and E.H. Jeffery. 1999. Variation of glucosinolates in vegetable crops of *Brassica oleracea*. J. Agric. Food Chem. 47: 1541-1548.

Lalla, J.G., V. Laura, A. Rodrigues, S. Seabra Jr., D.S. Silveira, V.H. Zago, and M.F. Dornas. 2010. Single-head broccoli cultivars evaluation in Campo Grande, Brazil. Hort. Brasileira. 28(3): 360-363.

Lasota, J.A. and L.T. Kok. 1989. Seasonal abundance of imported cabbageworm (Lepidoptear: Pieridae), cabbage looper (Lepidoptera: Noctuidae), and diamondback moth (Lepidoptera: Plutellidae) on cabbage in southwestern Virginia. J. Econ. Entomol. 82: 811-818.

Latheef, M.A. and R.D. Irwin.1979. The effect of companionate planting of lepidopteran pests of cabbage. Can. Entomol. 111: 863-864.

Latheef, M.A. and J.H. Ortiz. 1983a. The influence of companion herbs on egg distribution of the imported cabbageworm, *Pieris rapae* (Lepidoptera: Pieridae), on collard plants. Can. Ent. 115: 1031-1038.

Latheef, M.A. and J.H. Ortiz. 1983b. Influence of companion plants on oviposition of imported cabbageworm, *Pieris rapae* (Lepidoptera: Pieridae), and cabbage looper, *Trichoplusia ni* (Lepidoptera: Pieridae), on collard plants. Can. Entomol. 115: 1529-1531.

Lee, J.C. and G.E. Heimpel. 2005. Impact of flowering buckwheat on Lepidopteran cabbage pests and their parasitoids at two spatial scales. Bio. Control. 34: 290-301.

Leskovar, D.I. and A.K. Boales. 1996. Azadirachtin: Potential use for controlling lepidopterous insects and increasing marketability of cabbage. HortScience. 31(3): 405-409.

Levin, D.B., J.E. Laing, R.P. Jacques, and J.E. Corrigan. 1983. Transmission of granulosis virus of *Pieris rapae* (Lepidoptera: Pieridae) by the parasitoid *Apanteles glomeratus* (Hymentoptera: Braconidae). Environ. Entomol. 12: 166-170.

Lundgren, J.G., G.E. Heimpel, and S.A. Bomgren. 2002. Comparison of *Trichogramma brassicae* (Hymentopera: Trichogrammatidae) augmentation with organic and synthetic pesticides for control of cruciferous lepidoptera. Environ. Entomol. 31(6): 1231-1239.

Luther, G.C., H.R. Valenzuela, and J. DeFrank.1996. Impact of cruciferous trap crops on lepidopteran pests of cabbage in Hawaii. Environ. Entomol. 25: 39-47.

Mader, E. and N.L. Adamson. 2013. Organic-approved pesticides: Minimizing risk to bees. The Xerces Society for Invertebrate Conservation. 6 pp.

Maguire, L.A. 1984. Influence of surrounding plants on densities of *Pieris rapae* (L.) eggs and larvae (Lepidoptera: Pieridae) on collards. Environ. Entomol. 13: 464-468.

Mahr, S.E., D.L. Mahr, and J.A. Wyman. 1993. Biological control of insect pests of cabbage and other crucifers. NCR publication. No. 471: University of Wisconsin, Madison.

Maltais, P.M., J.R. Nuckle, and P.V. LeBlanc. 1994. Economic threshold for management of Lepidopterous larvae on broccoli in southeastern New Brunswick. J. Econ. Entomol. 87(3): 766-774.

Maltais, P.M., J.R. Nuckle, and P.V. LeBlanc. 1998a. Economic threshold for three Lepidopterous larval pests of fresh-market cabbage in southeastern New Brunswick. J. Econ. Entomol. 91(3): 699-707.

Maltais, P.M., J.R. Nuckle, and P.V. LeBlanc. 1998b. Seasonal occurrence of three Lepidoptera on broccoli and effectiveness of planting date as a pest management tool in southeastern New Brunswick. Phytoprotection. 79: 129-137.

Matter, M.M., M.A. Gesraha, A.A.I. Ahmed, and N.A. Farag. 2002. Impact of neem and chinaberry fruit extracts on the pest/parasitoid (*Pieris rapae/Hyposoter ebeninus*) interactions. J. Pest Science. 75: 13-18.

Maxwell, E.M. and H.Y. Fadamiro. 2006. Evaluation of several reduced-risk insecticides in combination with an action threshold for managing lepidopteran pests of cole crops in Alabama. Florida Entomol. 92(2): 117-126.

Metcalf, R.L. 1994. Insecticides in pest management. In: Metcalf, R.L. and W.H. Luckman [eds.], Introduction to Insect Pest Management. Wiley-Interscience Publication, New York, pp. 650.

Midwest Vegetable Production Guide for Commercial Growers: 2017. 2016. Accessed at: https://ag.purdue.edu/btny/midwest-vegetable-guide/Documents/2017/01_MWVegGuide_2017.pdf

Miles, C.I., M.L. del Campo, and J.A.A. Renwick. 2005. Behavioral and chemosensory responses to a host recognition cue by larvae of *Pieris rapae.* J. Comp. Physiol. 191: 147-155.

Morisak, D.J., D.E. Simonet, and R.K. Lindquist. 1984. Use of action thresholds for management of Lepidopterous larval pests of fresh-market cabbage. J. Econ. Entmol. 77: 476-482.

Moynihan, M. 2010. Status of organic agriculture in Minnesota: A report to the Minnesota legislature. Minnesota Department of Agriculture.

Moynihan, M. 2016. Status of organic agriculture in Minnesota 2015: A report to the Minnesota legislature. Minnesota Department of Agriculture.

Muller, R., M. de Vos, J.Y. Sun, I.E. Sonderby, B.A. Halkier, U. Wittstock, and G. Jander. 2010. Differential effects of indole and aliphatic glucosinolates on lepidopteran herbivores. J. Chem. Ecol. 36: 905-913.

Mumm, R., M. Burow, G. Bukovinszkine'Kiss, E. Kazantzidou, U. Wittstock, M. Dicke, and J. Gershenzon. 2008. Formation of simple nitriles upon glucosinolates hydrolysis affects direct and indirect defense against the specialist herbivore, *Pieris rapae.* J. Chem. Ecol. 34: 1311-1321.

NASS. 2014. Vegetables 2013 Summary. USDA, National Agricultural Statistics Service.

Oberholtzer, L., C. Dimitri, and C. Greene. 2005. Price premiums hold on as U.S. organic produce market expands. USDA Econ. Research Service. VGS-308-01.
Orzolek, M.D., W.J. Lamont Jr., L.F. Kime, and J.K. Harper. 2012. Broccoli production. Penn State Extension publication.

Parker, J.E., W.E. Snyder, G.C. Hamilton, and C. Rodriguez-Saona. 2013. Companion planting and insect pest control. InTech. 30 pp.

Peric, P, D. Marcic, M. Prijovic, I. Ogurlic, and G. Andric. 2009. Effectiveness of biorational pesticides for controlling some vegetable pests in Serbia. In: L. Krasteva and N. Panayotov [eds.], Proc. IVth Balkan Symp. On Vegetables and Potatoes. Acta Hort. 830: 531-538.

Pfiffner, L., H. Luka, C. Schlatter, A. Juen, and M. Traugott. 2009. Impact of wildflower strips on biological control of cabbage lepidopterans. Agri., Ecosystems, and Environ. 129: 310-314.

Philips, C.R., T.P. Kuhar, and D.A. Herbert. 2014a. Effect of buckwheat farmscapes on abundance and parasitism of *Pieris rapae* (L.) in Virginia collards. J. Entomol. Sci. 49(3): 273-284.

Philips, C.R., M. Rogers, and T. Kuhar. 2014b. Understanding farmscapes and their potential for improving IPM programs. J. Integ. Pest Mngmt. 5(3): 1-9.

Poelman, E.H., C. Broekgaarden, J.J.A. van Loon, and M. Dicke. 2008a. Early season herbivore differentially affects plant defence responses to subsequently colonizing herbivores and their abundance in the field. Mol. Ecol. 17: 3351-3365.

Poelman, E.H, R.J.F.H. Galiart, C.E. Raaijmakers, J.J.A. van Loon, and N.M. van Dam. 2008b. Performance of specialist and generalist herbivore feeding on cabbage cultivars is not explained by glucosinolates profiles. Entomologia Experimentalis et Applicata. 127: 218-228.

Poelman, E.H., N.M. van Dam, J.J.A. van Loon, L.E.M. Vet, and M. Dicke. 2009. Chemical diversity in *Brassica oleracea* affects biodiversity of insect herbivores. Ecology. 90(7): 1863-1877.

Radcliff, J.A.A. and R.K. Chapman. 1965. Seasonal shifts in the relative resistance to insect attack of eight commercial cabbage varieties. Ann. Entomol. Soc. Am. 58: 892-897.

Ratzka, A, H. Vogel, D.J. Kliebenstein, T. Mitchell-Olds, and J. Kroymann. 2002. Disarming the mustard oil bomb. Proceedings of the National Academy of Sciences of the USA. 99(17): 11223-11228.

Renaud, E.N.C., E.T.L. van Bueren, M.J. Paulo, F.A. van Eeuwijk, J.A. Juvik, M.G. Hutton, and J.R. Myers. 2014. Broccoli cultivar performance under organic and conventional management systems and implications for crop improvement. Crop Science. 54: 1539-1554.

Renwick, J.A.A. and K. Lopez. 1999. Experience-based food consumption by larvae of *P. rapae:* Addiction to glucosinolates? Entomologia Experimentalis et Applicata. 91: 51-58.

Renwick, J.A.A. and C.D. Radke. 1985. Constituents of host- and non-host plants deterring oviposition by the cabbage butterfly, *P. rapae.* Entomologia Experimentalis et Applicata. 39: 21-26.

Renwick, J.A.A., C.D. Radke, K. Sachdev-Gupta, and E. Stadler. 1992. Leaf surface chemicals stimulating oviposition by *Pieris rapae* (Lepidoptera: Pieridae) on cabbage. Chemoecology. 3: 33-38.

Richards, O.W. 1940. The biology of the small white butterfly (*Pieris rapae*), with special reference to the factors controlling its abundance. J. Animal Ecology. 9: 243-288.

Root, R.B. 1973. Organization of a plant-arthropod association in simple and diverse habitats, the fauna of collards (*Brassica oleracea*). Ecol. Monogr. 43: 95-120.

Rosa, E.A.S. and A.S. Rodrigues. 2001. Total and individual glucosinolate content in 11 broccoli cultivars grown in early and late seasons. HortScience. 36(1): 56-59.

Rosen, C.J., V.A. Fritz, G.M. Gardner, S.S. Hecht, S.G. Carmella, and P.M. Kenney. 2005. Cabbage yield and glucosinolate concentrations as affected by nitrogen and sulfur fertility. HortScience. 40(5): 1493-1498.

Santolamazza-Carbone, S., T. Sotelo, P. Velasco, and M.E. Cartea. 2016. Antibiotic properties of the glucosinolates of *Brassica oleracea* var. *acephala* similarly affect generalist and specialist larvae of two lepidopteran pests. J. Pest Sci. 89: 195-206.

Santolamazza-Carbone, S., P. Velasco, J. Selfa, P. Soengas, and M.E. Cartea. 2013. Intraspecific variation of host plant and locality influence the lepidopteran-parasitoid system of *Brassica oleracea* crops. J. Econ. Entomol. 106(3): 1134-1144.

Santolamazza-Carbone, S., P. Velasco, J. Selfa, P. Soengas, and M.E. Cartea. 2014. Bottom-up and top-down herbivore regulation mediated by glucosinolates in *Brassica oleracea* var. *acephala.* Oecologia. 174: 893-907.

Schellhorn, N.A. and V.L. Sork. 1997. The impact of weed diversity on insect population dynamics and crop yields in collards, *Brassica oleracea* (Brassicaceae). Oecologia. 111: 233-240.

Sears, M.K., R.P. Jacques, and J.E. Laing. 1983. Utilization of action thresholds for microbial and chemical control of lepidopterous pests (Lepidoptera: Noctuidae, Pieridae) on cabbage. J. Econ. Entomol. 76: 368-374.

Shelton, A.M., J.T. Andaloro, and J. Barnard. 1982. Effects of cabbage looper, imported cabbageworm, and diamondback moth on fresh market and processing cabbage. J. Econ. Entomol. 75: 742-745.

Shelton, A.M., C.W. Hoy, R.C. North, M.H. Dickson, and J. Barnard. 1988. Analysis of resistance in cabbage varieties to damage by Lepidoptera and Thysanoptera. J. Econ. Entomol. 81: 634-640.

Shelton, A.M., M.K. Sears, J.A. Wyman, and T.C. Quick. 1983. Comparison of action thresholds for Lepidopterous larvae on fresh-market cabbage. J. Econ. Entomol. 76: 196-199.

Stoner, K.A. 1990. Glossy leaf wax and plant resistance to insects in *Brassica oleracea* under natural infestation. Environ. Entomol. 19: 730-730.

Stoner, K.A. 1992. Density of imported cabbageworms (Lepidoptera, Pieridae), cabbage aphids (Homoptera, Aphididae), and flea beetles (Coleoptera, Chrysomelidae) on glossy and trichome-bearing lines of *Brassica oleracea*. J. Econ. Entomol. 85: 1023-1030.

Tabashnik, B.E. 1987. Plant secondary compounds as oviposition deterrents for cabbage butterfly, *P. rapae* (Lepidoptera: Pieridae). J. Chem. Ecol. 13: 309-316.

Tabashnik, B.E. 1994. Evolution of resistance to *Bacillus thuringiensis.* Annu. Rev. Entomol. 39: 47-79.

Textor, S. and J. Gershenzon. 2009. Herbivore induction of the glucosinolates-myrosinase defense system: Major trends, biochemical bases and ecological significance. Phytochem. Rev. 8: 149-170.

Theunissan, J. and H. den Ouden. 1980. Effects of intercropping with *Spergula arvensis* on pests of Brussels sprouts. Entomol. Exp. Appl. 27: 260-268.

Toth, N., S. Fabek, B. Benko, M.H. Custic, and I.R. Redovnikovic. 2014. Yield components and glucosinolate content of broccoli cultivars in summer-autumn growing cycle. In Proc. IIIrd IS on Human Health Effects of Fruits and Vegetables. B. Patil et al. [eds]. Acta Hort. 1040: 323-330.

Traw, M.B and T.E. Dawson. 2002. Reduced performance of two specialist herbivores (Lepidoptera: Pieridae, Coleoptera: Chrysomelidae) on new leaves of damaged black mustard plants. Environ. Entomol. 31(4): 714-722.

Vagen, I.M., T.S. Aamlid, and A.O. Skjelvag. 2007. Nitrogen fertilization to broccoli cultivars at different planting times: Yield and nitrogen use. Acta Agric. Scandinavica. 57(1): 35-44.

van Dam, N.M., C.E. Raaijmakers, and W.H. van der putten. 2005. Root herbivory reduces growth and survival of the shoot feeding specialist *Pieris rapae* on *Brassica nigra.* Entomologia Experimentalis et Applicata. 115: 161-170.

Walling, L.L. 2000. The myriad plant responses to herbivores. J. Plant Growth Reg. 19: 195-216.

White, A.J., S.D. Wratten, N.A. Berry, and U. Weigmann. 1995. Habitat manipulation to enhance biological control of *Brassica* pests by hover flies (Diptera: Syrphidae). J. Econ. Entomol. 23: 654-548.

Wittstock, U, N. Agerbirk, E.J. Stauber, C.E. Olsen, M. Hippler, T. Mitchell-Olds, J. Gershenzon, and H. Vogel. 2004. Successful herbivore attack due to metabolic diversion of a plant chemical defense. Proceedings of the National Academy of Sciences of the USA. 101(14): 4859-4864.

Workman, R.B., R.B. Chalfant, and D.J. Schuster. 1980. Management of cabbage worms with two damage thresholds and five insecticide sprays. Ibid. 73: 757-760.

Zhang, S. H. Huang, H. Shan, F. Zhang, F. Wan, and T. Liu. 2013. Defense against *Pieris rapae* in cabbage plants induced by *Besimia tabaci* biotype B. Entomologia Experimentalis et Applicata. 147: 293-300.

Zhao, J.Z., G.S. Ayers, E.J. Grafius, and F.W. Stehr. 1992. Effects of neighboring nectar-producing plants on populations of pest Lepidoptera and their parasitoids in broccoli plantings. Great Lakes Entomol. 25(4): 253-258.