THE EFFECTS OF GROUND-FLOOR MANAGEMENT ON BLUEBERRY MAGGOT (*RHAGOLETIS MENDAX* CURRAN) AND PREDATORY BEETLES IN HIGHBUSH BLUEBERRIES

by

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The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled "The Effects of Ground-Floor Management on Blueberry Maggot (*Rhagoletis mendax* Curran) and Predatory Beetles in Highbush Blueberries" by Justin M. Renkema in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

There is very low tolerance for blueberry maggot (*Rhagoletis mendax* Curran) in blueberries making it a serious pest in eastern North America. Control is focused on eliminating flies with insecticides before oviposition in fruit. Organic mulches may improve highbush blueberry production, but their effects on this pest are largely unknown. The research in this thesis evaluated compost and pine needle mulches on blueberry maggot (mature maggots, pupae, emerging flies) and predatory beetles that may consume these stages.

Mulches affected fly emergence and maggot pupation. Fly emergence was reduced by 80-100% and delayed ~8 days from pupae covered with 20 cm of pine needles compared to 1 cm of soil. Emergence from 20 cm of compost was lower in wet conditions, and 5 cm of mulch did not limit emergence in the field. Pupation at increased depth reduced emergence because flies did not eclose or, if eclosed, were unable to crawl to the surface. The delay was likely due to lower temperatures at depth. No flies emerged from 1 cm in pine needles in the field probably due to high temperatures. In the laboratory, maggots pupated more deeply in pine needles than other substrates, but ~30% did not pupate in dry pine needles. Saturated soil caused ~50% of maggots to pupate on the surface.

Mulching altered beetle diversity and activity/density and affected beetle predation on maggots and pupae. Compost plots in a highbush blueberry field attracted predatory carabids and staphylinids, although some staphylinids were frequent in pine needles and phytophagous carabids preferred unweeded compost plots. Attraction to compost, particularly for *Pterostichus melanarius* (Illiger), was due primarily to higher prey densities - earthworms and millipedes - than other treatments. This beetle consumed some maggots or pupae in soil or compost, but predation rates decreased when alternative prey was abundant.

Blueberry bushes in weedy compost plots attracted many flies, but infestation rates were higher in composted bushes only in the year mulch was applied. Overall, thick mulching with pine needles contributes most significantly to blueberry maggot management. Future research should explore integration of mulch with other tactics for *R. mendax* control.

LIST OF ABBREVIATIONS USED

ABS	acrylonitrile butadiene styrene
AITC	allyl isothiocyanate
AM	apple maggot
ANOVA	analysis of variance
C	carbon
°C	degree Celsius
CI	confidence interval
DCA	detrended correspondence analysis
DD	degree-days
DNA	deoxyribonucleic acid
GF-120	GF-120 NF Naturalyte* Fruit Fly Bait
h	hour
HSD	honestly significant difference
N	nitrogen
NH_4-N	ammonium-nitrogen
NO_3-N	nitrate nitrogen
R	correlation coefficient
R^2	coefficient of determination
RDA	redundancy analysis
SD	standard deviation
SE	standard error
SEM	standard error of the mean
T_{max}	maximum daily temperature
T_{min}	minimum daily temperature
V	volume
W	watt
WHC	water holding capacity

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CHAPTER 1 INTRODUCTION

1.1 GENERAL INTRODUCTION

Blueberry maggot (*Rhagoletis mendax* Curran, Diptera: Tephritidae) is a Nearctic carpophagous pest of blueberries. As with all fruit-feeding pests, there is low customer tolerance for maggot in fresh fruit; in unmanaged fields up to forty-five percent of highbush blueberries can be infested with maggot (Stelinski et al., 2004). In addition, blueberry maggot is a quarantine pest in Canada, and, to prevent its spread, commercial fruit exported beyond boundaries of infested provinces must be inspected to be maggot-free (CFIA, 2009). Growers primarily rely on conventional insecticides to kill flies before females oviposit in berries (Delbridge and Rogers, 2010). Management may be shifting towards more targeted insecticide use and reduced-risk products (e.g., Pelz et al., 2005), including GF-120 in organic production (DowAgroSciences, 2009). However, an integrated approach to control would be improved if soil-dwelling life stages were negatively affected by altering ground-floor management of highbush blueberries.

1.2 DISTRIBUTION, HOST RANGE, AND IDENTIFICATION

In Canada, blueberry maggot occurs in Nova Scotia, New Brunswick, and Prince Edward Island and has spread to municipalities and isolated farms in southern Quebec and Ontario (Vincent and Lareau, 1989; CFIA, 2009). It is not found in Newfoundland or British Columbia. In the eastern USA, the range of blueberry maggot extends from northern Florida, through North Carolina, New Jersey, and Maine and northwest to Michigan and southwest to the Ozark Plateau (Missouri/Arkansas) (Bush, 1966; Payne and Berlocher, 1995). Blueberry maggot is relatively monophagous, found in plantings of lowbush (*Vaccinium angustifolium* Aiton) and highbush (*V. corymbosum* L.) blueberries in the north and in rabbiteye blueberries (*V. ashei* Aiton) in the south. Blueberry maggot may also infest non-crop hosts, especially other *Vaccinium* spp., closely related huckleberries (*Gaylussacia* spp.) and also wintergreen (*Gaultheria procumbens* L.) in the north (Lathrop and Nickels, 1932; Smith et al., 2001). In the south, deerberry (*V. stamineum* L.) appears to be a primary non-crop host (Payne and Berlocher, 1995). When these species grow in close proximity to managed blueberry fields, they may provide a reservoir of flies that yearly re-infest field edges, although this is seldom documented (Payne and Berlocher, 1995).

Blueberry maggot is often considered a sibling species of *Rhagoletis pomonella* (Walsh), *R. zephyria* Snow, and *R. cornivora* Bush (the *pomonella* group). This group can be identified from other *Rhagoletis* spp. by characteristic black, F-shaped markings on clear wings and bicoloured halteres (Bush, 1966). Within the *pomonella* group, identification based on adult morphological characteristics is difficult, although blueberry maggot is typically smaller than *R. pomonella* (5 mm long with 8 mm wing span), females have shorter ovipositors, and there is a difference in wing band ratios and a lack of black shading on the posterior surface of femur I (Bush, 1966). Since the preferred hosts of sympatric *R. pomonella* (domestic apples and hawthorns) and *R. mendax* often grow in close proximity and there may be overlap in host acceptance and emergence timing, the possibility of hybridization exists. However, significant numbers of species-specific alleles have been found (Berlocher, 1980; Berlocher and Bush. 1982; Feder et al., 1989), and in combination with differences in host-plant use and host-odour recognition that may serve as pre-mating barriers or oviposition preferences that may be post-mating barriers (Averill et al., 1996; Bierbaum and Bush, 1990; Diehl and Prokopy, 1986; Frey and Bush, 1996; Frey et al., 1992), substantial gene flow is likely minimal. Unlike *R. pomonella*, *R. mendax* is relatively uniform in genetic structure across its geographic range with no consistent differentiation between specific host plants (Berlocher, 1995). Therefore, host race formation in blueberry maggot is unlikely.

1.3 GENERAL BIOLOGY

The general biology of blueberry maggot is relatively well known, with Lathrop and Nickels (1932) providing the first detailed natural history of flies infesting lowbush blueberries in Maine. Across its range, blueberry maggot is univoltine, but a portion of the population may overwinter for 2-4 years. Teneral adult flies emerge from soil puparia beneath host plants during fruiting period (Boller and Prokopy, 1976; Lathrop and Nickels, 1932). Occuring in northerly regions from late June, throughout July and sometimes into August, emergence is largely determined by soil temperatures. A degree-day model has been developed to predict emergence timing (Teixeira and Polavarapu, 2001c). Once capable of flight, flies disperse in search of food sources; homopteran honeydew, bird feces, and plant exudates provide ample carbohydrates for longevity and proteins for gonadal maturation (Boller and Prokopy, 1976; Christenson and Foote, 1960; Lathrop and Nickels, 1932). *Panotea (Enterobacter) agglomerans*, a diet-obtained symbiont identified from *Rhagoletis* spp., allows for the degradation of uric acid in the guts of adult fruitflies (Lauzon et al., 1998, 2000). Flies may be attracted to food sources containing this and other beneficial bacteria (MacCollom et al., 2009). After 1-2 weeks spent feeding, mating begins on plant leaves and also on fruit (Smith and Prokopy, 1982). Female movement and contrasting wing pattern colours may serve as male visual cues (Bush, 1966; Christenson and Foote, 1960; Smith and Prokopy, 1982). Males may also guard or perch on mating sites (berries), waiting for arriving females (Smith and Prokopy, 1981). Oviposition of a single egg just below the fruit surface is mainly elicited by fruit shape, size and colour (ripeness) (Bateman, 1972; Boller and Prokopy, 1976; Lathrop and Nickels, 1932), and fecundity can be greater than 300 eggs per season under optimal conditions. Females drag their ovipositors over the fruit surface and deposit a pheromone that deters multiple oviposition in a berry (Prokopy et al., 1976). First-instar maggots are found in berries in mid-July in Maine lowbush blueberries (Lathrop and Nickels, 1932) and develop through three instars by feeding on fruit pulp. Bacteria may also be important at this stage for breaking down fruit tissue and causing rot. Pre-pupal maggots exit from hanging or fallen fruit in early to mid-August and form a puparium in the upper 2-5 cm of the soil (Lathrop and Nickels, 1932). Pupae enter an obligatory diapause period until increasing spring temperatures initiate morphogenetic development (Teixeira and Polavarapu, 2001c). A late emerging cohort has been documented in New Jersey (Teixeira and Polavarapu, 2001b) caused by high summer soil temperatures inducing a period of quiescence (Teixeira and Polavarapu, 2005b,c,d).

1.4 MANAGEMENT

Accurate monitoring to determine when blueberry maggot flies are active is important for successful control. New knowledge about attraction to food-, and fruit-volatiles may help refine monitoring techniques and may also be important for trapping large numbers of flies. Pherocon®AM traps (Trécé, CA; yellow sticky boards containing ammonium acetate and protein hydrolysate) are commonly used and are most effective at capturing flies when placed sticky-side-out in a downwards-facing V position in lowbush blueberries (Gaul et al., 1995; Geddes et al., 1989) and when replaced every two weeks within the top 15 cm of the highbush blueberry canopy (Liburd et al., 2000). Baited green and red spheres can be more attractive than Pherocon®AM traps, but do not always capture more male and female flies throughout the flight period (Liburd et al., 1998b, 2000; Prokopy and Coli, 1978; Teixeira and Polavarapu, 2001a). Sugars are attractive to flies (Barry and Polavarapu, 2004), and flies are attracted to some of the volatile compounds identified from ripe host-fruits (Kwasniewska, 2009; Lugemwa et al., 1989; Stelinski, 2001). However, responses were typically not greater than to ammonium acetate and did not necessarily increase captures when incorporated into traps in the field (Liburd, 2004; Kwasniewska, 2009; Pelz-Stelinksi et al., 2005). If symbiotic bacteria are attractive, they could be useful for attracting and trapping flies (MacCollom et al., 2009). The success of mass-trapping or attract-and-kill strategies that could reduce insecticide use will largely depend on continued refinement of lures and traps for flies.

Currently, control of blueberry maggot is typically achieved by targeting flies with insecticides before females oviposit in fruit. This was achieved with arsenicals and DDT prior to organophosphates and carbamates (Boller and Prokopy, 1976), and now acetamiprid, dimethoate, phosmet, carbaryl, malathion, and spinosad (GF-120 NF Naturalyte Fruit Fly Bait) are registered and recommended for use in Canada (Delbridge and Rogers, 2010). GF-120, an organically acceptable product, was as effective as other insecticides in the laboratory (Barry and Polavarapu, 2005) and reduced maggot infestation in berries to 0.5% or less (Barry et al., 2005; Pelz et al., 2005). Insecticides are usually applied to entire blueberry fields 5 to 10 days following detection of first fly on baited traps. Acetamiprid and spinosad may be reapplied up to four and five times per season, respectively, with the latter reapplied at least every seven days and at shorter intervals in rainy conditions. The other insecticides should be reapplied only once if flies are continually captured on traps.

Recent research has focused on reducing or eliminating insecticide sprays on crops by incorporating mainly neonicotinoid insecticides into ammonium baited spheres for an attract-and-kill system. When imidacloprid, clothianidin, thiamethoxam, fipronil, and thiocloprid were coated on spheres fly mortality was greater than on untreated spheres, and imidacloprid tended to cause highest rates of mortality (Ayyappath et al., 2000; Barry et al., 2004; Liburd et al., 1999, 2003; Stelinski and Liburd, 2001; Stelinski et al., 2001). In order to reduce berry infestation to levels achieved with sprayed insecticides (Stelinski and Liburd, 2001) with minimal numbers of spheres/labour costs, deployment strategies will need to be optimized. The index for assigning distances between spheres developed by Prokopy et al. (2005) for apple maggot may serve as a good template for blueberry maggot. Spheres may primarily be used in fields with low infestations to control flies in areas, such as edges, with a history of higher infestations.

There are few other proven or adopted methods aimed at reducing blueberry maggot, but certain treatments and production practices may be valuable in an integrated management system. Kaolin clay can negatively affect fly behaviour on fruits, but infestation rates may not be lowered due to non-uniform coverage on fruit in the field (Lemoyne et al., 2008). Good weed management (Gaul et al., 1995) and removal of non-crop hosts around fields may lower immigration rates into fields. Shallow cultivation under bushes may damage and expose pupae to other mortality factors, as suggested for other *Rhagoletis* spp. (AliNiazee, 1974), and irrigation could alter survival of pupae in soil (Pearson and Meyer, 1990). Early ripening highbush cultivars, such as Bluetta and Earliblue, could be chosen, as they were reported to have fewer maggots than mid-season or late cultivars (Liburd et al., 1998a). Finally, post-harvest fumigation of fruit with CO_2 (Prange and Lidster, 1992) or gamma irradiation (Hallman and Thomas, 1999) may be effective for killing maggots in fruit and applicable for larger producers.

The potential of natural enemies – parasitoids, predators, or pathogens – against blueberry maggot has been suggested (Lathrop and Nickels, 1932), but there are no biological control tactics currently being used. The braconid parasitoid, *Diachasma alloeum* (Muesebeck) is attracted to volatiles emitted from infested blueberries (Stelinski et al., 2004, 2006), but parasitism rates declined when kaolin clay and imidacloprid were used (Stelinski et al., 2006). Specific predators of blueberry maggot have not been identified, but generalists such as ground and rove beetles, crickets, centipedes, ants and others destroyed many apple maggot pupae, and spiders may capture adult flies (Monteith 1971, 1972, 1976a,b). Interestingly, the dark F-shaped wing pattern combined with the quick, "jumping" nature of maggot flight, may mimic certain spiders and deter predators (Greene et al., 1987; Mather and Roitberg, 1987). Entomopathogens can infect multiple life stages of other *Rhagoletis* spp. (Cossentine et al., 2010; Daniel and Wyss, 2009; Yee and Lacey, 2005), but *Beauveria bassiana* did not provide significant control of maggot when applied in the field as a soil drench (Collins and Drummond, 2010).

1.5 BLUEBERRY PRODUCTION AND MULCHING

Blueberries are a popular fresh fruit because they contain high levels of beneficial antioxidants (Prior et al., 1998). In Canada, there were over 62 000 hectares of blueberries (2008) producing over 77 000 metric tonnes with a farm-gate value of $189 000 000 (2007), more than double the value in 2002 (StatsCan, 2008). In the United States, the world's largest producer, production and farm-gate values have increased substantially for most major blueberry producing states from 2000-2008 (USDA, 2010). However, increased worldwide production – Chile's production has increased to almost 10 000 hectares in 2007 – may be over-supplying demand, leading to lower prices in recent years (Banados, 2006; Fruit Growers News, 2011). Market-share and profitability may be sustained and increased by growers who are interested in new ideas and techniques for production.

Practices are changing in many areas of highbush blueberry production as opportunities for organic production are increasing (Sciarappa et al., 2008). For example, mulching and amending the soil is increasingly common. In young highbush plantings, Eck et al. (1990) recommended pine needle or sawdust mulch, and Burkhard et al. (2009) showed a significant yield increase after two years with seafood or manure compost mulch. In southern highbush, plant growth and yield was greatest when pine bark or white-over-black plastic was used as a mulch (Magee and Spiers, 1995), and peat mulch caused better growth compared to plastic and sawdust mulch in half-high blueberries (Starast et al., 2000). At-plant soil amendments with forest litter (Yang et al., 2002), low-cost materials containing pine products (such as telephone pole peelings) (Krewer and Ruter, 2000), and pine bark (Odneal and Kaps, 1990) all increased plant growth and yield. Pine needle mulch was particularly effective at decreasing the number of weeds (Burkhard et al., 2009).

Organic mulches, such as killed cover crops or straw, have often been applied in a variety of cropping systems to reduce pest pressure and plant damage by conserving natural enemies. (Brust, 1994; Halaj et al., 2000; Jackson and Harrison Jr., 2008; Johnson et al., 2004; Pullaro et al., 2006; Schmidt et al., 2004). Composted mulches may be particularly effective at attracting natural enemies because they encourage a detritial food chain that provides abundant food sources for predators (Bell et al., 2008; Mathews et al., 2004). Mulches may also directly affect pests (Vincent et al., 2003), but potential for control seems to be little studied. Mulches, as do altered tillage regimes, may affect moisture and humidity levels and thus ovisposition success or pupal survival (e.g., Brust and House, 1990; Keularts and Lindquist, 1989), or when applied may physically limit emergence of pests (Chen and Shelton, 2007). Mulches may also contain chemicals that may have a negative effect on pests (e.g., terpenes in pine needles). If mulches can be used successfully for weed control and improved yields in highbush blueberries, and also negatively effect blueberry maggot through physical and biological mechansisms, then their benefit to cost ratio will increase and organic production or reduced-risk insecticide programs may be more attractive to growers.

1.6 RESEARCH OBJECTIVES

The research undertaken in this thesis is primarily motivated by the need to develop an integrated management strategy for blueberry maggot in a highbush blueberry system where conventional insecticide use is reduced or completely eliminated in order to meet organic standards. Because of its proven effectiveness, GF-120 may be the primary tool against blueberry maggot in an organic system, and baited spheres and edge-based approaches appear to be emerging alternatives for lowering insecticide use in conventional systems (e.g., Stelinski and Liburd, 2001; Zaman et al., 2009). These methods primarily target flies, but a fully integrated management plan should include tools that target multiple life stages. Mortality factors of maggots, pupae, and emerging flies in soil are notoriously understudied, and mulches may alter biotic and abiotic environment so that survival is significantly affected. Therefore, mulch may reduce maggot pressure in fields, but could also have undesired consequences if mortality is lower than in soil.

There are numerous ways by which mulch application may directly or indirectly affect stages of the blueberry maggot life cycle. In this thesis I primarily explore mulch effects that have potential to negatively impact wandering and burrowing maggots, overwintering pupae, and post-diapause pupae and emerging flies. These stages occur briefly on or entirely in soil and the organic layer beneath blueberries.

In chapter two, laboratory and field experiments were used to test eclosion and emergence rates of flies when varying thicknesses of mulches were placed on pupae. I hypothesized that thickly applied mulch will reduce emergence compared to that in soil because eclosed flies would have difficulty reaching the surface. I also expected that low temperatures at increased mulch depth would delay emergence. In the third chapter, pupation depth in mulches at varying moisture levels was evaluated in the laboratory. I anticipated deeper pupation in mulches because they are less dense than soil and shallower pupation at high moisture levels in all substrates, except in porous pine needle mulch.

Chapter four examined how ground-dwelling predators (Carabidae and Staphylinidae) were affected by mulches and weedy vegetation in a highbush blueberry field. I hypothesized that predatory beetles, particularly the larger carabid *Pterostichus melanarius* (Illiger), would be attracted to mulches if food sources, particularly detritivorous prey, were abundant. The fifth chapter; therefore, evaluated *P. melanarius* as a predator of *R. mendax* maggots and pupae and the effect alternative prey had on predation rates in semi-field and field conditions. I expected this beetle to reduce numbers of *R. mendax* pupae in compost mulch, but that predation rates would decrease when alternative prey was abundant. Chapter six evaluated effects of mulch and weeds on blueberry maggot fly captures, fruit infestation rates, and plant growth over three years in one field. I anticipated that flies would be attracted to weedy plots resulting in higher infestation rates in berries from these plots. I also expected that bushes would grow faster rate in mulched than unmulched plots, and that these bushes would contain more ripe berries that would also attract more flies and increase infestation rates.

I concluded by estimating total mortality inflicted by abiotic or biotic factors at maggot, pupal, and adult stages in mulches versus soil as determined in experiments in this thesis. I then formulated practical recommendations taking into account mortality rates in different mulches and horticultural reasons for mulch application. Finally, I offered ideas for future research on further quantification of mortality of ground-dwelling life stages in mulches and how mulch may be compatible with other emerging techniques for integrated management of blueberry maggot.

CHAPTER 2 EMERGENCE OF BLUEBERRY MAGGOT FLIES (DIPTERA: TEPHRITIDAE) FROM MULCHES AND SOIL AT VARIOUS DEPTHS

2.1 ABSTRACT

Control of blueberry maggot (*Rhagoletis mendax* Curran) is typically achieved with insecticides targeting adult flies prior to oviposition in ripening fruit. Management strategies targeting other life stages, such as the use of mulches to inhibit emergence from soil, have received less attention. Effects of adding compost or pine needle mulches on eclosion and emergence of blueberry maggot were tested in laboratory and field conditions. Pupae that were deeply buried (20 cm) under pine needles had consistently lower emergence rates than most shallowly buried pupae (1 cm), partially due to difficulty of flies reaching the surface after eclosion. Pupae buried 20 cm in compost had low emergence rates under wet conditions. Eclosion failure was associated with high moisture levels in compost or with high temperatures and dry conditions near the surface of other substrates, particularly pine needles. Cooler temperatures at depth also delayed fly emergence. Thus, mulch application under bushes after maggots have dropped from berries can reduce emergence success of buried pupae, but the level of control will depend on mulch depth and type and may vary with rainfall and temperature.

2.2 INTRODUCTION

Blueberry maggot (*Rhagoletis mendax* Curran) is a major pest of highbush blueberries in eastern North America. Females lay eggs in ripening berries where developing maggots feed and cause soft, disfigured fruit. Pre-pupal maggots leave fruit, burrow shallowly into soil, and form a puparium in which they overwinter and complete diapause. Insecticides are used mainly to kill adult flies before females oviposit, and multiple sprays in a season are not uncommon (Delbridge and Rogers, 2010). Customer demands for organic produce, lower pesticide residue limits on food (EU, 2008), and concerns about environmental impacts of chemical inputs are driving the search for alternative management strategies for blueberry maggot.

Little is known about mortality factors affecting soil-dwelling pre-pupal maggot and pupal life stages of blueberry maggot. However, studies with other tephritid pupae have shown that exposure to very dry or moist conditions reduces fly emergence (Hou et al., 2006; Hulthen and Clarke, 2006; Montoya et al., 2008; Trottier and Townshend, 1979). High temperatures can also reduce emergence success and alter timing (Fitt, 1981; Rwomushana et al., 2008; Salles et al., 1995; Teixeira and Polavarapu, 2005d). Entomopathogenic fungi caused significant mortality of emerging *Rhagoletis* spp. flies (Yee and Lacey, 2005) and moderate mortality when maggots and pupae were exposed to conidia of fungi (Cossentine et al., 2010; Daniel and Wyss, 2009). Predation by beetles and ants (Aluja et al., 2005; Hennessey, 1997; Urbaneja et al., 2006) and infection by entomopathogenic nematodes (Sirjani et al., 2009) may also be significant sources of pupal mortality. Ground-floor management and irrigation regimes may affect moisture, temperature, pathogens, and predators in highbush blueberries, and it could be useful to determine if and how blueberry maggot fly emergence is affected.

Organic mulches in highbush blueberries are recommended to growers for increased plant productivity and weed suppression (Burkhard et al., 2009; Kuepper and Diver, 2010). Mulch applied thickly in late fall after blueberry maggot pupation or early spring would bury pupae deeper than the normal pupation depth of 1-3 cm (Lathrop and Nickels, 1931, 1932). The following summer, eclosed flies may have difficulty reaching the surface causing reduced emergence. Lathrop and Nickels (1932) reported that flies were able to emerge through 10 cm of sand or peaty loam; however, deep pupation was thought to reduce emergence of another tephritid (Siddiqi and Ashraf, 2002) and lowers emergence of a leaf miner and swede midge (Chen and Shelton, 2007; Keularts and Lindquist, 1989).

There is no information on effects of mulching on blueberry maggot emergence. Management, especially in organic cropping systems, could be improved if mulching has deleterious effects on the buried pupae or emerging flies. In this study, I evaluated the effects of mulches on fly eclosion and emergence when pupae were placed at different depths. I anticipated that fewer flies would emerge from more deeply buried pupae because eclosed flies would have difficulty reaching the surface and because temperature and moisture levels in mulches may affect eclosion rates.

2.3 MATERIALS AND METHODS

2.3.1 Pupae And Mulch Sources

Pupae were obtained from a population of blueberry maggot in a highbush blueberry field near Rawdon, Nova Scotia (45N 3’37’’, 63W 42’21’’). Infested blueberries were picked in summers previous to experiments and held on wire screens over wooden bins of moist sand to allow maggots to drop and pupate. In the fall, pupae were floated from sand and immediately used (2009 experiment) or were allowed to overwinter and complete diapause in moist sand in Petri dishes at 2-3°C until used the following spring (2010 experiments).

Mulches were composted hardwood processing waste (Louisiana-Pacific Ltd., Chester, NS) (Envirem Organics Inc., 2003) and uncomposted fallen pine needles, *Pine strobus* L., raked from a campground (Coldbrook, NS). Mulches were collected in September 2008 (spring 2009 experiment) and March 2010 (spring 2010 experiments) from plots under rows of highbush blueberries (Rawdon, NS). Samples of clay loam soil were collected from the top 5-10 cm in the same field. All materials were held in covered plastic tubs at 4°C until used in experiments. Sub-samples were dried at 105°C for 24 h to determine initial moisture by weight (Table 2.1).

Table 2.1 Physical characteristics of substrates used for *R. mendax* growth chamber and field emergence studies

Substrate[a]	Bulk density (g/cm^3)	Initial % moisture (w^b/w)	
	2009 and 2010	2009	2010
Compost	0.50	57.7 ± 0.7	54.2 ± 0.3
Pine needles	0.05	27.3 ± 1.9	11.6 ± 2.0
Soil	1.10	22.1 ± 0.2	16.8 ± 0.6

[a]compost was from wood processing waste, pine needles were uncomposted, and soil was from a highbush blueberry field

[b]w = weight

2.3.2 Mulch Effects On Fly Emergence In A Growth Chamber

Effects of mulch or soil and burial depth on fly emergence and eclosion were tested under controlled conditions in a growth chamber in 2010. Treatments were three substrates (compost mulch, pine needle mulch, and soil) and three depths (1, 5, and for mulch only, 20 cm). I did not test 20 cm in soil, as maggots do not burrow that deep in soil, and growers do not apply 20 cm of soil as mulch. Each mulch and depth combination was replicated four times in a completely randomized design. The growth chamber was kept at a constant 23°C, 16:8 L:D, and 60-65% RH. The experiment was initiated on 31 May 2010.

For each replicate, 20 pupae were placed on 1 cm of mulch or soil in the centre of a plastic dish (DM8R-0090, Solo®, Toronto ON). A black ABS plastic tube (diameter = 5.3 cm) with a Mason jar band glued to the top was placed over each dish of pupae and then filled to the appropriate depth with a pre-weighed amount of each treatment to obtain the correct bulk density (Table 2.1). The initial weight of the filled tube and dish was recorded.

Twice weekly until the end of the experiment, tubes and dishes were weighed and water dripped over surfaces to replace that lost to evaporation. On 24 June 2010, Mason jars (125 mL) were screwed into the bands and checked twice weekly for emerging flies. Flies, including any dead on substrate surfaces, were counted and removed until there was no emergence from any tube for three consecutive checks (~10 days). Soil or mulch from tubes was then examined, and pupae that were found were classified as having had a fly eclose (exit hole present) (Lathrop and Nickels, 1932), rotten (discolored, bluish-black), or intact (normal golden yellow color).

2.3.3 Mulch Effects On Fly Emergence In The Field

Effect of mulch type and depth on blueberry maggot emergence was tested two years in the field within mulch plots previously applied in highbush blueberries near Rawdon, NS. In 2009, treatments were compost and pine needle mulches at 5 and 20 cm and soil at 5 cm. In 2010, additional treatments of 1 cm of mulches and soil were included. The varying depths were replicated eight times in 2009 and six times in 2010 within one 18 m row of compost, pine needles or soil. Groups with one replicate of each depth were placed 1.5 – 2.0 m apart on the south side of the bush row. The 2009 experiment was initiated with diapausing pupae on 19 Sep 2008, and the 2010 experiment began on 17 Apr 2010 with pupae that had completed diapause.

For each replicate, 20 pupae were placed on pieces of plastic window screening fitted in the bottom of a Mason jar band (Fig. 2.1). A small amount of mulch or soil was placed in the band to prevent the pupae from moving and the band with pupae was placed into a nylon stocking with the toe (under the band) filled with mulch or soil. The stocking was placed inside an ABS tube to maintain its shape and filled with a pre-weighed substrate to the appropriate depth. The stocking was then slid from the tube and placed into a pre-dug hole in mulch or soil beneath a bush so that the substrate at the top of the stocking was flush with the mulch or soil surface. Holes were backfilled so that the stocking was snug with mulch or soil. Stockings were cut ~10 cm above the surface and covered with a piece of hardware cloth to prevent damage during winter and spring.

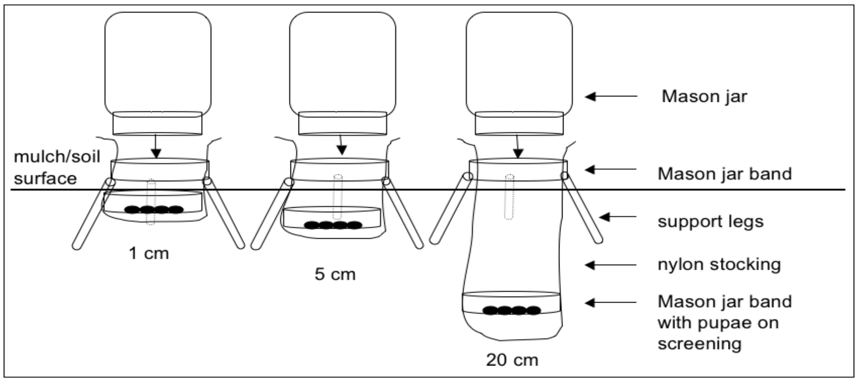

Figure 2.1 Pupae cages used in field experiments to assess effect of pupal depth on emergence of *R. mendax* flies.

Soil and mulch moisture levels were monitored in stockings without pupae from 21 April until 31 July in 2009 and 2010. Soil and mulch temperatures were recorded in 2010 only, beginning April 17. Volumetric moisture (m^3/m^3) levels were obtained from ECH$_2$0 Dielectric Aquameter probes (S-SMC-M005 sensors, Decagon Devices Inc., Pullman, WA; calibrated to -0.1 m^3/m^3 in air) attached to a HOBO Weather Station (Onset Computer Corp., Pocasset, MA), and hourly temperatures were recorded with

HOBO U12 data loggers (TMC50-HD sensors, Decagon Devices Inc., Pullman, WA). Sensors were placed at 1, 5, or 20 cm below soil or mulch surfaces in one stocking of each treatment, except moisture levels were not recorded in the 1 cm pine needle treatment due to difficulty placing a sensor. Degree-days accumulating at varying depths in substrates were calculated as $(T_{max} - T_{min})/2 - 4.7$ºC; T_{max} was 30 when daily maximums exceeded 30ºC in the field (Teixeira and Polavarapu, 2001b). Mean moisture levels for the entire monitoring period were determined.

On 25 June 2009 and 2010 hardware cloth pieces were removed. The top of each stocking was pulled through a Mason jar band, and a glass Mason jar (500 mL) was screwed into the band to capture flies (Fig. 2.1). Each jar and band was supported a few centimeters above the surface on four to five large nails pushed into the mulch or soil to minimize heating of mulch or soil through the jar. Jars were checked every third day, and emerged flies and those found dead on the surface were counted and removed until there was no emergence from any stocking for nine consecutive days. In 2010, stockings were brought to the laboratory at the end of the experiment, and pupae were categorized as in the growth chamber experiment.

2.3.4 Data Analysis

Analysis of variance (ANOVA) was used to analyze effects of substrate and pupal depth on number of flies emerged, eclosion success, and number of emerged flies divided by the number of eclosed flies. In the growth chamber experiment I tested two hypotheses: (1) emergence differs between soil and mulches in the range of depths at which pupation normally occurs (1-5 cm), and (2) emergence success is lower under deep (20 cm) mulch compared to shallow (1 or 5 cm) mulch. Separate ANOVAs were used for each substrate in the field, as replicates for the mulch treatments were not spatially interspersed.

The linear model of Teixeira and Polavarapu (2001c) describing development rate of blueberry maggot pupae was used to predict the date of 50% emergence in each treatment using field-recorded temperatures and 23ºC for the growth chamber. The observed number of days to 50% emergence in the field was extrapolated from cumulative fly emergence plots and calculated from linear regressions of cumulative emergence in the growth chamber. Degree-day estimates were calculated by using the observed day to 50% emergence in equations from linear regressions that described relationships between days and cumulative degree-days in treatments. JMP software (SAS, 2009) was used for analyses at $\alpha = 0.05$.

2.4 RESULTS

Emergence of flies in the growth chamber was significantly affected by pupal burial depth but not substrate type (Table 2.2). More flies emerged when pupae were buried at 1 cm compared to 5 cm for all substrates, and no flies emerged from compost or pine needles when pupae were buried at 20 cm (Fig. 2.2A). Flies failed to eclose from approximately 50-75% of buried pupae in the growth chamber (Fig. 2.2B). The number of failed eclosions was greater in compost than pine needles and increased significantly with burial depth (Table 2.2; Fig. 2.2B). Burial depth also affected the likelihood of emerging after successful eclosion, but this did not vary among substrate type (Table 2.2). The fly emergence to eclosion ratio was significantly lower at 5 cm compared to 1 cm for all substrates (Table 2.2; Fig. 2.2C). An average of four to seven flies per replicate eclosed when buried 20 cm in compost or pine needles, but no flies emerged (Fig. 2.2C).

In the field in 2009, deep burial of pupae at 20 cm reduced emergence compared to 5 cm in both compost (8.3 ± 0.7 and 3.2 ± 0.5 flies, respectively) and pine needles (10.5 ± 1.8 and 1.0 ± 0.5 flies, respectively) (Table 2.3). The number of flies that emerged from 5 cm of soil (1.0 ± 0.5) was similar to that emerging from 20 cm of pine needles.

In the field in 2010, burial depth did not affect fly emergence in compost, but significantly more flies emerged from pine needles when pupae were buried at 5 cm compared to 1 or 20 cm (zero flies emerged from 1 cm). In soil, more flies emerged from 1 compared to 5 cm pupal burial (Table 2.3; Fig. 2.3A). Burial depth strongly affected eclosion success in pine needles, but had much weaker effects in soil or compost (Table 2.3; Fig. 2.3B). In pine needles, pupal failures were very high when buried 1 cm deep compared to 5 or 20 cm, with most pupae appearing intact rather than rotted (Table 2.3; Fig. 2.3B). Emergence success following eclosion was significantly affected by pupal burial depth in all substrates (Table 2.3). Fly emergence to eclosion ratios declined with pupal burial depth, except in 1 cm of pine needles where no pupae eclosed (Fig. 2.3C).

Table 2.2 Results of analysis of variance for effects of substrate type (compost, pine needles, soil) and pupal depth (1, 5, 20 cm) on *R. mendax* fly eclosion and emergence in a growth chamber

Treatments analyzed	Model factors		No. flies emerged		No. flies not eclosed		No. emerged / No. eclosed	
		df	*F*	*P*	*F*	*P*	*F*	*P*
All substrates 1 & 5 cm	Substrate	2	2.48	0.1117	7.34	0.0047	3.46	0.0537
	Depth	1	85.53	0.0001	8.80	0.0083	28.64	0.0001
	Substrate*depth	2	1.99	0.1655	3.24	0.0626	2.18	0.1418
	Error	18						
Compost & pine needles all depths	Substrate	1	1.18	0.2928	18.17	0.0005	2.75	0.1159
	Depth	2	95.76	0.0001	6.30	0.0090	60.76	0.0001
	Substrate*depth	2	1.94	0.1744	2.11	0.1518	0.72	0.4999
	Error	18						

Table 2.3 Results of analysis of variance for effects of substrate type and pupal depth on *R. mendax* fly eclosion and emergence in a highbush blueberry field

Substrates and depths[a]	2009			2010						
		No. flies emerged			No. flies emerged		No. flies not eclosed		No. emerged / No. eclosed	
	df	*F*	*P*	*df*	*F*	*P*	*F*	*P*	*F*	*P*
Compost	1	35.97	0.0001	2	1.53	0.2513	3.28	0.0679	7.90	0.0050
Pine needles	1	27.24	0.0001	2	33.61	0.0001	89.70	0.0001	21.01	0.0002
Soil	-	-	-	1	18.59	0.0015	4.05	0.0719	11.21	0.0074
Error	15			15 & 10 for soil						

[a] Depths of 5 and 20 cm for 2009; 1, 5 and 20 cm in compost and pine needles, 1 and 5 cm in soil for 2010

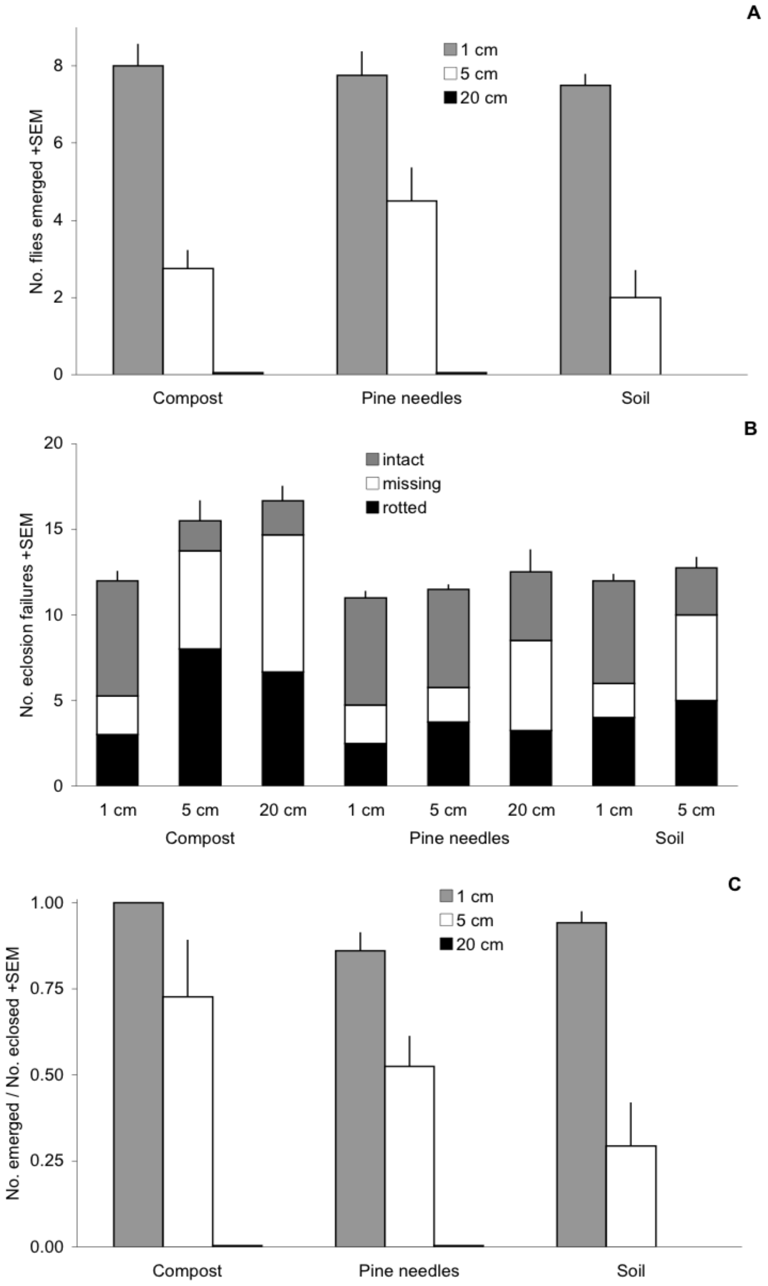

Figure 2.2 Fly emergence (A), eclosion failure (B), and proportion emerged following successful eclosion (C) of *R. mendax* in a growth chamber when 20 pupae were placed 1, 5, or 20 cm below surfaces of compost, pine needles or soil in 2010.

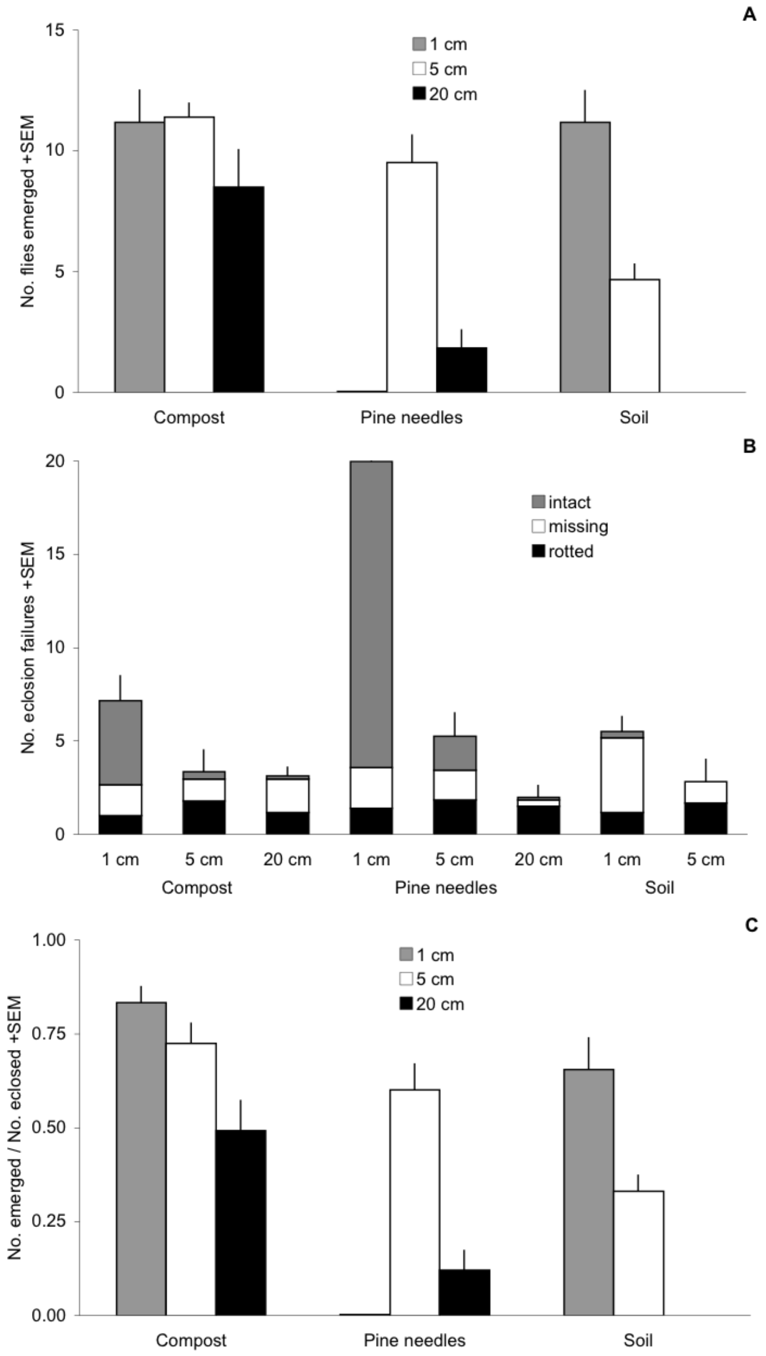

Figure 2.3 Fly emergence (A), eclosion failure (B), and proportion emerged following successful eclosion (C) of *R. mendax* in a highbush blueberry field when 20 pupae were placed 1, 5, and 20 cm below surfaces of compost, pine needles or soil in 2010.

In the growth chamber, fly emergence began about 28 days after transfer from cold storage to the chamber and ended by 45 days for all substrates and depths. Linear models fit to emergence data were significant for all treatments ($P < 0.0001$), but the rate of emergence was slower in soil and compost at 5 cm than in other treatments (Fig. 2.4). Fifty-percent emergence occurred on average 15 days earlier than predicted by the linear model of Teixeira and Polavarapu (2001c) (Table 2.4). Earlier emergence corresponded to fewer degree-days required for 50% emergence (637-703) than 934 DD predicted by Teixeira and Polavarapu (2001c) (Table 2.4).

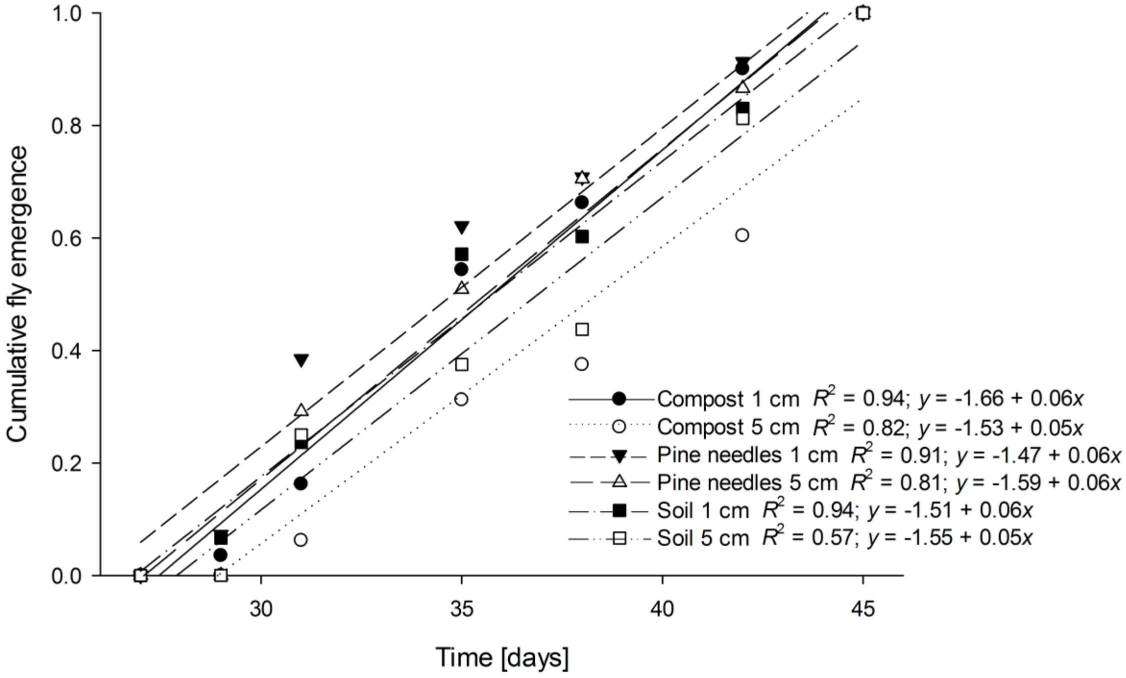

Figure 2.4 Linear regressions for *R. mendax* fly emergence in a growth chamber from pupae placed 1 or 5 cm beneath the surface of compost, pine needles, or soil. Day 1 was 31 May 2010 when pupae were transferred from 4°C to 23°C, 60-65% RH, and 16:8 L:D.

Table 2.4. The effect of substrate and pupal depth on days predicted for 50% *R. mendax* fly emergence in the field, differences between observed and predicted days to 50% emergence, and degree-day (DD) accumulation. Day 1 was 31 May and 18 April 2010 in the growth chamber and field, respectively.

Substrate	Depth (cm)	Growth chamber		Field		
		Observed – predicted (d)[a]	DD 50% emergence[b]	Predicted 50% emergence (days)	Observed[c] – predicted (days)	DD 50% emergence[d]
Compost	1	-15.8	653.3	91	-13	797.5
	5	-13.1	702.7	93	-12	770.1
	20	-	-	97	-9	814.0
Pine needles	1	-16.7	636.8	91	-	-
	5	-15.9	651.5	93	-10	826.7
	20	-	-	104	-15	770.2
Soil	1	-16.1	655.1	95	-14	727.2
	5	-14.6	675.3	95	-9	840.0
Mean ±SD		-15.4±1.3	662.5±23.2	-	-11.7±2.4	792.2±39.2

[a] Observed calculated from equations in Fig. 2.4

[b] DD for 50% emergence = 18.3 DD per day x (Days observed for 50% emergence)

[c] Extrapolated from Fig. 2.5

[d] DD for 50% emergence = Cum. DD on day 74 + DD per day x (Days observed for 50% emergence - 74)

In the field, predicted number of days to 50% fly emergence increased with depth in compost and pine needle mulches because temperatures were cooler at depth (Table 2.4, Fig. 2.5). As in the growth chamber, emergence in all treatments was earlier (9-15 days) than predicted (Table 2.4). Again, this corresponded to fewer degree-days required for 50% emergence (727-840) than the predicted 934 DD (Table 2.4).

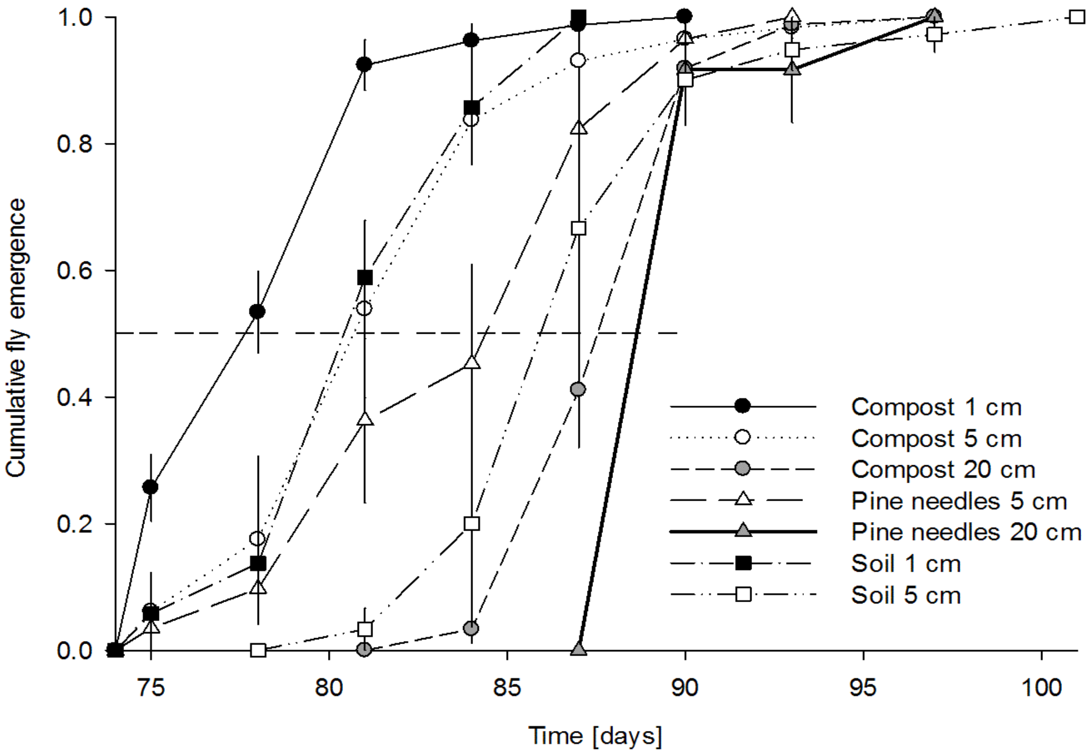

Figure 2.5 *Rhagoletis mendax* fly emergence (±SEM) in a highbush blueberry field from pupae covered with 1, 5, or 20 cm of compost, pine needles, or soil. Day 1 was 18 April 2010 when pupae were transferred from 4°C to the field.

From unreplicated temperature sensors in 2010, maximum daily temperatures exceeded 30ºC at 1 cm on 25 days in pine needles and on 22 days in compost. Similar high temperatures occurred at 5 cm in pine needles on 12 days. Maximum daily temperatures of 25-29.9ºC were common at 1 and 5 cm depths for all substrates but were not detected at 20 cm in either compost or pine needles.

Volumetric moisture levels in the growth chamber after water was added were 0.0071 m^3/m^3 in pine needles, 0.2134 m^3/m^3 in soil, and 0.2725 m^3/m^3 in compost at all depths. In both years, field mean moisture levels were similar to those in the growth chamber for pine needles (-0.0225 – 0.0522 m^3/m^3) but were drier for compost (0.0092 – 0.2346 m^3/m^3) and soil (0.0594 – 0.2544 m^3/m^3). Overall, moisture levels tended to increase with depth and were higher in 2009 than 2010.

2.5 DISCUSSION

Blueberry maggots dropping from ripe berries typically burrow 1-3 cm into soil, but occasionally as deep as 5 cm (Lathrop and Nickels, 1931, 1932). Pupae must contend with abiotic and biotic stressors in soil for over 10 months. If they survive until summer, flies eclose from underground puparia and then ascend to the soil surface. I anticipated that placement of a thick layer of mulch under highbush blueberries would decrease pupal survival and prevent or delay emergence of blueberry maggot flies, potentially providing a new tool in integrated management.

Fly emergence success was consistently lower with increasing pupation depth in all substrate types in both field and laboratory experiments. The depth effect was likely due to several factors. When pupae are buried deeply, eclosed flies must crawl a greater distance, and if energy reserves are insufficient some flies may fail to reach the surface. Inhibition of phototaxis could also play a role. Most tephritid flies are positively phototactic (e.g., Robacker and Fraser, 2002), and if no light penetrates to 20 cm, they may experience some disorientation. Substrate density was also likely important since in dense materials with smaller pore spaces fly ascents can be more difficult (Eskafi and Fernandez, 1990). In these experiments, emergence success was lower from 5 cm soil (the densest substrate) than from 5 cm of compost and pine needles.

However, deep coverage with low-density substrates may hinder emergence in other ways. Emergence success of pupae buried 20 cm in pine needles was much lower compared to compost in the field (2010) even though pine needles are considerably less dense. Temperature or moisture did not appear to account for the effect. Since temperatures in 5 cm of pine needles in the field did not greatly inhibit emergence, high temperatures are unlikely to have been a factor at 20 cm. Moisture levels were not excessive at any depth in pine needles and probably were not deficient at 20 cm, given that they were adequate for emergence at 5 cm. I speculate that poor emergence from beneath 20 cm of pine needles was due to the large amount of free air space and shifting of individual needles that may have provided limited traction for crawling flies.

Failed eclosion was largely responsible for low overall emergence in the growth chamber. Moisture levels in soil and compost were considerably higher in the growth chamber than in the field and this may have created hypoxic or anoxic conditions and/or promoted pathogens. Eskafi and Fernandez (1990) attributed mortality of *Ceratitis capitata* (Wiedemann) to low oxygen levels in soils with high moisture and bulk density. Entomopathogens can be important sources of mortality for tephritids; *Beauvaria bassiana*, *Metarhizium anisopliae*, and *Isaria fumosorosea* infected 4-20% of pre-pupal maggots and pupae in *Rhagoletis* spp. (Cossentine et al., 2010; Daniel and Wyss, 2009). Pathogen presence in substrates used in this study was not assessed, but initial numbers may have been greater in compost than in pine needles or soil. Further research is needed to confirm pathogen effects on blueberry maggot (Collins and Drummond, 2010) and to determine their potential for inducing significant mortality at different moisture levels and temperatures in mulches such as composted wood waste.

Eclosion was more successful in the field. Variation in eclosion success among substrates was low, except for pupae buried 1 cm in pine needles where high failure was likely due to moisture deficits and heat stress. In other studies, high temperatures (>30ºC) at increased moisture and humidity caused expression of heat shock proteins in blueberry maggot, which inhibited development and induced a period of quiescence (Teixeira and Polavarapu, 2005b,c,d) and were thought to explain the occurrence of a late emerging cohort of blueberry maggot flies in New Jersey (Teixeira and Polavarapu, 2001b). It would be interesting to determine whether high surface temperatures in compost or pine needles can induce a late emerging cohort of flies from shallowly buried pupae.

In all experiments, 50% fly emergence occurred earlier and at fewer accumulated degree-days than what was predicted by Teixeira and Polavarapu (2001c) for populations in New Jersey. In the growth chamber, pupae were held at low temperatures until 31 May, a much longer period of exposure to constant cold than normally experienced in the field. Lengthy exposure to low temperatures can shorten post-diapause periods for *Rhagoletis pomonella* Walsh and *R. mendax* pupae (Teixeira and Polavarapu, 2005a) resulting in emergence at fewer accumulated degree-days (Meck et al., 2008; Smith and Jones, 1991). In the field, it is likely that few degree-days accumulated prior to 18 April. Degree-day estimates from this study were lower by 94-207 compared to 934 predicted degree-days for populations in New Jersey. Cooler spring temperatures naturally experienced by pupae in more northerly Nova Scotia may shorten the post-diapause period and reduce cumulative degree-days until emergence.

The number of degree-days required for 50% emergence did not vary considerably between treatments in the growth chamber, and most variability was due to treatments with pupae placed under 5 cm of compost and soil. In all treatments, conditions were kept quite moist by adding water twice per week, but more water was retained at 5 than 1 cm. Under moister conditions pupae would have experienced more evaporative cooling, thereby slowing their development and delaying emergence from 5 cm of soil or compost. Effects of evaporative cooling would occur less in pine needles since air spaces would allow faster drying between rewetting. Slower emergence may increase susceptibility of flies to pathogens that kill flies before they emerge or reduce fitness after emergence.

In the field, the variability in the number of degree-days required for 50% emergence and the differences of observed minus predicted days between treatments may be explained by a number of factors. Numbers of days predicted for 50% emergence are based on one sensor per treatment in the field and variability in substrate temperatures could not be measured. For example, 50% emergence was predicted ninety-five days after 18 April in soil at both depths, even though flies emerged from 1 cm much earlier than predicted (5 days) than those at 5 cm. Thus, the degree-day estimate for soil at 1 cm is much lower than for other treatments (712) likely because of the unreplicated temperature recordings. Second, the linear development model cannot account for reduced rates of development at high temperatures (> 25 – 30°C) (Teixeira and Polavarapu, 2001c). Temperatures in and above this range were frequent at shallow depths, particularly at 1 cm in compost. An upper development threshold of 30°C was used in degree-day calculations, but a biophysical development model may have given more accurate predictions above 30°C because it includes a high temperature inhibition parameter (Teixeira and Polavarapu, 2001c). These authors reported smaller error between predicted and observed emergence dates with the linear model, but may not have recorded temperatures as high or as frequently as those in this study because their pupae were placed at 2.5 cm in sand in shaded locations. Finally, the time spent by flies crawling to the surface may have been slightly longer from 20 cm and also contributed to increasing the difference between observed and predicted days to 50% emergence.

In summary, a thick layer application of pine needle mulch below blueberry bushes in fall or spring significantly reduced emergence of blueberry maggot flies. Application of 20 cm of compost; however, did not consistently suppress fly emergence below levels observed for soil and is not likely to have significant inhibitory effects in years when spring moisture levels are lower than average. Average mid-April to late-June total rainfall for the region is 253 mm (1971-2000) (Environment Canada, 2010), and in 2009 and 2010, 264 and 225 mm were recorded, respectively. Although a thin layer of pine needles or any thickness of compost did not suppress blueberry maggot, there was also no evidence that mulching enhanced fly emergence. Thus, these results do not preclude the use of these ground management practices for other reasons (plant nutrition or weed suppression) (Burkhard et al., 2009).

Suppressive effects of mulch on blueberry maggot will likely be greatest in the year that thick mulch is applied, but there may be other mulch benefits for maggot control in successive years. The conditions of the mulch for burrowing maggots and the depth chosen by burrowing maggots could be detrimental for successful pupation. I show in the next chapter, for example, that some maggots avoid burrowing or die in dry pine needles before successfully pupating, and that wet conditions in soil and compost tend to induce very shallow pupation (< 1 cm), which is likely to reduce pupal survival. The extent to which these factors also affect mortality rates compared to those when pupae are buried deeply will help determine mulch choice, application depth, timing, and reapplication frequency. Strategies for maggot suppression integrated with other mulch uses will therefore benefit from more information on how particular mulches influence maggot burrowing behaviour, pupation success and fly emergence rates in particular substrates under different environmental conditions.

This chapter has been submitted to the journal Environmental Entomology for consideration for publication.

CHAPTER 3 MULCH TYPE AND MOISTURE LEVEL AFFECT PUPATION DEPTH OF BLUEBERRY MAGGOT (DIPTERA: TEPHRITIDAE) IN THE LABORATORY

3.1 ABSTRACT

Mulching can be beneficial for organic highbush blueberry production, but its effects on insect pests have received little attention. For pests that pupate in soil, pupation depth may affect emergence success due to differences in temperature and moisture, mortality factors such as predation, or efficacy of controls such as insecticidal soil drenches. I examined how mulch type and moisture affect pupation depth for blueberry maggot, *Rhagoletis mendax* Curran, an important pest of blueberries. In laboratory studies, pupation depth was measured in wood waste compost, uncomposted pine needles, soil and sand, each at dry, field moisture levels, and wet, 99% water holding capacity, conditions. Pupating maggots tended to burrow deeper, and pupation depth was more variable in pine needle mulch compared to compost mulch, soil, or sand. Approximately 50% of maggots pupated on the surface of wet soil, but pupation occurred more deeply in wet than in dry pine needles. Moisture level did not significantly affect pupation depth in compost or sand. Some maggots unexpectedly escaped the stacks of cups used to assess pupation depth or died before forming a puparium. Less than 70% of stacks with dry pine needles contained pupae, and fewer pupae were recovered from wet than dry compost and soil. These results suggest that mulch material and/or moisture levels can have significant impacts on blueberry maggot pupation depth with potential implications for its management.

3.2 INTRODUCTION

Blueberry maggot, *Rhagoletis mendax* Curran, is often the most serious insect pest of highbush and lowbush blueberries in eastern North America (Crozier, 1995; Kuepper and Diver, 2010). Females oviposit in ripening or ripe fruit and developing maggots feed on the fruit. Blueberry maggot is a quarantined pest, and there is zero-tolerance for maggots in fruit exported from infested areas within or imported into Canada (CFIA, 2009). Local sales are not subject to stringent inspections, but meeting customer satisfaction requires maintaining low maggot thresholds. Currently, insecticides are the main control for *R. mendax* flies with an application recommended 7-10 days after the first detection of a fly on a sticky trap in highbush blueberries (Delbridge and Rogers, 2010); however, growing markets for organic produce and stricter limits and guidelines for pesticide residues on exported fruit (EU, 2008) require the development of effective alternative management strategies. Successful maggot pupation may depend on factors such as moisture, temperature, predation, or disease, and these factors vary with depth. Thus the depth at which pupation occurs is likely to influence survival and could be manipulated as a management tool.

When *R. mendax* maggots are mature, they exit berries, drop to and burrow into soil, and form a puparium. Lathrop and Nickels (1931, 1932) found 70% of pupae in the top 12.5 mm and nearly 100% in the top 25 mm of soil and organic material extracted from lowbush blueberry fields. The authors suggested that the dense layer of blueberry roots probably impeded deeper pupation. In other studies on tropical tephritid flies, Caribbean and olive fruit flies responded to increased moisture and soil density or compactness by pupating closer to the surface (Hennessey, 1994; Tsitsipis and Papanicolaou, 1979), and Oriental fruit fly pupated on the surface of saturated soil (Hou et al., 2006). However, in other studies, pupation depth increased when soil or sand moisture levels were increased (*Bactrocera* spp. and Mediterranean fruit fly) (Alyokhin et al., 2001; Dimou et al., 2003; Jackson et al., 1998). It is likely that pupation depth is reduced at very low or high moisture levels, and the effect depends on the density, compactness, or porosity of the soil or material.

Mulching in highbush blueberries is recommended for building organic matter (Kuepper and Diver, 2010). Weed growth can be suppressed by pine needle mulch, and greater plant vigor and growth has been observed with seafood and manure composts (Burkhard et al., 2009). The physical properties of mulches are usually quite different than that of soil, particularly in terms of bulk density and water holding capacity, and this may alter the pupation depth of *R. mendax*.

Shallow pupation could increase pupal mortality through more frequent encounters with ground dwelling predators (Guillén et al., 2002; Urbaneja et al., 2006) and other natural enemies, or through increased exposure to freezing in winter and flooding during heavy rainfall in spring and summer. In addition, shallow pupation could increase the effectiveness of soil drenches of reduced-risk insecticides or biopesticides. On the other hand, lower temperatures and increased moisture at greater depths could reduce pupal survival and emergence success by promoting pathogens (Cossentine et al., 2010) or alter timing of adult fly emergence the following summer.

The objectives of this experiment were to determine: (1) if wood waste compost and uncomposted pine needle mulches alter pupation depth of *R. mendax* compared to soil or sand, and (2) if the addition of moisture has consistent effects on pupal depth in all materials. I hypothesized that pupation depth would be negatively related to bulk densities of materials, with greatest depths occurring in pine needles and shallowest pupation in sand. I predicted that high moisture levels would reduce pupation depth in most materials but would have less effect in pine needles where pore size is large and moisture levels were expected to be lower.

3.3 MATERIALS AND METHODS

Pupation depth was assessed in the laboratory using four materials: compost mulch, pine needle mulch, soil, and sand, each under dry and wet conditions. Compost mulch was wood processing waste (Louisiana-Pacific Ltd., Chester NS) and fallen pine needles were from a campground (Coldbrook, NS). Mulches were collected in August 2008 and 2009 from plots in a highbush blueberry field (Rawdon, NS). Samples of clay loam soil were collected from the top 5-10 cm in the same field (23% sand, 46% silt, 31% clay). All materials were held in covered plastic tubs at 4°C until use in experiments in the spring of 2009 and 2010. Sterile sand was purchased (PlaySand, Shaw Resources, Shubenacadie, NS). Percent moisture, bulk density, and water-holding capacity (WHC) of materials were determined both years (USDA-USCC 2001). Materials were prepared to 99% WHC by weight (wet moisture level) by mixing with distilled water in plastic jars. The same weights of field-collected materials were put in jars (dry moisture level). Since sand was at 0% moisture, the dry moisture level was prepared to 10% WHC by mixing with distilled water. Pine needles were cut into smaller pieces at this time to facilitate their future placement into cups. Terpenes emitted by broken needles may potentially affect burrowing maggots, but most are emitted from needles within 24 h of being damaged (Loreto et al., 2000). Jars were sealed and stored in a refrigerator for at least 2-3 days before first use.

Stacks of modified cups similar to those used by Jackson et al. (1998) were filled with materials to test pupation depth. Bottoms of clear plastic cups (#9051, Bioserv, Frenchtown, NJ; 30mL, bottom diameter = 27 mm, height = 40 mm) were removed and each was replaced with a disk of 2.0 mm mesh plastic screen glued over the hole. Each stack had 12 cups, and cups were separated by 5 mm layers of material between the screened bottoms giving a total treatment depth of 60 mm. Each layer was weighed to achieve the correct density. Large clumps of compost or soil were broken and stones were removed. Assembled stacks were covered with plastic to minimize evaporation, placed in a refrigerator, and used in experiments within 24 h.

Blueberry maggot pupae used in experiments were collected from harvested berries held on screens over large wooden boxes containing sand. Maggots that emerged from berries dropped into the boxes and borrowed into the sand to pupae. Pupae were subsequently floated from sand and put in Petri dishes with moist sand at 4°C to complete diapause for approximately four months. In the 2009 experiment, pupae were from lowbush blueberries grown beside highbush blueberries and picked in August 2007 and reared through one generation in the laboratory. In 2010, pupae were from highbush blueberries harvested in August 2009.

Experimental maggots were obtained using the following protocol. Pupae were placed in moist sand in a screen cage located in a growth chamber (23°C, 60-70% RH, 16:8 L:D). Emerged flies were given water, sugar, and a vitamin and protein food source. Batches of 'Ida Red' apples were placed in the cage for approximately one week and then removed and placed on hardware cloth over a shallow plastic tray lined with moist paper towel in the growth chamber. Maggots that fell on the moist paper towel were individually transferred to the material surface in the top cup of a stack (one maggot per stack). Maggots were assigned to treatments at random until all eight stacks of one replicate were filled. This process was repeated as maggots dropped from apples over a period of two to three weeks. The number of replicates per treatment was determined by numbers of maggots obtained (range of 64 to 71 replicates per treatment over both years). Maggots dropped primarily in the first 3-4 h of photophase, and the paper towel was checked for maggots every 0.5 h during this period. However, some maggots dropped later during photophase and during scotophase; these maggots were transferred to stacks the next day.

Cup stacks were kept in shallow plastic trays in the growth chamber and held together by slipping pieces of nylon stocking under the bottom cups and over the rims of the top cups. After eight days, stacks were removed from the growth chamber and taken apart cup-by-cup. Pupation depth was recorded as the mid-depth at each 5 mm layer using the formula $D = L(5) – 2.5$, where D = total depth and L = layer number. Pupae visible on the surface of the first layer were recorded as pupating at 0 mm. Pupae were not found in all stacks even though they were covered with nylon stocking. I observed a few maggots in trays containing stacks indicating some maggots were able to climb up the inside wall of the top plastic cup and slide between the rim of the cup and stocking. Therefore, the number of stacks in each treatment without pupae was recorded as an indication of the suitability of the material for pupation.

Mixed model analysis of variance (ANOVA) was used to analyze effects material and moisture level on pupation depth and percent missing pupae, with year included as a random block effect. Pupation depth data were transformed log (x + 1) to normalize error variance. Means were compared using Tukey’s HSD test at α= 0.05. JMP software (SAS, 2009) was used for analyses.

3.4 RESULTS

Initial moisture and bulk density were lowest, but water-holding capacity by weight was highest, in pine needles (Table 3.1). Low bulk density of pine needles meant that the amount of water held by pine needles was less than compost, soil or sand. Soil and sand were considerably denser than compost with lower water-holding capacities (Table 3.1).

Table 3.1 Physical characteristics of materials used for assessing pupation depth of *R. mendax* in laboratory studies.

	Initial moisture (% w/w)[a]		Bulk density (g/cm^3)		Water-holding capacity (% w)	
	2009	2010	2009	2010	2009	2010
Compost	58	55	0.31	0.32	66	65
Pine needles	6	12	0.04	0.04	71	73
Soil	25	19	0.90	1.03	33	27
Sand	0	0	1.58	1.58	16	16

[a] %w/w calculated as [1 - (dried weight/collected weight)] x 100

Mean pupation depth differed significantly with material type (Table 3.2), being deeper in pine needles than other materials in both dry and wet conditions (Fig. 3.1). In dry compost, pupation depth was greater than in dry soil or sand. Most pupation occurred at 0-20 mm in compost, soil, and sand, but pupae in pine needles were more evenly distributed throughout 10-60 mm (Fig. 3.2). The effect of moisture on pupation depth was significant, but this also varied with material (Table 3.2; material*moisture interaction). Pupation was deepest in wet pine needles, whereas wet soil caused shallowest pupation (Fig. 3.1). Nearly 50% of maggots pupated on the surface of wet soil compared to 0% in dry soil (Fig. 3.2). Moisture did not significantly affect pupation depth in compost or sand (Fig. 3.1), although the distribution of pupae appeared somewhat skewed towards more shallow pupation in wet compared to dry compost (Fig. 3.2).

Table 3.2 Results of mixed model analysis of variance for effect of material type (compost, pine needles, soil, or sand) and moisture level (dry or wet) on *R. mendax* pupation depth and loss of pupae.

		Pupation depth		Missing pupae	
	df	*F*	*P*	*F*	*P*
Material	3	151.73	<0.0001	5.96	0.0243
Moisture level	1	6.30	<0.0124	0.54	0.4879
Material * moisture level	3	32.78	<0.0001	6.95	0.0166

Error *df* = 475 and 7 for pupation depth and lost pupae, respectively

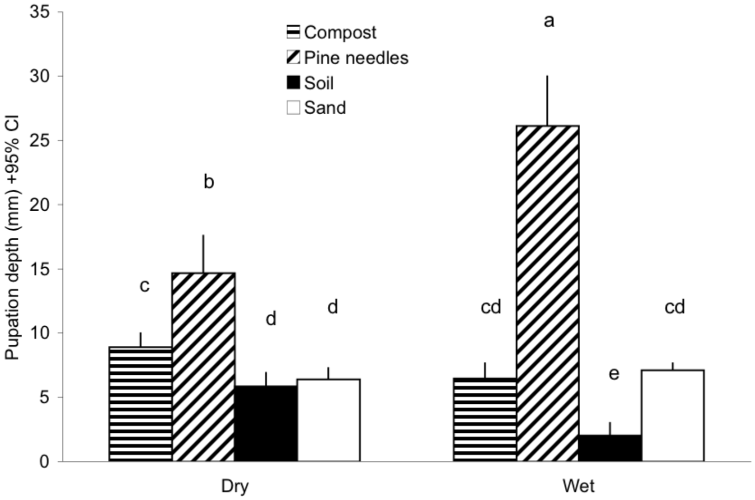

Figure 3.1 Mean pupation depth of *R. mendax* in four materials at two moisture levels in the laboratory. 'Dry' indicates moisture level at field conditions and 'wet' at 99% water holding capacity. Data were log ($x + 1$) transformed for analysis; back-transformed means with same letter are not significantly different on the transformed scale (Tukey's HSD, $\alpha = 0.05$).

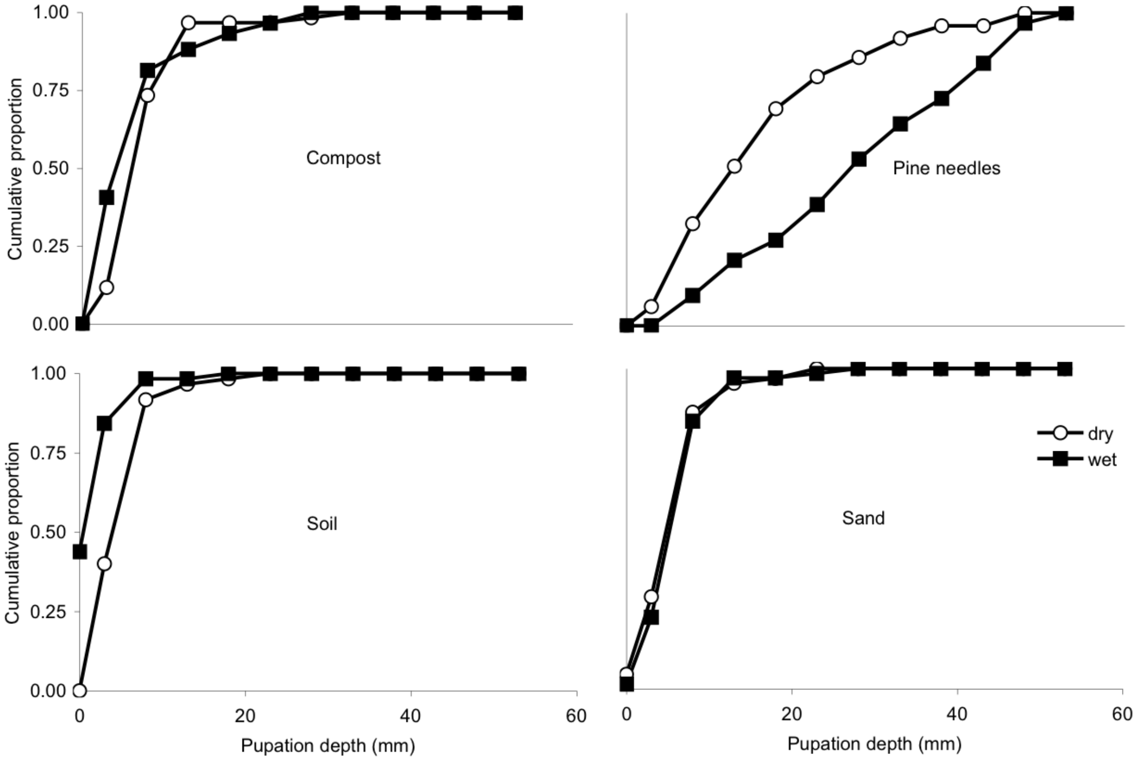

Figure 3.2 Cumulative proportion of *R. mendax* pupae found at increasing depths in four materials, under dry and wet moisture levels in the laboratory.

The effect of moisture on the percent of pupae missing from the stacks also differed among materials (Table 3.2). Adding water greatly decreased the number of missing pupae in pine needles, but tended to increase (non-significant effect) the number in compost and soil (Fig. 3.3).

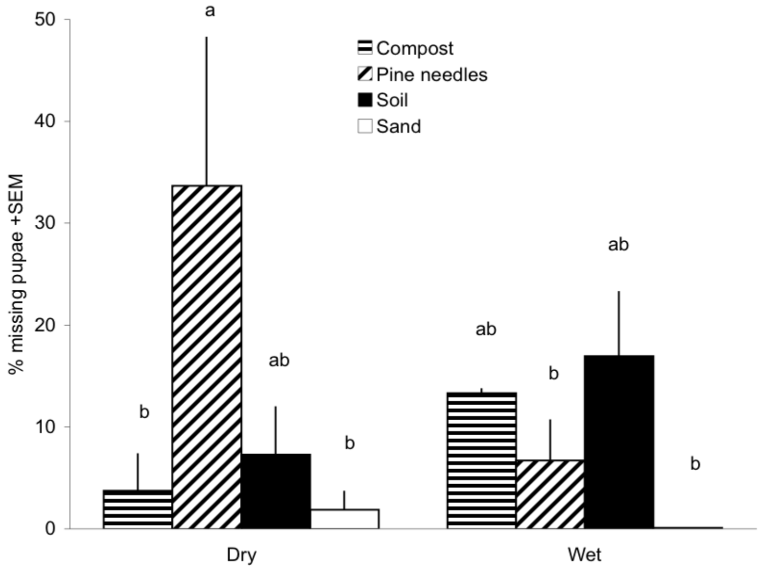

Figure 3.3 Percent *R. mendax* pupae missing eight days after maggots were placed on the surface of four materials at two moisture levels in the laboratory. ‘Dry’ indicates moisture level at field conditions and ‘wet’ at 99% water holding capacity. Means with same letter are not significantly different (Tukey’s HSD, $\alpha = 0.05$).

3.5 DISCUSSION

Mulching in highbush blueberries is increasingly being used in organic systems for building organic matter, providing fertility, and controlling weeds, but effects of mulches on blueberry maggot, a key pest of blueberries, are largely unknown. Shallow pupation of maggots due to mulching could increase mortality by desiccation, freezing, increased rates of predation or better efficacy of insecticidal drenches. On the other hand, deep pupation would protect pupae from these hazards but could promote pathogens in wet conditions or delay emergence due to lower temperatures. Knowledge of pupation behavior in different mulch and moisture conditions will help determine whether these factors can promote blueberry maggot management.

The range of pupation depths recorded in this study, 0-50 mm, is comparable to depths found by others, 5-40 mm, in laboratory tests using tropical tephritids (Dimou et al., 2003; Hennessey, 1994; Hou et al., 2006; Jackson et al., 1998; Tsitsipis and Papanicolaou, 1979). The depths are also similar to those reported in the field for other tephritids including *Rhagoletis* spp. (Cameron and Morrison, 1974; Hodgson et al., 1998; Ibrahim and Mohamad, 1978; Lathrop and Nickels, 1932), although Western cherry fruit fly pupae were found up to 100 mm deep (AliNiazee, 1974).

Pupae were found slightly deeper in dry compost and much deeper in pine needles (up to 50 mm) than soil or sand, indicating that physical properties of these materials have an effect on burrowing maggots. The larger particle size, lower specific gravity, and greater free air space of compost and pine needles result in lower bulk densities (0.30 and 0.04 g/cm^3) and likely facilitate maggot movement. Similarly, Dimou et al. (2003) showed pupation depth for olive fruit fly was greater in soil with lower bulk density, and Hennessey (1994) reported that increased compaction of soil caused shallower pupation of Caribbean fruit fly. Arrestment of maggot burrowing and initiation of pupation may be determined partially by negative phototaxis. Light would penetrate more deeply through pine needles, and possibly compost, than denser materials. Deeper pupation in mulches could alter emergence timing of flies due to differences in temperature, possibly increasing susceptibility of pupae to pathogens or anoxia due to greater moisture levels. However, deeper pupation could also be an advantage, sheltering pupae from extreme temperature fluctuations and from surface dwelling predators.

High moisture levels had opposite effects on pupation depth in soil and pine needles. Maggots in wet soil pupated very shallowly, corroborating other reports suggesting very high moisture in soil causes shallow pupation of tephritids (Hou et al., 2006; Siddiqui and Ashraf, 2002). This clay loam soil at 99% WHC was completely saturated and had no remaining free air space. Maggots would be physically and aerobically restricted from burrowing deeper and forced to pupate on or very near the surface. Furthermore, 15% of stacks did not contain pupae, suggesting maggots escaped due to unfavourably wet conditions, or high levels of pathogens killed them before forming a puparium. When given a choice, other tephritid maggots also avoided soil with moisture extremes (Alyokhin et al., 2001; Hulthen and Clarke, 2006).

In pine needles maggots burrowed deeper in wet compared to dry needles. Wet pine needles do not restrict burrowing through physical restrictions or anaerobic conditions because large pore spaces are retained and not filled with water as in soil. Therefore, maggots are able to easily penetrate wet pine needles until they reach a depth where they are sufficiently buried at a suitable moisture level. Dry pine needles, on the other hand, appear an unfavourable environment for pupation as evidenced by the fact that more than 30% of stacks did not contain pupae. Maggots were able to escape the stacks, but a limited capacity for osmoregulation in edaphic arthropods (Villani et al., 1999) and low initial moisture level of dry pine needles (6-14% w/w) may cause maggots to dehydrate and die before forming a puparium or cause rapid energy loss and result in shallower pupation by some individuals.

Mean pupation depth was not significantly affected by moisture in compost or sand, but the distribution of pupae was skewed to more shallow pupation in wet compared to dry compost. Sand has a high density but low water holding capacity, so the difference in moisture between 10 and 99% WHC is small. Similarly, compost at field moisture levels was 55-58% which is close to 65-66% moisture at 100% WHC. Slightly shallower pupation in wet compost was likely caused by physical and aerobic restrictions on burrowing maggots as in wet soil.

I examined effects of mulch and moisture on blueberry maggot in a controlled environment, but a number of factors could lead to alternate results in the field. If burrowing maggots respond to temperature, the fluctuating daily temperatures and rates of heating and cooling in the field could lead to pupation depths and success that are different than those observed at constant temperatures in the laboratory (Dimou et al., 2003). Pine needles, in particular, reach high temperatures at the surface on sunny afternoons, and depending on the response of maggots to temperature, may promote lower success rates and more shallow pupation. However, if most maggots exit berries in the morning, then extreme temperatures would be avoided and more successful, deeper pupation may be the result.

Presence of vegetation that affects shade and humidity, thick litter layers, dense and shallow plant roots, and general irregularity of conditions in the field will also alter pupation depths. These same biotic and abiotic factors will influence predators that in turn may affect pupation depth. For example, the presence of ants caused deeper pupation of *Anastrepha* spp. (Aluja et al., 2005). In the field maggots would normally begin burrowing soon after exiting berries and contacting soil. In our study, maggots that had dropped from berries later in the day or during scotophase wandered on moist paper towel for up to 12 h. This activity may have depleted their energy and had an effect on burrowing depth once placed in the stacks of cups (Dimou et al., 2003). In addition, pupal size is probably related to maggot size, but may be altered by maggot wandering time and differ between mulches and moisture levels. Smaller pupae may not overwinter as successfully and measuring pupal size may provide another indication of how mulches could be used to negatively impact *R. mendax* survival.

Mulch selection in highbush blueberries is usually determined by its ability to control weeds or increase plant productivity, but its implications for maggot pupation depth and success should also be considered. Compost mulch did not have a large effect on pupation depth compared to soil and may buffer deeply buried pupae from moisture extremes because it is neither as dry as pine needles nor does it easily saturate like soil. There is potential for shallower pupation in wet compost that may result with heavy rainfall or irrigation. Pine needle mulch creates a habitat that causes a greater degree of variability in pupation success and depth. Hot, dry pine needles may cause desiccation of maggots before pupation or increase mortality of more shallowly buried pupae; freezing temperatures in winter may also kill some pupae. Pine needles that are sufficiently moist, however, will probably cause deeper burrowing that will protect pupae from extremes of temperature and moisture. Overall, it appears that pine needles in dry conditions could create conditions more unfavourable than soil for burrowing maggots and overwintering pupae, but that compost may not negatively affect maggots or pupae because conditions are similar to those in soil.

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SUMMARY OF CHAPTERS 2 & 3; TRANSITION TO CHAPTERS 4 & 5

In these chapters I have shown that mulches can affect burrowing maggots and developing pupae by altering temperature, moisture, physical habitat (e.g., density), and possibly pathogen levels. Overall, pine needles may be better than compost when compared to soil for increasing mortality during these stages. Further control benefits may be gained if mulches favour predators, and if predators consume blueberry maggot maggots and/or pupae. The following two chapters explore mulch effects on predatory beetles and consumption of blueberry maggot by a common beetle species.

CHAPTER 4 GROUND AND ROVE BEETLES (COLEOPTERA: CARABIDAE AND STAPHYLINIDAE) ARE AFFECTED BY GROUND-FLOOR MANAGEMENT IN HIGHBUSH BLUEBERRIES

4.1 ABSTRACT

Ground-floor management can influence pest control in agriculture through direct effects on weeds and insect pests and indirectly through changes in the invertebrate community and soil environment. In this chapter mulch effects on beneficial beetles (Carabidae and Staphylinidae) were examined in a highbush blueberry field in Nova Scotia, Canada. Beetle communities were compared in weeded and unweeded plots with no mulch, pine needle or composted mulch from late July through September in 2008 and 2009. Taxonomic richness of both families was highest in compost, and predatory taxa were captured more frequently in compost in 2009. Captures of *Pterostichus melanarius* were highest in compost both years, and numbers were positively correlated with abundance of small earthworms in late July and early August but not later in the season in 2009. In 2009, composted plots had more weeds than other treatments that negatively affected captures of *P. melanarius*. Captures of phytophagous carabids were higher in unweeded compost probably due greater availability of weed seeds. Compost mulch may be a valuable tool for conserving carabids and staphylinids in highbush blueberries, and it does so by creating a favourable habitat with increased abundance of prey for predatory taxa.

4.2 INTRODUCTION

Naturally occurring generalist predators in agroecosystems can provide some control of important crop pests (Symondson et al., 2002b). Ground and rove beetle families (Carabidae and Staphylinidae) are large and diverse with many predatory species that are common in temperate agroecosystems. Within these families, some carabids consume mainly plant seeds (Lundgren, 2009), and some staphylinids consume fungi, decaying organic material, or are parasitoids (Newton, 1990). By grouping beetles into feeding guilds or assessing functional diversity (Clough et al., 2007; Cole et al., 2002;

Woodcock et al., 2010), more robust predictions about beetle response to agricultural practices with implications for biological control can be made.

Agriculture relies heavily on ecosystem services – such as pest control by predators – and growers may attempt to manage crops so that predators are conserved. Increasing alternative prey is an important way to promote generalist beetle predators that are typically food limited. Practices that appear to benefit carabids and staphylinids by increasing prey (and through other positive effects) include organic farming or reduced pesticide use (Andersen and Eltun, 2000; Koss et al., 2005), altering tillage practices (Andersen, 2003; Clark et al., 1993), increasing vegetative growth through cover cropping or reduced weed control (Lys et al., 1994; Thorbek and Bilde, 2004), or mulching with organic materials (Purvis and Curry, 1984; Rämert, 1996). Before these practices are adopted on a broad scale, outcomes should be predictable and the benefits clearly greater than any negative effects (Gurr et al., 2004). In this chapter, I focus on the effect of mulches on carabids and staphylinids in highbush blueberries.

Mulches have been shown to have variable effects on ground and rove beetles in perennial fruit systems. Straw and compost mulch increased the numbers of carabids but not staphylinids in vineyards (Thomson and Hoffmann, 2007). In apples, straw mulch increased diversity and richness of staphylinids but decreased carabid diversity and richness (Miñarro and Dapena, 2003; Miñarro et al., 2009), while compost mulch caused small increases in all predator, carabid and staphylinid numbers (Brown and Tworkoski, 2004; Matthews et al., 2004). Mulching may only cause shifts in predator spatial distribution within the field. For example, more large carabids, mostly *Pterostichus* spp., were collected in all organic mulches compared to row spaces in strawberry fields, but densities did not increase overall (Tuovinen et al., 2006). The effects of mulches on predatory beetles have not been evaluated in highbush blueberries, but O'Neal et al. (2005a,b) found that clover and ryegrass planted in aisles and a reduced-risk insecticide program had positive effects on two carabid species.

Variations in predator diversity and numbers may be due in part to differing effects of mulches on potential prey. Some mulch types may encourage rich detritivore communities that will significantly affect higher trophic levels such as predatory carabids and staphylinids (Halaj and Wise, 2002). Generalist, including omnivorous, feeding tendencies of many predatory species means that the abundance of all possible food, rather than a single prey species, may be a more important determinant of predator distribution. However, certain prey taxa may have large effects due to local abundance, ease of capture, or nutritional superiority. Identification and quantification of these taxa will aid in making ecological predictions about the distribution and abundance of predatory beetles. For example, the diet and preferences of the large, common carabid, *Pterostichus melanarius* (Illiger) are well studied (see references in 'Materials and Methods'), making it a good model to study mulch effects on alternative prey. The diets of many other carabids are also known but prey preferences of most staphylinid species require further study.

In organic highbush blueberries, mulch is increasingly being recommended for increased productivity (Burkhard et al., 2009; Kuepper and Diver, 2010), and alternatives to conventional insecticides are being sought for management of blueberry maggot (*Rhagoletis mendax* Curran), a major pest in eastern North America. In this study, I evaluated the effects of two organic mulches on the numbers and composition of the Carabidae and Staphylinidae and on the abundance of potential prey for *P. melanarius* in a highbush blueberry field in eastern Canada. If mulches benefit predatory beetles, then they may also be used to promote biological control of soil-dwelling life stages of blueberry maggot.

4.3 MATERIALS AND METHODS

4.3.1 Study Area

The experimental site was a 0.75 ha highbush blueberry (*Vaccinium corymbosum* L; cultivars Bluejay, Bluetta and Bluecrop) field near Rawdon, NS (45N 3'37'', 63W 42'21''). The surrounding vegetation was mixed forest, an abandoned field, and a lowbush blueberry field (Fig. A.1). Within the field no fertilization, irrigation, or weed control, and only minimal pruning and control of mummy berry were done in years prior to our experiment. Grasses, sweet fern, broadleaf herbaceous annuals, young birch and maple trees were also growing in the field.

A randomized complete block design with four replications was used to test two factors: ground-cover type (compost, pine needles, soil) and weed control (weeded or unweeded). Blocks were 18 rows x 18 m separated by a 3 m buffer. Each factor combination was randomly assigned to a plot of 3 rows x 18 m (approximately 12 bushes) within a block. Each block had 9 rows (3 plots) with 'Bluejay' and 'Bluetta' bushes and 9 rows with 'Bluecrop' bushes.

Approximately 70% of the field was mowed and vegetation between blueberry bushes cut low to the ground with a gas-powered lawn trimmer in late May 2008. The compost source was hardwood processing waste from Louisiana-Pacific Ltd. (Chester, NS) (Envirem Organics Inc., 2003) and uncomposted pine needles, *Pinus strobus* L., were from a campground (Coldbrook, NS). From 6-19 June 2008, mulches were applied by hand (shovels, pitchforks and wheelbarrows) to 1.5 m wide by 15-20 cm deep layers centered on bush rows. Grassed aisles between rows were 1.5 m wide. Unmulched, weeded plots were shallowly roto-tilled (2-3 cm) once on 19 June to break-up the surface and remove vegetation not removed by the lawn trimmer. This was done so initial weed-free status was similar to weeded mulch treatments. No additional mulch was added or tilling done in 2009.

Weeded compost and pine needle plots were maintained mainly by hand weeding 7-8 times during the season in 2008 and 2009. Weeded soil plots were maintained by removing some weeds by hand, but also by using the lawn trimmer 3-4 times per season to keep vegetation short (< 5 cm) where it was re-growing. Grassed aisles were mowed on the same days the lawn trimmer was used. In unweeded plots, weeds were identified to family and counted in three 50 x 50 cm quadrats placed randomly near pitfall traps in the central plot rows. Quadrat locations were marked on the first date each year so successive counts were made in identical locations. Weeds were counted five times in 2008 and 2009 between late July and late September.

Soil and mulch temperatures and moisture levels were monitored each year over four day intervals when beetles were trapped in August and September. Sensors were not available for the July collection period each year. Hourly temperatures were recorded with HOBO U12 data loggers (TMC50-HD sensors, Decagon Devices Inc., Pullman, WA) and volumetric moisture (m^3/m^3) levels were obtained from ECH_20 Dielectric

Aquameter probes (S-SMC-M005 sensors, Decagon Devices Inc., Pullman, WA; calibrated to -0.1 m^3/m^3 in air) attached to a HOBO Weather Station (Onset Computer Corp., Pocasset, MA). One temperature and one moisture sensor was placed approximately 5 cm below soil or mulch surfaces in each plot of one block. A second temperature sensor was set in unweeded pine needles and unweeded compost plots in an adjacent block. The average daily means, maxima, and minima were determined for each four-day period. Air temperature and rainfall were recorded hourly at a nearby site with a temperature probe (107) and a tipping bucket rain gauge (TE525M) attached to a CR200-series datalogger (Campbell Scientific Corp., Edmonton AB).

4.3.2 Sampling

Dry pitfall traps were used to capture live Carabidae and Staphylinidae. Each trap was an outer plastic cup (top diameter = 9 cm, bottom diameter = 6 cm, height = 13 cm) with a hole punched in the bottom to facilitate water drainage and a removable inner cup of the same size into which specimens fell. A 10 x 10 cm piece of wood supported by thin wires 5 cm above each trap was used as a rain cover. Plastic mesh (2 x 2 mm openings) was inserted 2 cm from the bottom of the inner cup to separate large and small beetles and prevent predation in the trap. Rims of traps were flush with the compost and soil surfaces, but traps had to be pushed slightly into pine needles to maintain good contact between needles and rims of traps.

Seven pitfall traps were placed in the centre row of each plot. Three of the traps were placed in-line with the bush row with one trap at the mid-point (9 m) and the other two at three metres from each row end. The other four traps were placed at the mulch-aisle edge, with two on each side of the row and all at 6 m from the row ends. To increase captures in these four traps, four pieces of plastic lawn edging (1m by 10 cm) were dug 3-5 cm into the soil parallel to the row so that each trap rim touched one piece of edging at its midpoint. On each side of the row, one trap was on the mulch side of the edging and the other on the aisle side. Traps and lawn edging were placed on 12 and 15 July 2008 and 2009. A ~10 cm radius around traps was weeded in unweeded plots during trapping periods.

Pitfall traps were monitored daily 20 times in 2008 and 19 times in 2009 (one day missed due to heavy rainfall) between late July and mid September because this is the time blueberry maggot maggots leave berries and pupate in the soil where they may be susceptible to ground predators. Collection occurred over four consecutive days for five periods each year, and between collection periods, plastic lids were put on traps. Inner cups of traps were collected each morning and replaced before midday. All live carabids were immediately identified to genus or species and returned to outer rows of plots from which they were trapped. Staphylinids and any dead carabids in the pitfall trap were saved for identification. Captures were recorded per plot per day and averaged for each year. Carabids were designated as being either predominantly predatory (carnivorous) or phytophagous (granivorous) using descriptions in Larochelle and Larivière (2003) and Lundgren (2009). Staphylinids were designated as predatory, fungivorous, detritivorous, myrmecophilous, or unknown using groupings of Clough et al. (2007), Good and Giller (1991), and advice from Adam Brunke, staphylinid taxonomist, University of Guelph.

Due to high captures of *P. melanarius* and observations of many small earthworms in compost plots in 2008, abundance of potential prey was assessed in 2009 using chemical extraction with dilute allyl-isothiocyanate (AITC), a component of hot mustard, and from samples extracted in Berlese funnels. AITC brings earthworms and slugs to the soil surface (Gavin et al., 2005; Zaborski, 2003), is readily available and safe, and allows rapid assessment. However, this technique may not provide good estimates in pine needles due to the physical characteristics of pine needles (e.g. high porosity, lack of a defined surface). Organisms may not be irritated by AITC to move to the surface, thereby limiting ability of the experimenter to see organisms that remain under the top layer of needles. Therefore, only the numbers of potential prey extracted in Berlese funnels were analyzed in this study because I expected the sample type to have minimal effect on the accuracy of the extraction process. Berlese funnels are generally not recommended for earthworm sampling (Reynolds, 1977), but in at least one study, earthworm estimates from funnels were similar to those from hand-sorting soil (Smith et al., 2008). AITC extraction methods are described below, and analysis and mean numbers of earthworms and millipedes extracted are in Appendix B for comparison. *P. melanarius* is a slug predator in cereal crops (Symondson et al., 2002a) but is also highly polyphagous, feeding on many invertebrates (Prasad and Snyder, 2004; Sunderland, 1975) including earthworms (Symondson et al., 2000) and the millipede, *Cylindroiulus caeruleocinctus* (Wood) (family Julidae) (Brunke et al., 2009).

Samples for Berlese funnel extractions were collected from all plots on 22 July, 11 and 26 August, and 10 and 22 September. A 20 x 20 cm metal frame was hammered 5 cm deep into one outside plot row near the midpoint of the plot. The compost or soil was removed from around the frame so that a spade could be inserted under the frame to lift it from the plot. Pine needles were hand-grabbed from inside the frame because they could not be neatly removed using a spade. The sample in the frame was immediately transferred to a plastic bag that was then sealed and placed in a cooler.

On the same day as collection, samples were placed on hardware cloth in large plastic funnels (top diameter = 25 cm, middle diameter = 10 cm, bottom diameter = 2 cm, height = 32 cm) in the laboratory. Metal screening placed in the funnel near the bottom prevented excess debris from falling with specimens into collecting jars containing 70% ethanol. The jar lids were sealed with Parafilm® M barrier film to the bottoms of the funnels. Samples were dried under 60 W incandescent light bulbs for 14 days.

AITC (Fisher Scientific; 94%; density 1.0175) was diluted with isopropanol (Fisher Scientific; 100%; density 0.785) to provide a stock solution of 5 g L^{-1}. Stock solution was prepared a few days prior to use in the field and stored in brown glass bottles in the fridge. In the field immediately before use, stock solution was diluted with tap water to 100 mg L^{-1} in 20 L plastic jugs. Jug contents were shaken and thoroughly stirred.

AITC sampling occurred in three blocks during the afternoons and early evenings on the days after collecting Berlese funnel samples. A square white plastic pail with bottom removed (21 x 21 cm; 441cm^2) was hammered 5 cm deep into each outside plot row near the midpoint of the plot. Each pail was irrigated with 880 mL of dilute AITC (Zaborski, 2003), watched for 8 minutes, irrigated with a second 880 mL, and again watched for 8 minutes. Pine needles were compressed by hand prior to irrigation to reduce air space and increase retention of AITC. All macroinvertebrates coming to the surface were removed with tweezers and saved per plot (880 cm^2) in a jar containing 70%

ethanol. Jars from both extraction methods were stored at 5°C until identification and counting. Earthworm lengths were measured (mm).

4.3.3 Data Analysis

Mixed model analyses of variance (ANOVA) were used to analyze the effects of mulch type and weeding on number of genera or species, on beetle feeding groups, and on weed density in unweeded plots. Year was included as a fixed effect and block and bush cultivar as random effects. Cultivars and interaction terms were initially tested as fixed effects because they could influence the ecology of the field (e.g., larger bushes create more shade), but there were no significant effects (P >0.05) in any analysis. The cultivars Bluejay and Bluetta were grouped in the models because there were few 'Bluetta' bushes and because they were more similar in size and phenology to each other than to 'Bluecrop'.

Repeated measures ANOVAs were used to test effects of mulch type and weeding on *P. melanarius*, small earthworms and julid millipedes in 2009. The sampling date for prey and pitfall captures averaged over each four-day collection period for *P. melanarius* were the repeated measures. Relatedness of *P. melanarius* captures to numbers of these abundant prey taxa was tested by a separate correlation analysis for each taxa and capture period/sampling date. Data were transformed where necessary to meet assumptions of normality and homogeneity of variance. Differences between means were tested with Tukey’s HSD test. Analyses were performed using JMP software (SAS, 2009) at $\alpha = 0.05$.

Constrained ordination statistics were used to examine effects of environmental variables on beetle community composition in both years. Only genera and species (log [x + 1] transformed) with 20 or more total captures each year were used. Environmental variables were mulches entered as binomial variables and weed family counts (log [x + 1] transformed) averaged over dates. Blocks were used as covariables. Initially, detrended correspondence analysis (DCA) was used to measure eigenvector length of genera and species (Leps and Smilauer, 2003). Redundancy analysis (RDA) was performed and a Monte Carlo test with 1000 permutations used to test significance of first axis and all four axes. Biplots of genera and species with environmental variables showed associations along the two axes accounting for the majority of variation. CANOCO (ter Braak and Smilauer, 2009) was used for analysis and CanoDraw for biplots.

4.4 RESULTS

4.4.1 Effects Of Mulching On Weed Growth, Temperature, And Moisture

Weed density was higher in compost than pine needles or soil, but only in 2009 (mulch*year interaction; $F_{2,14} = 4.92$, $P = 0.0245$). There were over 250 weeds per square meter in compost in 2009, whereas only 15-125 weeds per square meter were found in all other treatments in both years.

Daily mean temperatures averaged over August and September intervals were similar across all plot types and between years ranging 16.4 – 19.0ºC. Daily minimum and maximum temperatures were more variable, ranging 11.0 – 14.1ºC and 20.7 – 25.6ºC, respectively, in pine needles compared to minimums of 13.9 – 17.1ºC and maximums of 18.2 – 21.6ºC in compost and soil. Weeds appeared to have little effect on temperatures in plots. Mean air temperature (late July to late September) was similar in 2008 (17.1ºC) and 2009 (17.3ºC).

Moisture levels averaged over August and September intervals both years were 0.08 – 0.01m^3/m^3 in pine needles (sensors calibrated to -0.1 m^3/m^3 in air) and 0.10 – 0.24 m^3/m^3 in compost and soil. There was 70 mm more rainfall from late July to late September in 2008 (359.3 mm) than in 2009 (289.1 mm).

4.4.2 Effects Of Mulching On Numbers Of Beetle Genera Or Species And Community Composition

A total of 3508 carabids and 1255 staphylinids were captured in pitfall traps from late July to late September in 2008 and 2009 (Tables 4.1, 4.2). Number of carabid genera or species was higher in compost, but only in 2009 (Table 4.3, Fig. 4.1). Number of staphylinid genera or species was greater in compost and pine needles than in soil in 2008 but only in compost in 2009 (Table 4.3, Fig. 4.1).

Table 4.1 Total numbers and feeding guild designation for Carabidae captured from late July to late September in pitfall traps beneath highbush blueberries. Pr = predatory; Ph = phytophagous. Percent calculated from total carabid captures each year.

Genus or Species	2008 (%)	2009 (%)	Total	Feeding Guild
Pterostichus melanarius (Illiger)	1118 (70)	910 (47)	2028	Pr
Dicheirotrichus cognatus (Gyllenhal)	12 (1)	294 (15)	304	Ph
Poecilus lucublandus (Say)	114 (7)	143 (7)	257	Pr
Harpalus rufipes (DeGeer)	47 (3)	100 (5)	147	Ph
Dyschirius globulosus (Say)	11(1)	87 (5)	98	Pr
Syntomus americanus (Dejean)	36 (2)	46 (2)	82	Pr
Anisodactylus spp.[a]	50 (3)	28 (1)	78	Ph
Blemus discus (Fabricius)	21 (1)	51 (3)	72	Pr
Amara spp.[b]	39 (2)	32 (2)	71	Ph
Harpalus affinis (Schrank)	33 (2)	30 (2)	63	Ph
Clivina fossor (Linné)	21 (1)	19 (1)	40	Pr
Harpalus somnulentus Dejean	13 (1)	26 (1)	39	Ph
Agonum spp.[c]	6 (<1)	27 (1)	33	Pr
Pseudamara arenaria (LeConte)	4 (<1)	22 (1)	26	Ph
Notiophilus spp.[d]	7 (<1)	15 (1)	22	Pr
Chlaenius emarginatus Say	4 (<1)	8 (<1)	12	Pr
Pterostichus pensylvanicus LeConte	5 (<1)	7 (<1)	12	Pr
Synuchus impunctatus (Say)	5 (<1)	6 (<1)	11	Ph
Stomis pumicatus (Panzer)	2 (<1)	9 (<1)	11	Pr
Atranus pubescens (Dejean)	1 (<1)	9 (<1)	10	Pr
Harpalus pensylvanicus (DeGeer)	9 (1)	1 (<1)	10	Ph
Cicindela sexguttata Fabricius	7 (<1)	2 (<1)	9	Pr
Bembidion spp.	1 (<1)	8 (<1)	9	Pr
Cymindis neglectus Haldeman	1 (<1)	8 (<1)	9	Pr
Notiobia terminata (Say)	0	9 (<1)	9	Ph
Bradycellus lugubris (LeConte)	1 (<1)	6 (<1)	7	Ph
Patrobus longicornis (Say)	3 (<1)	2 (<1)	5	Pr
Carabus nemoralis O.F. Müller	2 (<1)	3 (<1)	5	Pr
Carabus granulatus hibernicus Lindroth	1 (<1)	2 (<1)	3	Pr
Elaphropus anceps (LeConte)	2 (<1)	0	2	Pr
Pterostichus commutabilis (Motschulsky)	0	2 (<1)	2	Pr
Harpalus sp.	1 (<1)	0	1	Ph
Pterostichus luctosus (Dejean)	0	1 (<1)	1	Pr
Unidentified	14 (1)	3 (<1)	17	
TOTAL	1592	1916	3508	

[a] *A. nigerrimus* (Dejean) and *A. nigrita* Dejean frequently captured

[b] *A. communis* (Panzer) frequently captured. *A. aenea* (DeGeer), *A aulica* (Panzer), *A. laevipennis* Kirby, *A. lunicollis* Schiodte, *A. obesa* (Say), *A. otiosa* Casey, *A. patreulis* Dejean, *A. cupreolata* Putzeys also captured

[c] *A. cupripenne* (Say) and *A. muelleri* (Herbst) frequently captured

[d] *N. palustris* (Duftschmid), *N. aeneus* (Herbst), and *N. semistriatus* Say captured

Table 4.2 Total numbers and feeding guild designation for Staphylinidae captured from late July to late September in pitfall traps beneath highbush blueberries. P = predatory, D = detritivorous, F = fungivorous, M = myrmecophilous, U = unknown. Percent calculated from total staphylinid captures each year.

Genus or Species or Subfamily	2008 (%)	2009 (%)	Total	Feeding Guild
Stenus erythropus Melsheimer	225 (37.9)	159 (24.0)	384	P
Xantholinus linearis (Olivier)	34 (5.7)	117 (17.7)	151	P
Mocyta fungi (Gravenhorst)	35 (5.9)	28 (4.2)	63	F
Drusilla canaliculata (Fabricius)	7 (1.2)	53 (8.0)	60	P
Philonthus carbonarius (Gravenhorst)	49 (8.3)	6 (0.9)	54	P
Ochthephilum fracticorne (Paykull)	34 (5.7)	15 (2.3)	49	P
Stenus semicolon LeConte	17 (2.9)	32 (4.8)	49	P
Anotylus rugosus (Fabricius)	13 (2.2)	35 (5.3)	48	D
Gabrius picipennis (Mäklin)	15 (2.5)	27 (4.1)	42	P
Oxypoda nigriceps Casey	10 (1.7)	23 (3.5)	33	P
Dinarea angustula (Gyllenhal)	15 (2.5)	12 (1.8)	27	P
Mycetoporus horni Berhauer & Schubert	9 (1.5)	17 (2.6)	26	F
Unidentified Aleocharinae	24 (4.0)	1 (0.1)	25	
Amischa analis (Gravenhorst)	0	21 (3.2)	21	P
Ilyobates bennetti Donisthrope	2 (0.3)	17 (2.6)	19	P
Mycetoporus consors LeConte	14 (2.4)	1 (0.1)	15	F
Strigota ambigua (Erichson)	0	15 (2.3)	15	P
Quedius curtipennis Bernhauer	10 (1.7)	3 (0.5)	13	P
Cordalia obscura (Gravenhorst)	4 (0.7)	6 (0.9)	10	P
Tachyporus nitidulus (Fabricius)	0	10 (1.5)	10	P
Sepidophilus testaceus (Fabricius)	8 (1.3)	0	8	P
Unidentified Paederinae	8 (1.3)	0	8	
Unidentified Steninae	0	8 (1.2)	8	P
Pella glooscapi Klimaszewski & Majka	6 (1.0)	0	6	M
Gabrius microphthalmus Horn	0	5 (0.8)	5	P
Quedius peregrinus Gravenhorst	1 (0.2)	4 (0.6)	5	P
Stenus mammops mammops Casey	2 (0.3)	3 (0.5)	5	P
Platydracus cinnamopterus (Gravenhorst)	1 (0.2)	3 (0.5)	4	P
Unidentified Tachyporinae	3 (0.5)	1 (0.1)	4	
Astenus discopunctatus (Say)	2 (0.3)	1 (0.1)	3	P
Habrocerus capillaricornis Gravenhorst	0	3 (0.5)	3	U
Sepidophilus marshami Stephens	0	3 (0.5)	3	P
Pella loricata (Casey)	3 (0.5)	0	3	M
Rugilus angustatus (Geoffrey)	2 (0.3)	1 (0.1)	3	P
Astenus cinctus (Say)	2 (0.3)	0	2	P
Bryoporus rufescens LeConte	0	2 (0.3)	2	F
Philonthus concinnus Gravenhorst	0	2 (0.3)	2	P
Philonthus lomatus Erichson	0	2 (0.3)	2	P
Philonthus sericans (Gravenhorst)	2 (0.3)	0	2	P
Stenus colonus Erichson	0	2 (0.3)	2	P
Aleochara verna Say	0	1 (0.1)	1	P
Dalotia coriaria (Kraatz)	1 (0.2)	0	1	P
Heterothops fusculus LeConte	1 (0.2)	0	1	P
Liogluta sp.	1 (0.2)	0	1	P
Lithocharis ochracea (Gravenhorst)	1 (0.2)	0	1	P
Lithocharis thoracica (Casey)	1 (0.2)	0	1	P
Oligota sp.	0	1 (0.1)	1	P
Philonthus varians (Paykull)	0	1 (0.1)	1	P
Philonthus viridanus Horn	1 (0.2)	0	1	P
Phyllodrepa humerosa (Fauvel)	1 (0.2)	0	1	U
Scopaeus notangulus Casey	1 (0.2)	0	1	P
Stenus carinicollis Casey	1 (0.2)	0	1	P
Tasgius melanarius (Heer)	0	1 (0.1)	1	P
Unidentified	27 (4.6)	20 (3.0)	47	
TOTAL	593	662	1255	

Table 4.3 Results of analysis of variance for the effect of ground-cover type (compost, pine needles, soil), weed control (weeded, unweeded) and year on numbers of captured Carabidae and Staphylinidae genera or species and numbers of captured *Pterostichus melanarius* and the major feeding groups in a highbush blueberry field.

		Carabidae genera or species		Staphylinidae genera species		*P. melanarius*		Phytophagous Carabidae		Predatory Carabidae[a]		Predatory Staphylinidae	
	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Year	1	15.72	0.0004	3.40	0.0744	2.57	0.1186	124.82	0.0001	27.17	0.0001	5.73	0.0227
Mulch	2	17.57	0.0001	22.02	0.0001	132.51	0.0001	79.43	0.0001	25.44	0.0001	14.18	0.0001
Weeds	1	0.03	0.8627	0.83	0.3704	10.87	0.0024	97.91	0.0001	2.67	0.1132	0.16	0.6929
Year*Mulch	2	5.80	0.0071	6.26	0.0051	11.27	0.0002	81.58	0.0001	24.37	0.0001	18.04	0.0001
Year*Weeds	1	0.05	0.8302	0.85	0.3634	17.96	0.0002	88.15	0.0001	1.82	0.1862	0.01	0.9873
Mulch*Weeds	2	2.53	0.0957	0.07	0.9333	0.15	0.8586	79.86	0.0001	0.12	0.8860	0.10	0.9065
Year*Mulch*Weeds	2	0.61	0.5507	0.90	0.4157	13.72	0.0001	52.27	0.0001	1.78	0.1857	0.42	0.6639
Error	32												

[a] excluding *P. melanarius*

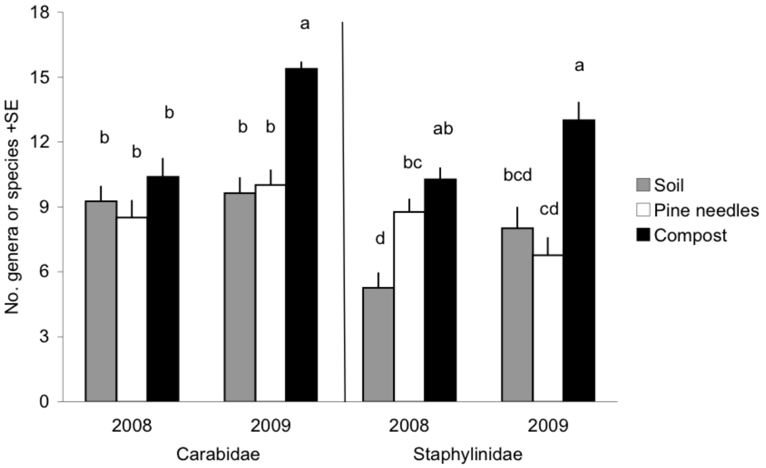

Figure 4.1 Effect of ground cover type (composted mulch, pine needle mulch, soil) on mean number of genera or species of Carabidae and Staphylinidae captured in pitfall traps in a highbush blueberry field between late July and late September. For each family, means with different letters are significantly different (Tukey's HSD, $\alpha = 0.05$).

Effects of mulches and weeds (by family) on beetle species composition were examined in RDA biplots for 2008 and 2009 (Fig. 4.2). In 2008, the eigenvalues were 0.302, 0.125, 0.043, and 0.034 for a significant first axis ($F = 6.40$, $P = 0.02$) and all four axes ($F = 2.34$, $P = 0.02$). Species-environment correlations were 0.946 and 0.924 for the x and y axes, respectively. Vectors for mulches tended to be longer than those for weed families (Fig. 4.2), indicating that mulches accounted for more variation than weeds in beetle captures. Compost mulch closely corresponded to the x-axis while differences due to soil or pine needle mulch could be partially explained by the y-axis. Different weed families were associated with the mulch treatments. Grasses and ferns grew primarily in compost, while the asters, roses, sheep sorrel (Polygonaceae), and sweet ferns (Myricaceae) were found more in soil. Beetle taxa differed in their association with mulch treatments and weed families. Among the most obvious trends, *P. melanarius*, *Harpalus rufipes* (DeGeer), and *Poecilus lucublandus* (Say) were associated with compost, grasses and ferns, while *Amara* spp. tended to be collected more in weedy unmulched plots. Two species, *Stenus erythropus* Melsheimer and *Mocyta fungi* (Gravenhorst), were strongly associated with pine needles while *Ochthephilum fracticorne* (Paykull) was found in both compost and pine needles.

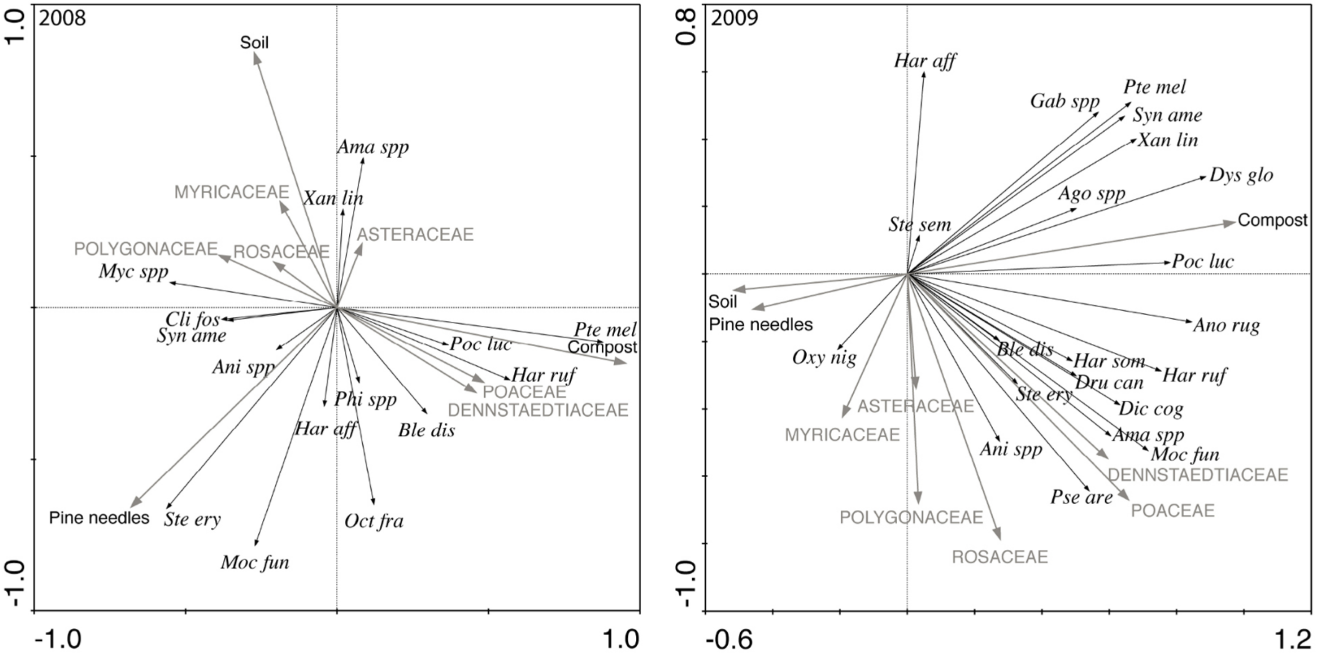

Figure 4.2 Relationships of frequently captured beetle taxa with ground cover treatments (compost, pine needles, soil) and weed densities as shown by redundancy analyses (RDA) biplots. Weed families include Asteraceae, Dennstaedtiaceae, Myricaceae, Poaceae, Polygonaceae, Rosaceae. Carabid taxa are: **Ago spp** = *Agonum* spp., **Ama spp** = *Amara* spp., **Ani spp** = *Anisodactylus* spp., **Ble dis** = *Blemus discus*, **Cli fos** = *Clivina fossor*, **Dic cog** = *Dicheirotrichus cognatus*, **Dys glo** = *Dyschirius globulosus*, **Har aff** = *Harpalus affinis*, **Har ruf** = *Harpalus rufipes*, **Har som** = *Harpalus somnulentus*, **Poc luc** = *Poecilus lucublandus*, **Pse are** = *Pseudamara arenaria*, **Pte mel** = *Pterostichus melanarius,* **Syn ame** = *Syntomus americanus*. Staphylinid taxa are: **Ano rug** = *Anotylus rugosus*, **Dru can** = *Drusilla canaliculata*, **Gab spp** = *Gabrius* spp., **Moc fun** = *Mocyta fungi*, **Myc spp** = *Mycetoporus* spp., **Oct fra** = *Ochthephilum fracticorne*, **Oxy nig** = *Oxypoda nigriceps*, **Phi spp** = *Philonthus* spp., **Ste ery** = *Stenus erythropus*, **Ste sem** = *Stenus semicolon*, **Xan lin** = *Xantholinus linearis*.

In 2009, the eigenvalues were 0.384, 0.144, 0.050, and 0.026 for a significant first axis ($F = 9.28$, $P = 0.02$) and all four axes ($F = 3.94$, $P = 0.02$). Species-environment correlations were 0.986 and 0.971 for the x and y axes, respectively. There was a strong contrast between compost and pine needles/soil that closely aligned with the x-axis (Fig. 4.2). Vectors for weed families were typically longer than in 2008 and more closely aligned with the y-axis, indicating that they explained variation not accounted for by the mulch treatments. Almost all beetle taxa associated at least somewhat with compost but differed in their association with weed families. Captures of some taxa (e.g., *P. melanarius* and *P. lucublandus*) were higher in compost without weeds while others (e.g., *H. rufipes*, *Pseudamara arenaria* (LeConte), and *Amara* spp.) tended to associate with weed families, particularly grasses and ferns in compost. *S. erythropus* and *M. fungi* did not strongly associate with pine needles in 2009 as they did in 2008. *Harpalus affinis* (Schrank) avoided weeds in both years and was not collected more frequently in any mulch treatment.

4.4.3 Effects Of Mulching On Beetle Feeding Groups

The most numerous carabid species in both years was *P. melanarius* comprising 70 and 47% of total carabid captures in 2008 and 2009, respectively (Table 4.1). Captures of *P. melanarius* were higher in compost than pine needles or soil in 2008 and were higher in weeded than unweeded compost plots in 2009 (Table 4.3, Fig. 4.3A).

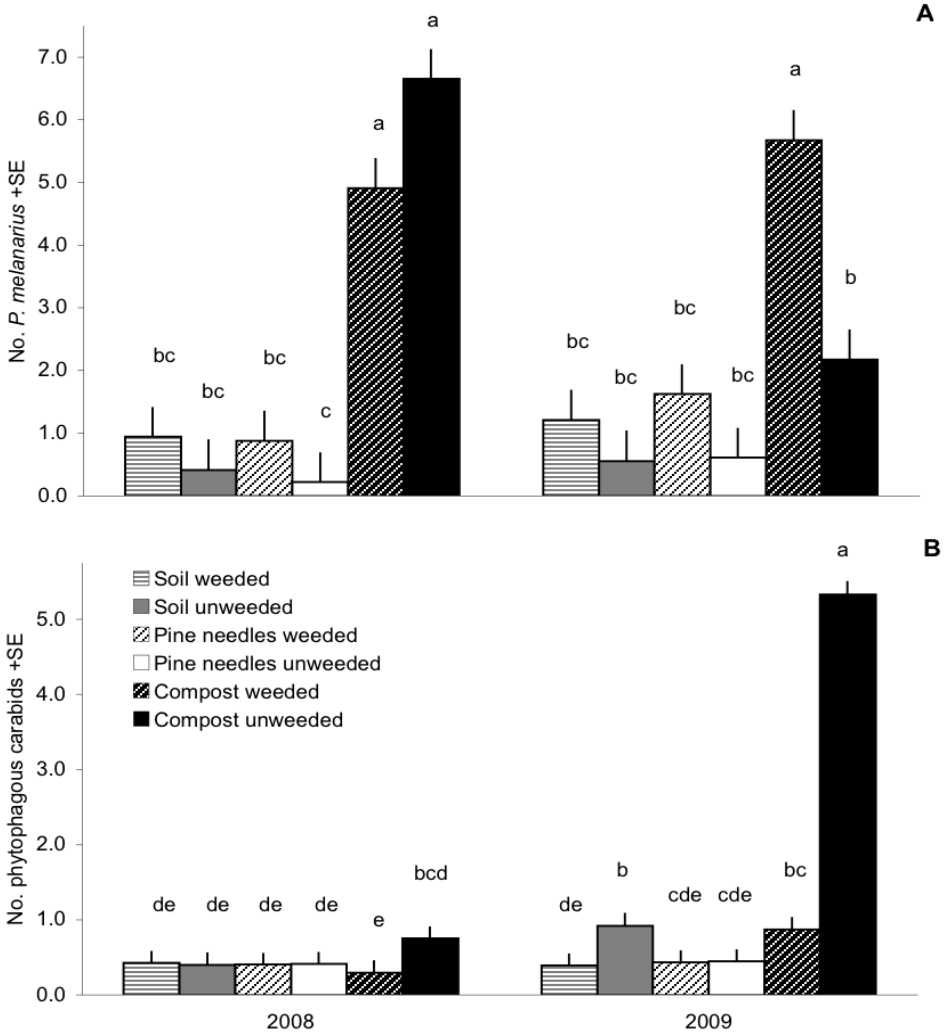

Figure 4.3 Effects of ground cover types (composted mulch, pine needle mulch, soil) on mean number of *Pterostichus melanarius* (A) and phytophagous carabids (B) captured in pitfall traps in a highbush blueberry field. Values are the averages of five sampling periods of four consecutive days each between late July and late September. Differences between means with the same letter were not significant (Tukey's HSD, $\alpha = 0.05$).

Captures of phytophagous carabids were significantly higher in unweeded compost in 2009 compared to 2008 (Table 4.3, Fig. 4.3B). The increase was largely due to two species; *Dicheirotrichus cognatus* (Gyllenhal) increased from 12 to 294 captures and *H. rufipes* increased from 47 to 100 captures.

Captures of predatory carabids (excluding *P. melanarius*) and predatory staphylinids were higher in compost in 2009, and neither group was affected by weeding (Table 4.3, Fig. 4.4A). Significantly more predatory staphylinids were captured in pine needles than soil in 2008 (Fig. 4.4B). Captures of all common predatory carabids and most predatory staphylinids were higher in 2009 compared to 2008, except *Philonthus* spp., *S. erythropus*, and *O. fracticorne*. (Table 4.2).

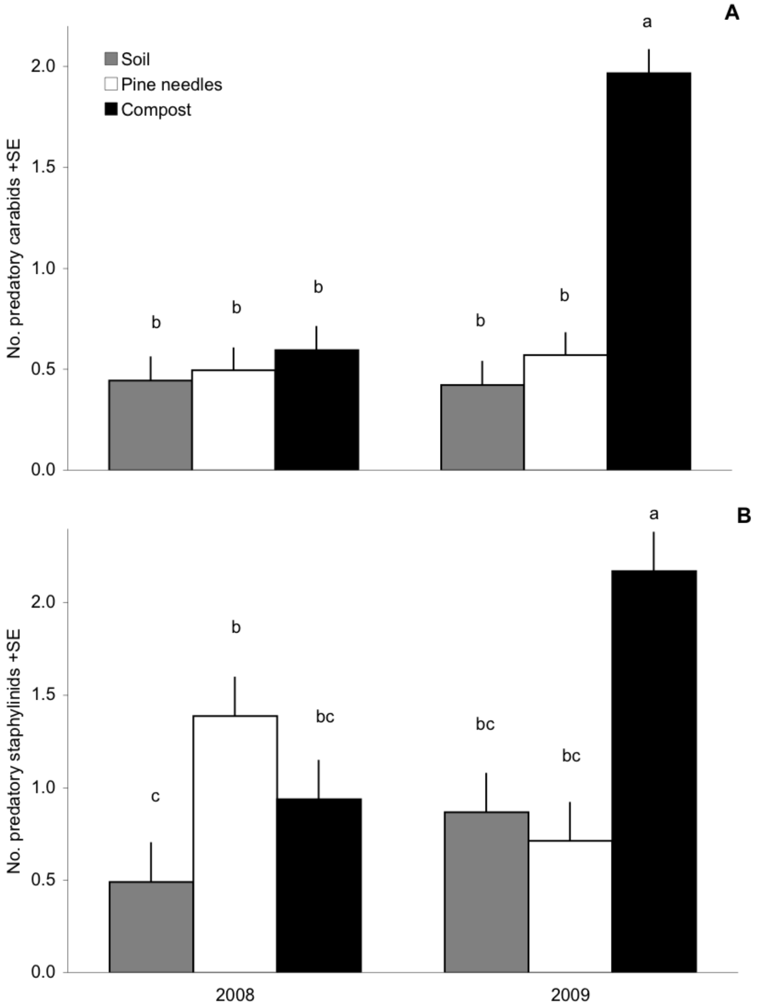

Figure 4.4 Effects of ground cover types (composted mulch, pine needle mulch, soil) on mean number of predatory carabids (A) and predatory staphylinids (B) captured in pitfall traps in a highbush blueberry field. Values are the averages of five sampling periods of four consecutive days each between late July and late September. Differences between means with the same letter were not significant (Tukey's HSD, $\alpha = 0.05$).

4.4.4 Effects Of Mulching On Potential Prey

In 2009, captures of *P. melanarius* declined from late July to late September in all ground cover treatments (Table 4.4, Fig. 4.5A). A total of 2023 macroinvertebrate potential prey was extracted using Berlese funnels, of which small earthworms (14%) and julid millipedes (55%) were most abundant. Seasonal patterns of small earthworm abundance varied among mulch types (Table 4.4). A large decline occurred from late July to early August in compost, whereas numbers increased through the season in pine needles (Fig. 4.5B). The abundance of julid millipedes increased in both mulches over the season (Fig. 4.5C). The significant mulch*weeding effect on julid millipedes (Table 4.4) was due to higher abundances in unweeded than weeded soil, with abundance in compost or pine needles not affected by weeding (data not shown). Soil plots contained low, fairly constant numbers of small earthworms and julid millipedes through the season (Fig. 4.5B,C). Other less common potential macroinvertebrate prey included other millipede taxa, maggots, coleopteran maggots, lepidopteran maggots, slugs, and isopods.

Pitfall captures of *P. melanarius* were significantly correlated with the numbers of small earthworms early but not later in the summer (Table 4.5). There were no significant correlations between *P. melanarius* and numbers of julid millipedes.

Table 4.4 Results of repeated measures analysis of variance on the effect of mulch type (compost, pine needles, no mulch) and vegetation (weeded, unweeded) on *P. melanarius* captures, small earthworms, and julid millipedes extracted from samples in Berlese funnels. Capture period or sampling day was the repeated measure, ‘date’. Data square root transformed (x + 0.5) for analysis.

	Model Factors	*df*	*P. melanarius*		Small earthworms <30 mm		Julid millipedes	
			F	*P*	*F*	*P*	*F*	*P*
Between Factors	Mulch	2, 14	71.17	0.0001	12.22	0.0001	27.52	0.0001
	Weeds	1, 14	84.90	0.0001	0.76	0.3848	0.24	0.6236
	Mulch*Weeds	2, 14	3.96	0.0244	1.01	0.3671	4.52	0.0137
Within Factors	Date	4, 11	29.92	0.0001	0.83	0.5094	2.78	0.0317
	Date*Mulch	8, 22	1.01	0.4405	2.30	0.0276	1.46	0.1860
	Date*Weeds	4, 11	0.69	0.6014	2.71	0.0357	1.89	0.1191
	Date*Mulch*Weeds	8, 22	0.48	0.8655	1.23	0.2921	1.37	0.2219

Table 4.5 Correlations between pitfall trap captures *P. melanarius* and numbers of abundant prey (small earthworms and julid millipedes) extracted from compost, pine needle, and soil plots.

Sampling Period	Small earthworms <30 mm		Julid millipedes	
	R	*P*	*R*	*P*
Late July	0.50	0.0141	-0.17	0.4450
Early August	0.64	0.0008	-0.06	0.7707
Late August	-0.07	0.7554	0.24	0.2657
Early September	-0.06	0.7729	0.07	0.7628
Late September	-0.28	0.1896	0.16	0.4476

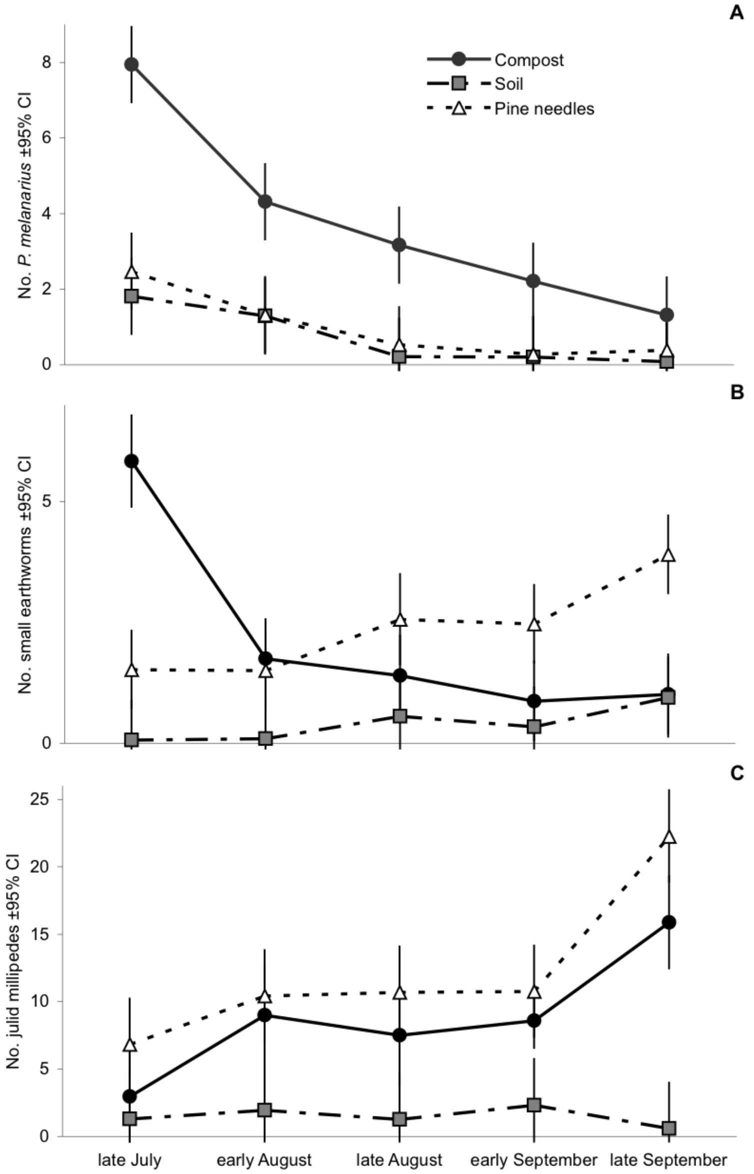

Figure 4.5 Effect of ground cover type (compost, pine needles, bare soil) on mean number of *P. melanarius* captured in pitfall traps (A), and small earthworms (B) and julid millipedes (C) extracted with Berlese funnels from 400 cm^2 samples in a highbush blueberry field (2009). Means and confidence intervals are back-transformed from data that was square root transformed (x + 0.5) for analysis.

4.5 DISCUSSION

Mulching is becoming increasingly important in highbush blueberries, especially in organic production, for weed control, building organic matter, and increasing plant growth and yield (Burkhard et al., 2009; Kuepper and Diver, 2010). Certain mulches and the density of weeds growing in them will also impact densities of epigeic arthropods, including beneficial predatory beetles and their prey. Mulches that benefit beetles may contribute to conservation efforts and promote biological control of pests.

The response to mulches and weeds varied among beetle genera and species but with few exceptions the differences may be best explained by feeding preferences. Predatory Carabidae and Staphylinidae responded strongly to mulching and were captured more frequently in weeded and unweeded compost mulch than in pine needle mulch or soil, particularly in the second year of the experiment. The response of predatory beetles to the compost mulch in the first year was likely due almost entirely to immigration from surrounding habitat, particularly for mobile *P. melanarius* (Raworth and Choi, 2001). Reproductive responses may have contributed to greater densities in compost in the second year, as most taxa have a one-year life cycle. The contribution of immigration to increased beetle numbers will decrease as the area mulched increases, and so it will be important to know the magnitude of the numerical response and stability of associations with mulches over the long term.

The effect that mulching has on the availability of prey accounts for some of the observed variation in *P. melanarius* captures between mulches, and likely contributes to determining the spatial distribution of other species as well (e.g., *P. lucublandus* is a medium-sized carabid that feeds on small earthworms, millipedes, and dipteran maggots, Larochelle and Larivière, 2003). The positive relationship between *P. melanarius* and small earthworms in late July and early August suggests that these beetles may be attracted to compost at this time because it contains high densities of this prey. An aggregative response to high prey densities, as in field studies with slugs (Bohan et al., 2000; Symondson et al., 2002a) and preference for small over larger prey because they are easier to capture or handle is consistent with observations in the laboratory (McKemey et al., 2001; Oberholzer and Frank, 2003). Large earthworms (> 30 mm) were not considered acceptable prey because beetles had difficulty feeding on them in the laboratory (J. Renkema, personal observation). However, *P. melanarius* is capable of reducing the densities of small and large slugs under semi-field conditions (McKemey et al., 2003). Slugs may be the primary diet of *P. melanarius* in cereal fields (Symondson et al., 1996), but they appear to have been less common in this field. Only two slugs were extracted using Berlese funnels and a few dozen emerged using AITC. In contrast, almost 40 small earthworms per 880 cm^2 of compost were extracted with AITC in late July (Table B.1, Fig. B.1). Since these beetles are highly polyphagous they likely aggregate to areas of high densities of acceptable prey rather than to particular prey taxa.

Prey abundance did not totally explain the spatial variation in *P. melanarius* captures later in the season. *P. melanarius* numbers remained high in compost even though densities of small earthworms and julid millipedes were equal or greater in pine needles. A similar spatial pattern has been shown where slugs were the main prey; beetles were associated with slugs early in the season, but later in the season there were fewer slugs where there were many beetles (Bohan et al., 2000). The dynamic nature of beetle and prey associations may be explained by attractiveness of high prey densities to beetles early in the season and a decline in prey numbers due to predation. *P. melanarius* may tend to remain in the same areas over the season because satiation (estimated to last up to 23 days; Bohan et al., 2000 from Symondson and Lidell, 1993) reduces activity. In the present study, millipedes remained abundant in compost later in the season, and thus beetles did not need to move from compost plots to find food.

P. melanarius may select areas of high prey density directly or by responding to the abiotic environment. Measurements with a limited number of temperature and moisture probes suggested that pine needle mulch was much drier with more extreme daily temperatures (higher in the day and colder at night) near the surface than compost. The extremes in pine needles may have deterred prey-searching beetles from entering these plots at any time in the season. Females choose oviposition sites based on appropriate wetness, with higher soil moisture preferable (Holland et al., 2007). If females laid more eggs in compost in 2008, then more adult beetles would have emerged in 2009 in compost in close proximity to abundant prey early in the season (before late July). Similarly, female beetles may have remained in compost later in the season in 2009 to oviposit. *P. melanarius* maggots, as do adults, detect slugs using chemical cues

(McKemey et al., 2004; Thomas et al., 2008). Depending on whether earthworms or millipedes are also detected by maggots, the range of detection, and the movement of maggots, adult beetles could be abundant in compost because maggots were attracted from other areas with low prey density.

High densities of weeds in compost reduced captures of *P. melanarius* in the second year, even though prey abundances were not less than in weeded compost plots. The most likely explanation involves change in the activity and/or movement patterns of *P. melanarius* in response to high densities of weeds. Individual beetle movement may be restricted in dense vegetation leading to fewer captures in pitfall traps that do not necessarily reflect lower densities (Thomas et al., 2006). However, *P. melanarius* may also avoid entering dense vegetation, such as hedgerows around cereal fields (Holland et al., 2005), or move out of these areas at night to forage (Chapman et al., 1999). Weed control could thus be important to maintaining higher activity of *P. melanarius* within fields.

The presence of weeds tended to have larger effects on phytophagous taxa than on predatory taxa. The response of phytophagous carabids to weedy compost in 2009 was most likely due to the abundance of seeds produced by annual plants; weeds were much more abundant in second year compost mulch plots than in any other treatment. Seed-eating carabids are known to aggregate to patches of weed seeds (Honek and Martinkova, 2001; Honek et al., 2005) and to field areas where weed density and cover is high (Heggenstaller et al., 2006; Meiss et al., 2010). *Harpalus* spp., *Amara* spp., and *Anisodactylus* spp. all eat seeds from plants that are in the annual weed families identified in this study (Honek et al., 2003, 2005, 2007). *D. cognatus* may feed on *Calluna* (Ericaceae) seeds or lepidopteran eggs (Larochelle and Larivière, 2003), but likely eats seeds of plants found in this study as no Ericaceae (other than blueberry bushes) were recorded. *H. affinis* was the only phytophage not associated with weedy compost and appears to prefer bare, open habitat. My results suggest that a habitat rich in food – compost more so than pine needles - will attract and perhaps maintain most predatory beetles, and that above ground weedy vegetation will further benefit phytophagous carabids because it provides additional sources of food.

An exception to high predator captures in compost in the second year was *S. erythropus*, found in high numbers in pine needle mulch in 2008. *Stenus* spp. prey primarily on Collembola (Betz, 1998), and the fresh pine needles coupled with higher rainfall in 2008 may have provided a favourable habitat for springtails (Badejo et al., 1998). In addition, the staphylinid, *Philonthus* spp. was abundant in fresh compost and pine needles the first year but scarce the second year. These beetles are attracted to fresh manure where they prey on fly eggs and maggots (Wingo et al., 1974), and year-old compost was probably less attractive to flies and beetles.

Macroinvertebrates deemed likely prey in this study are largely or wholly detritivorous and were abundant in compost or pine needles because they offered a good source of food. Most of the small earthworms were *Dendrobaena octaedra* (Savigny), a widespread, epigeic species common in low pH and high organic matter habitats (Reynolds, 1977). Julid millipedes that feed on organic matter can be pests of root vegetables (Allen and Filotas, 2009) and were increasingly abundant in pine needles and compost as the season progressed. Seasonal fluctuations in abundance were likely driven by predation or life history seasonality and by temperature and moisture. For example, increased moisture due to lower temperatures and few predatory *P. melanarius* may have allowed the increase in earthworms in pine needles in September.

From a ground cover management perspective, a possible negative effect of adding this compost mulch was the greater weed growth that occurred in the second year. High weed densities did not benefit and possibly inhibited some of the predatory Carabidae. Phytophagous carabids were the only group to obviously benefit, and while these taxa will feed on some weed seeds, it seems unlikely that they would reach densities high enough to suppress weeds below economically damaging levels. However, certain grasses and other vegetation are important for overwintering success of carabids and staphylinids (Thomas et al., 2002), and a moderate level of certain weeds may be a desirable management objective. Mixing or layering mulches to promote predatory beetles (compost) and suppress weeds (pine needles) might optimize both objectives.

Mulching is typically used in crop production for nutrient and/or weed management (Burkhard et al., 2009) but also has a strong influence on invertebrate communities. I showed that compost can increase the diversity and abundance of predatory beetles in a highbush blueberry field, and that this effect may increase in the second year after application. Increases seem to be driven largely by corresponding increases of prey in compost, but factors such as temperature and moisture levels may also be important. Most importantly for crop production, high beetle densities could lead to increased predation on *R. mendax* when maggots drop from berries and burrow into soil or mulch to pupate during late summer. However, the extent to which potential predation is affected by beetle feeding preferences or behaviours and the abundance of non-pest prey in mulches needs to be determined. Trade-offs between potentially improved pest management and increased weed densities due to compost mulching need to be considered and a general crop management strategy developed that takes into account the multiple effects of mulch on an agroecosystem.

CHAPTER 5 PREDATION BY *PTEROSTICHUS MELANARIUS* (ILLIGER) (COLOPTERA: CARABIDAE) ON IMMATURE BLUEBERRY MAGGOT (DIPTERA: TEPHRITIDAE) IN SEMI-FIELD AND FIELD CONDITIONS

5.1 ABSTRACT

Practices that enhance abundance and diversity of generalist predators are often employed with the objective of improving biological control of insect pests. Ground beetles and other predators can prey on blueberry maggot, an important pest of blueberries, when mature larvae pupate in the ground. Mesocosm and field experiments were conducted to determine if *Pterostichus melanarius*, a common predatory ground beetle, lowers maggot numbers in compost mulch or when predator and alternative prey abundances are manipulated. At background (field) densities of alternative prey, increasing densities of *P. melanarius* did not significantly reduce pest numbers in mesocosms containing compost or soil. When alternative prey were removed from compost, beetles reduced pest numbers by up to 35%. In field experiments, maggot numbers were higher when beetles and other predators were excluded from soil plots, but beetle exclusions had no effect in compost plots where both predator and alternative prey numbers were high. These results indicate that there can be some reduction of blueberry maggot by *P. melanarius* and other potential predators when there are few alternative prey. However, despite attracting large numbers of predators compost mulch did not lead to a significant reduction in maggot; in fact, the high abundance of alternative food associated with compost appeared to interfere with beetle predation on blueberry maggot.

5.2 INTRODUCTION

The goal of conservation biological control in agriculture is to suppress and/or regulate crop pests using habitat management to enhance natural enemies (Ferro and McNeil, 1998; Snyder et al., 2005). Natural enemy populations may benefit from selective insecticide use (O'Neal et al., 2005a; Ruberson et al., 1994) or numbers may be increased by providing habitat that is limiting through managing field margins or establishing beetle banks to create favourable overwintering sites (Frank and Reichart, 2004; MacLeod et al., 2004; Thomas et al., 1992). Many predators are food-limited; therefore, practices that augment alternative prey, such as mulching or increasing pollen sources (Brust, 1994; Coll and Bottrell, 1992), can also be important for maintaining or increasing predator levels. Interest in conserving natural enemies through habitat management is growing, but research on the "right" type of habitat manipulation for optimal control of particular pests is still needed in many agroecosystems (Landis et al., 2000).

Tolerance for blueberry maggot (*Rhagoletis mendax* Curran) in eastern North America is extremely low because fresh product imported to or moved within Canada must be maggot-free (CFIA, 2009). Female *R. mendax* flies oviposit in ripe and ripening berries. Maggots eat fruit tissue and when mature (typically August) leave berries and pupate in the ground over winter (Lathrop and Nickels, 1932). Insecticides are often recommended following detection of a single fly on a sticky trap (Delbridge and Rogers, 2010). Growers may use relatively expensive organically approved GF-120 NF Naturalyte Fruit Fly Bait (spinosad) (Barry et al., 2005; Pelz et al., 2005) or Surround WP (kaolin) (Lemoyne et al., 2008), but there are few other proven control options for organic or non-chemical blueberry production.

Mulches are increasingly recommended in highbush blueberries for plant nutrition and weed control (Burkhard, 2007; Burkhard et al., 2009) and have potential to impact blueberry maggot in several ways. The abiotic environment created by mulches may inhibit pupation when maggots drop to the ground (Chapter 3), reduce pupal survival, and/or interfere with successful emergence of adult flies (Chapter 3). Furthermore, compost mulches have been shown to attract generalist predatory beetles, including Carabidae, particularly *Pterostichus melanarius* (Illiger), and Staphylinidae (Chapter 4; Renkema et al., submitted). If predatory beetles consume significant numbers of maggots and/or pupae, compost mulches could contribute to blueberry maggot control.

There are no examples to date where conservation of natural enemies alone has suppressed tephritid flies below economic thresholds (Aluja and Rull, 2009). However, there is some evidence that generalists prey on tephritids and can contribute to lowering population levels. For example, crickets, ground beetles, and rove beetles destroyed large numbers of apple maggot pupae (Monteith, 1971, 1972, 1976a, 1976b) and, along with ants, contributed 20–65% mortality of mature larvae (Cameron and Morrison, 1977). *Pterostichus melanarius* (Illiger) has been shown to feed on apple maggot (Allen and Hagley, 1990), and other carabids, *Harpalus distinguendus* (DeGeer) and *Pseudophonus rufipes* (Wiedemann) ate Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), larvae and pupae (Monzó et al., 2011; Urbaneja et al., 2006). Ants, wireworms, and earwigs preyed on wandering Caribbean fruit fly larvae, *Anastrepha suspensa* (Loew) (Hennessey, 1997), and ants, spiders, rove beetles, and deer mice preyed on Mediterranean and Mexican fruit fly, *Anastrepha ludens* (Loew) (Thomas, 1993, 1995; Urbaneja et al., 2006).

Predatory efficacy is critically dependent on consumption rate, which can vary with preferences and availability of alternative prey. Compost mulch attracts predatory beetles, but also contains large numbers of detritivorous macroinvertebrates (Chapter 4). An abundant and diverse detritivore community in compost attracts and benefits generalist predators (Harwood et al., 2009; Settle et al., 1996; van Baalen et al., 2001). It has been proposed that attracting generalist predators through provision of alternate prey may reduce numbers of the target pest through ‘apparent competition’ (Holt, 1977; Symondson et al., 2002). However, the outcome is expected to depend on predator preferences and overall prey abundance, as predators that prefer non-pest prey or are rapidly satiated may actually consume fewer target pests (Harmon and Andow, 2004). For example, abundant alternative prey in compost has been shown to reduce carabid predation on codling moth pupae in apple orchards (Matthews et al., 2004), addition of fly pupae lowered predation on black cutworms (Frank, 2007), and slug consumption by *P. melanarius* was reduced when earthworms and dipteran larvae were present (Symondson et al., 2006).

In this study, I examine how compost mulch and alternative prey influence beetle predation on blueberry maggot. I focused on the carabid *P. melanarius* because it is a common and nearly ubiquitous predatory beetle in eastern North America agroecosystems that is known to respond to conservation techniques such as mulching.

In addition, it is highly polyphagous, has been shown to consume the closely related apple maggot, and is active in August when blueberry maggot drops to the ground and pupates.

5.3 MATERIALS AND METHODS

5.3.1 Mesocosm Experiments

Effects of mulch and presence of alternative prey on predation of blueberry maggot pre-pupal maggots and pupae were examined over two years. In 2009, factors tested were substrate type (compost, soil) and beetle numbers per mesocosm (0, 1, 4). In 2010, factors were alternative prey (present, absent) and beetle number (0, 4) with only compost as the substrate. Each year a full factorial randomized design was used, with five replicates in 2009 and seven replicates in 2010.

Experiments were conducted in mesocosms consisting of circular, galvanized stainless steel rings (diameter = 76 cm, height = 30 cm) placed on landscape fabric on a fine gravel base. These were filled to a depth of 10 cm (2009) or 8 cm (2010) with composted wood-processing waste (0.7 g ml^{-1}, Louisiana-Pacific Ltd., Chester, NS) or soil (1.2 g ml^{-1}) from a highbush blueberry field (Rawdon, NS). Compost and soil temperatures and moisture levels were recorded every 2-3 days in each mesocosm with a pocket digital thermometer (9878E, Taylor Precision Products, Oak Brook IL) and a soil moisture meter (HH2 Moisture Meter & Dynamax TH20, Delta-T Devices Ltd., Cambridge UK). Water was added to mesocosms once in 2009 and four times in 2010 during periods of no rainfall. Mesocosms were enclosed with chicken-wire fencing and covered with bird netting.

P. melanarius adults were obtained in late July from dry pitfall traps placed in a grassy field (Bible Hill, NS). Collected beetles were held individually in plastic containers with moist peat. One week before starting the experiment, beetles were put in a Petri dish and allowed to feed for 24 h on 3-4 red wigglers (*Eisenia foetida* Savigny) having a combined weight of 150 mg. Beetles that did not feed were not used in experiments. Fed beetles were returned to containers with moist peat for seven days and weighed immediately before being put in mesocosms. Only female beetles (185 ± 4 mg) were used in 2009, and two females (194 ± 5 mg) and two males (162 ± 3 mg) per mesocosm were used in 2010.

On the same day in early-mid August, beetles were placed in and maggot-infested blueberries were placed above each mesocosm (Fig. 5.1). Berries were held on a hardware cloth screen in a wooden frame and supported by wooden posts. Emerging pre-pupae were directed through a funnel attached below the screen (aperture, 15 x 15 cm) to the centre of the mesocosm. A wooden square (10 x 10 cm) was placed on the mulch or soil surface to provide refuge for beetles, and a wooden doweling (20 cm) on the surface directed beetles from the edge to the centre. The upper inner and outer rims of mesocosms were painted with Insect-a-slip (Bioquip, USA) to prevent invertebrates from crawling in or out.

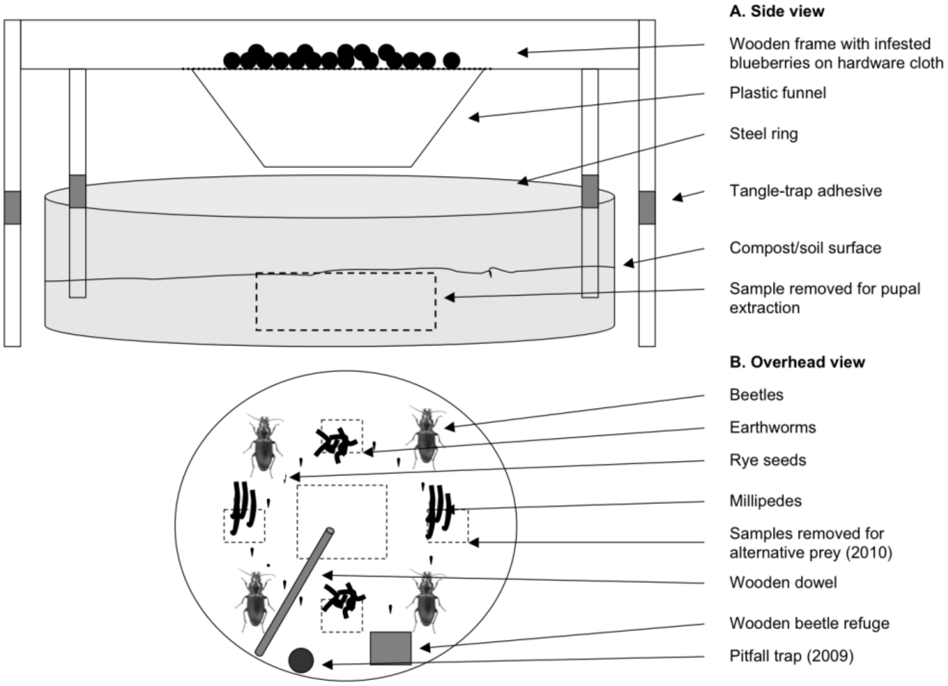

Figure 5.1 Mesocosm design showing initial placement of alternative prey (2010), beetle predator (*P. melanarius*), and area into which blueberry maggot maggots dropped from infested blueberries.

The experiments ran five (2009) and four weeks (2010). At the end of each experiment, beetles were removed and weighed. A 20 x 20 cm section of soil or mulch below the opening of the funnel was removed and shaken through a stack of sieves.

Material between 1.2 and 4.0 mm was hand-sorted to find pupae. Samples were further checked by re-sorting (compost) or soaking in water to float pupae (soil 2009).

In 2009, blueberry maggots were obtained from berries picked from a highly infested highbush blueberry field (Rawdon, NS). Berries were mixed and divided among the 30 mesocosms (6.5 kg fresh weight/mesocosm). The relative abundance of alternative prey in compost versus soil was assessed by placing a pitfall-trap along the inside edge of each mesocosm. Pitfall-traps were plastic cups (diameter = 9 cm, height = 4 cm) partially filled with water and a drop of detergent to reduce surface tension dug into compost or soil so rims were flush with the surface. Traps were opened for two consecutive nights at the beginning and end of the experiment. All captured specimens were preserved in 70% ethanol.

In 2010, flies reared in the laboratory were allowed to oviposit for five days in fresh highbush blueberries from an uninfested field (Sheffield Mills, NS). Blueberries were then mixed and divided among the 28 mesocosms (35 berries/mesocosm). Prior to the experiment, compost was dried at 85ºC for 24 h to kill macroinvertebrates, and then re-wetted to the original moisture content. Prey, consisting of 50 small earthworms (*E. foetida*, < 30 mm long), 40 millipedes (Julidae) and 1g of rye seed (*Secale cereale* L.) were then added to 14 mesocosms one day before beetles were added. Prey abundance at the end of the experiment was determined by hand-sorting millipedes and earthworms from four 10 x 10 cm compost samples from each mesocosm and from the 20 x 20 cm sample sorted to find *R. mendax* pupae. Rye seedlings were counted in the entire mesocosm.

5.3.2 Field Experiment 2010

Predation on *R. mendax* was also assessed using open and predator exclusion containers in a highbush blueberry field where mulch plots (20 cm of compost) were established in 2008 (Chapter 4). Plots (3 rows by 18 m) were randomly assigned within four blocks. Three pairs of containers were equally spaced (approx. 6 m apart) along the central row of a weeded compost mulch plot and a bare soil plot for a total of 12 container pairs in each plot type. Containers in each pair were 15-20 cm apart.

Containers were 22 x 22 cm clear plastic tubs with five holes in the bottom covered with screening to allow drainage (Fig. 5.2). Open containers were 5 cm tall and allowed ground predators to enter. Exclusion containers were 20 cm tall and prevented entry by predators. All containers were filled 5 cm deep with compost or soil (0.60 and 1.15 g cm$^{3\ -1}$, respectively) from the surrounding plots that was quickly hand-sorted to remove any larger potential predators. On 23 July, filled containers were dug 5 cm deep into plots so that material was flush and containers fit snugly and uniformly with the surrounding compost or soil. Compost or soil was placed over the lips (~1 cm wide) of the 'open' containers so no plastic was exposed.

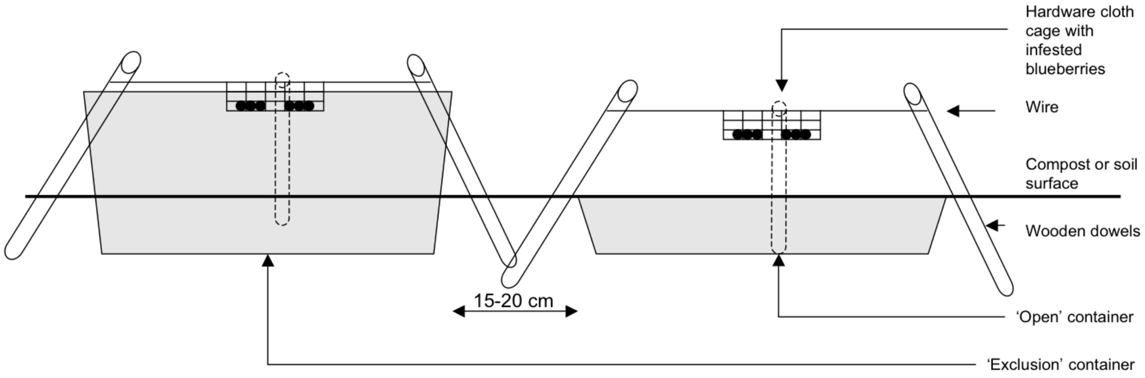

Figure 5.2 Design of containers that were open to or excluded to potential ground predators of blueberry maggot maggots and pupae.

A hardware cloth cage (8 x 8 x 2 cm) was put above each container by suspending it from wires attached to the vertical side of the cage and to three wooden dowels pushed into the compost or soil and angled over the container. Tops of cages were hinged, and a piece of window screen was glued over the top. Maggot infested berries removed from the growth chamber containing flies (same procedure as that described above for the 2010 mesocosm experiment) were mixed and randomly divided so that each cage received 20 berries on 30 July, 30 on 6 August, and 38 on 12 August. Any berries on bush branches hanging above containers were removed so potential extra maggots would not fall into containers.

Pitfall traps were used to assess levels of predator and alternative prey activity and density near containers. Each trap was an outer plastic cup (diameter = 9 cm, height

= 10.5 cm) with a hole punched in the bottom to facilitate water drainage and a removable inner cup of the same size into which specimens fell. A 10 x 10 cm wooden rain cover was supported by thin wires 5 cm above each trap. The inner cup was one-third filled with water, a drop of detergent to reduce surface tension and a small piece of salt to preserve specimens. Rims of traps were flush with the surface.

There were two pitfall traps near each pair of containers, each being 20-30 cm from one side of the containers. Traps were activated one night per week from 29 July to 9 September for a total of seven nights. Traps were covered with lids and a thin layer of compost or soil when not open. Contents of trap pairs were saved together in 70% ethanol for sorting and identification. Numbers of collected predators and prey were averaged for an early period (first four trapping nights) when most maggots were dropping from berries and a late period (last three trapping nights) when mainly pupae would be found.

Soil and compost temperatures and moisture levels were monitored from July 29 to September 10. Hourly temperatures were recorded with HOBO U12 data loggers (TMC50-HD sensors) and volumetric moisture (m^3/m^3) levels were obtained from ECH_20 Dielectric Aquameter probes (S-SMC-M005 sensors calibrated at -0.1 m^3/m^3 in air, Decagon Devices Inc., Pullman, WA) attached to a HOBO Weather Station (Onset Computer Corp., Pocasset, MA). One temperature and one moisture sensor was placed 1-5 cm below soil and compost surfaces in each plot of two blocks. Data was averaged between plots and the hourly mean and daily maxima and minima reported.

On 10 September, containers (except a pair in soil that was damaged during the experiment) were removed from the field and weighed. From 17 to 24 September, pupae were recovered as described above in methods for mesocosms.

5.3.3 Data Analysis

Analysis of variance (ANOVA) was used to test the effects of beetles and mulch on *R. mendax* pupae, alternative prey difference (number captured at beginning minus at end), and weight gain of recovered beetles in mesocosms in 2009. In mesocosms in 2010, effects of beetles and alternative prey on *R. mendax* pupae, effect of beetles on recovered alternative prey, and effect of alternative prey on recovery rates and weight gain of male and female beetles were tested with ANOVA. In the 2010 field experiment, effects of plot type on predators and alternative prey and effects of plot type and container type on *R. mendax* pupae were tested with a mixed model ANOVA with block as a random effect. Data were log ($x + 1$) transformed when assumptions of normality and homogeneity of variance were not met. Differences between means were tested with Tukey's HSD test. Analyses were performed using JMP software (SAS, 2009) at $\alpha = 0.05$.

5.4 RESULTS

5.4.1 Mesocosm Experiments

In 2009, mulch did not affect mean temperature in the mesocosms (compost: 21.8 ± 0.3ºC; soil: 22.2 ± 0.4ºC) ($F_{1, 298} = 0.62$, $P = 0.4311$), but soil was significantly (16.4 ± 0.5%) drier than compost (22.7 ± 0.5%) ($F_{1, 298} = 78.05$, $P < 0.0001$). Temperature averaged over all dates was 22.6 ± 0.2ºC and mean moisture was 17.1 ± 0.5% in 2010.

Mulch did not affect the number of blueberry maggot pupae remaining in the mesocosms at the end of the experiment in 2009 (Table 5.1). Number of beetles also did not significantly influence the number of pupae remaining in 2009 (Table 5.1); however, almost eight fewer pupae were recovered from soil with 4 beetles compared to soil with 0 beetles (Fig. 5.3). However, in 2010 blueberry maggot pupae recovery was significantly lower in mesocosms with four beetles than in those without beetles ($F_{1,22} = 23.30$, $P < 0.0001$; Fig. 5.4), and more pupae tended to be found in mesocosms where alternative prey had been added ($F_{1,22} = 4.18$, $P = 0.0531$).

Table 5.1 Results of analysis of variance for the effect of substrate (compost, soil) and number of beetles (0, 1, 4) on the number of blueberry maggot pupae, number of alternative prey at the beginning and end of the experiment, and percent weight gain of *P. melanarius* in mesocosms (summer 2009).

	Number of pupae			Alternative prey		Percent weight gain		
	df	*F*	*P*	*F*	*P*	*df*	*F*	*P*
Substrate	1	0.62	0.4402	10.74	0.0032	1	7.55	0.0252
Beetles	2	1.06	0.3635	0.64	0.5370	1	0.10	0.7595
Substrate*beetles	2	0.41	0.6691	0.02	0.9799	1	0.12	0.7373
Error	22					8		

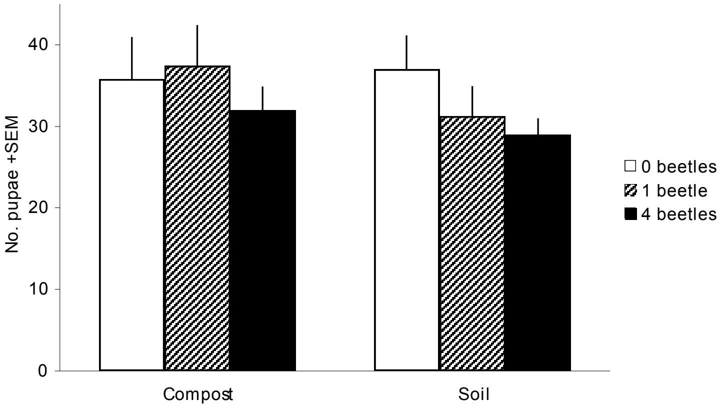

Figure 5.3 Mean number of blueberry maggot pupae from samples of compost or soil taken from mesocosms with 0, 1, or 4 *P. melanarius* beetles (summer 2009).

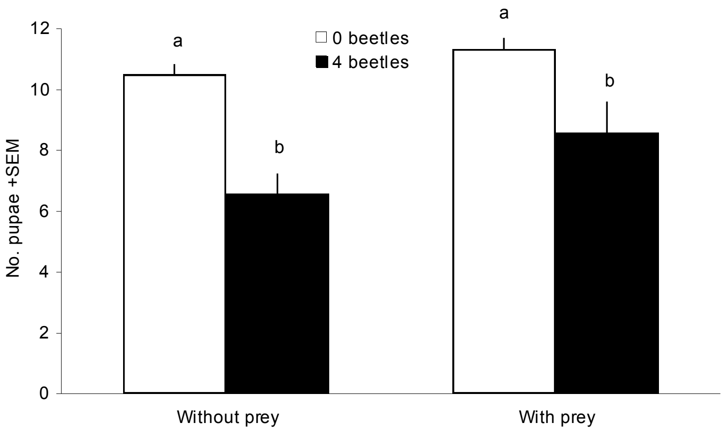

Figure 5.4 Mean number of blueberry maggot pupae from samples of compost taken from mesocosms with or without alternative prey and with 0 or 4 *P. melanarius* beetles (summer 2010). Means not connected by same letter are significantly different (Tukey's HSD, $\alpha = 0.05$).

There was significant decline in alternative prey (2009) in compost compared to soil, but the decline was not affected by beetles in either material (Table 5.1; Fig. 5.5). The number of recovered millipedes in 2010 was significantly greater in mesocosms without beetles (4.4 ± 0.4 vs. 1.3 ± 1.0) ($F_{1,12} = 8.11$, $P = 0.0147$), but there was no difference in the number of earthworms ($F_{1,12} = 0.98$, $P = 0.3413$) or rye seedlings ($F_{1,12} = 1.06$, $P = 0.3238$).

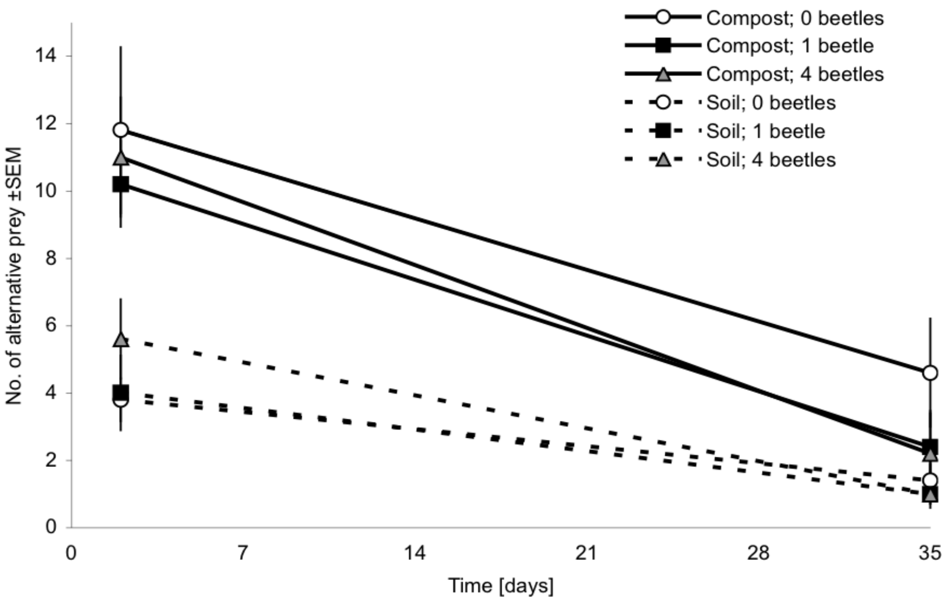

Figure 5.5 Mean number of alternative prey captured in pitfall traps in mesocosms with compost or soil and 0, 1, or 4 *P. melanarius* beetles (summer 2009).

Mulch did not significantly affect the number of beetles recovered from mesocosms in 2009 (one beetle treatment: 0.2 ± 0.2 in soil vs. 0.6 ± 0.2 in compost; $F_{1,8} = 1.60$, $P = 0.2415$; four beetle treatment: 1.6 ± 0.8 in soil vs. 3.4 ± 0.4 in compost; $F_{1,8} = 3.95$, $P = 0.0821$). In 2010, the number of beetles recovered was not significantly affected by alternative prey, but significantly fewer male (0.8 ± 0.2) than female beetles (1.7 ± 0.1) were recovered (Table 5.2).

Beetles, whether placed singly or in groups of four in mesocosms, gained significantly more weight in compost (44 ± 3%) than in soil (24 ± 5%) during the experiment in 2009 (Table 5.1). The alternative prey provided did not cause significant greater weight gain in 2010, but females gained significantly more weight (23 ± 1%) than males (4 ± 1%) (Table 5.2).

Table 5.2 Results of analysis of variance for the effect of alternative prey and beetle sex on the number and percent weight gain of recovered *P. melanarius* in mesocosms (summer 2010).

		Number of beetles			Percent weight gain	
	df	*F*	*P*	*df*	*F*	*P*
Prey	1	0.02	0.8964	1	0.01	0.9844
Sex	1	9.72	0.0050	1	7.81	0.0130
Prey*sex	1	0.01	0.9654	1	0.19	0.6673
Error	21			16		

5.4.2 Field Experiment 2010

The mean hourly temperature averaged over both sensors in compost was 21.1 ± 0.2ºC (range = 11.3 – 39.9ºC) and in soil was 20.2 ± 0.1ºC (range = 8.8 – 28.9ºC). The mean hourly moisture level averaged over both sensors in compost was 0.0025 ± 0.0023 m^3/m^3 (range = -0.1537 – 0.2570 m^3/m^3) and in soil was 0.0526 ± 0.0024 (range = -0.0418 – 0.3042 m^3/m^3).

There were significantly higher captures of *P. melanarius*, other carabids and staphylinids, and ants in compost plots than soil plots during both the early (July 30 – August 13) and late trapping periods (August 20 – September 10) (Table 5.3, Fig. 5.6A). Captures of other predators were only significantly greater in compost during the early period (Table 5.3). Captures of alternative prey – julid millipedes and all others – were also significantly greater in compost than soil plots during both early and later periods (Table 5.3, Fig. 5.6B).

Table 5.3 Results of analysis of variance for the effect of plot type (compost or soil) on predators and alternative prey captured in pitfall traps in a highbush blueberry field (summer 2010). Capture periods are separated into early, when the pest, blueberry maggot pre-pupal maggots were exiting blueberries, and late, when maggots had pupated. Predator captures were log ($x + 1$) transformed for analysis.

		Predators								Prey			
Capture period		*P. melanarius*		Other carabids and staphylinids		Ants		Others[a]		Julid millipedes		Others	
	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Early	1	47.81	0.0001	120.73	0.0001	5.89	0.0253	29.35	0.0001	21.48	0.0002	30.65	0.0001
Late	1	21.53	0.0002	25.52	0.0001	7.05	0.0156	1.81	0.1801	14.28	0.0013	67.37	0.0001
Error	19												

[a] Crickets, spiders, harvestmen, beetle maggots, centipedes, earwigs

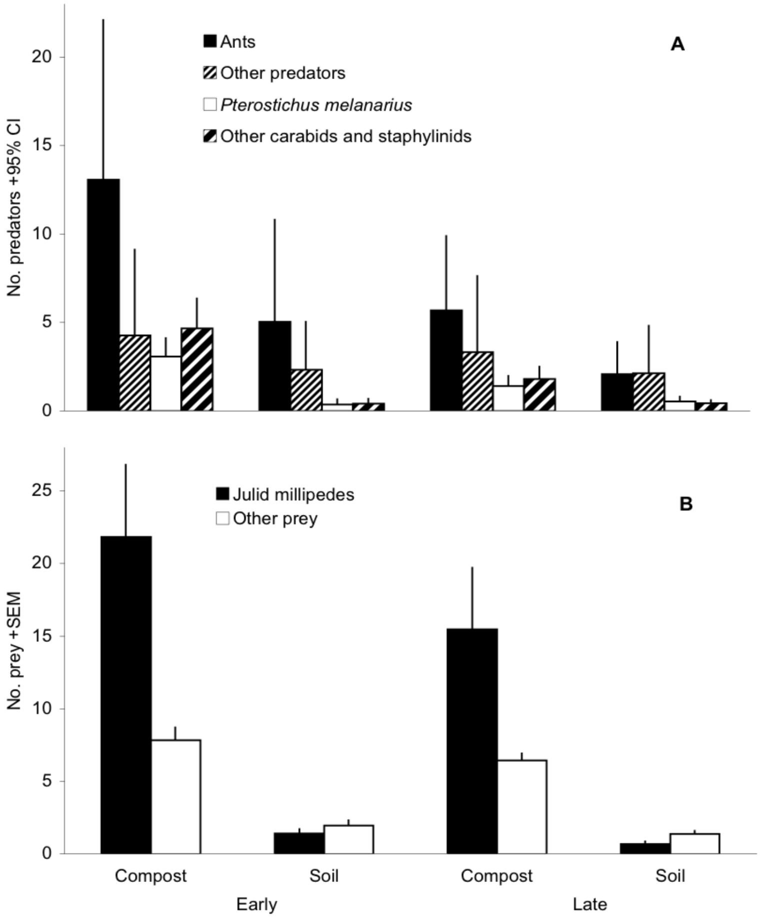

Figure 5.6 Mean number of predators (A) and alternative prey (B) captured per 24h in two pitfall traps in plots of compost or soil in highbush blueberries during early and late trapping periods (summer 2010). Predator means are back-transformed from analysis on log (*x* + 1) transformed data.

There was no significant effect of container type ($F_{1,39} = 1.21$, $P = 0.2790$) or plot type ($F_{1,39} = 3.10$, $P = 0.0862$) on the number of blueberry maggot pupae recovered, but the interaction of these terms was significant ($F_{1,39} = 7.11$, $P = 0.0111$), with fewer pupae being found in open containers in soil plots than other treatments (Fig. 5.7).

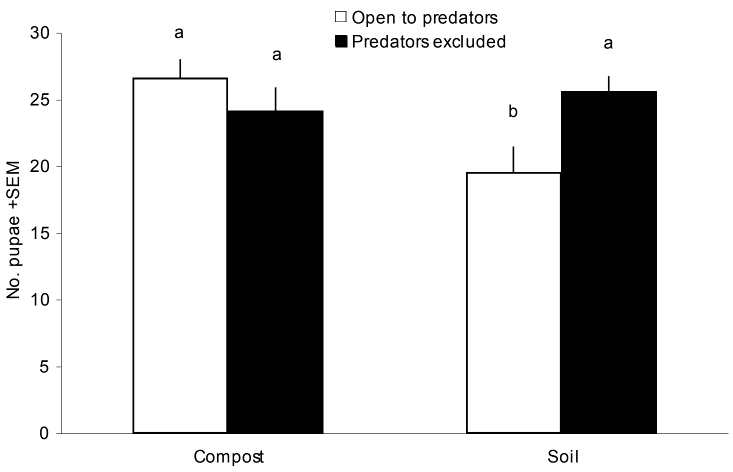

Figure 5.7 Mean number of blueberry maggot pupae from containers that were open to or excluded ground predators in compost or soil plots in a highbush blueberry field and removed in late summer 2010. Means not connected by same letter are significantly different (Tukey's HSD, $\alpha = 0.05$).

5.5 DISCUSSION

Agricultural practices such as mulching have potential to benefit ground-dwelling predators by providing habitat or enhancing food sources, and higher densities of predators have the potential to suppress pests and reduce population outbreaks. However, as I show in this study, the outcome for pest control depends on the relationships between predator, alternative prey, and pest. I have shown that numbers of blueberry maggot can be reduced in the presence of the predatory carabid *P. melanarius* and other potential predators when levels of other prey are low. When alternative prey is abundant, however, as is the case when compost mulch is added to a highbush blueberry field, even high abundances of predators do not lead to significantly lower number of maggots.

Levels of alternative prey in each study most easily explain variability in predator effects between my mesocosm and field studies. There was no evidence that high beetle densities in 2009 mesocosms containing compost lowered maggot numbers, but there was some indication that beetles were consuming maggots in soil mesocosms that had fewer alternative prey. Similarly, under field conditions, maggot numbers were not reduced in compost where both predator and alternative prey levels were high, but were reduced in soil where both predator and alternative prey levels were low. These results agree with findings in other systems where the presence of abundant alternative prey has been shown to reduce effectiveness of natural control agents (Koss and Snyder, 2005; Symondson et al., 2006).

The inhibitory effects of alternative prey on the potential for a predatory beetle to control blueberry maggot were most clearly demonstrated in 2010 mesocosm experiments. *P. melanarius* significantly reduced numbers of maggots when the alternative prey were eliminated by drying, indicating that the presence of other food was reducing the effectiveness of *P. melanarius* rather than some physical characteristic of the compost mulch.

Patterns of weight gain also indicate the importance of other prey for *P. melanarius*. Weight gain was much higher in compost than in soil in 2009. Weight gains were also lower in 2010 when prey were added back to compost after drying. I likely did not achieve live prey densities similar to those in the previous year due to low prey survival even in the absence of beetles (ca. 50% for millipedes and earthworms), and this was reflected in a 23% weight for female beetles compared to 44% the previous year. I suspect that the re-wetted compost was either still too dry for prey survival or was not a good food source after it had been dried. In 2010, similar weight gain for beetles in mesocosms with no prey added, but containing alternative prey killed by drying compost, and those in mesocosms with live alternative prey added may be due to scavenging by *P. melanarius* (Foltan et al., 2005). However, scavenged prey may not be as beneficial as live prey, as beetle weight gain was lower in 2010.

I have interpreted the significant reductions in maggot numbers in the field experiments as being most likely due to consumption. However, I cannot exclude the possibility that maggot behaviour prior to pupation contributed to lower recovery in soil containers open to predators. Without vegetation cover or a humus layer, maggots had to pupate in dry soil that had a high bulk density. Therefore, mature larvae may have had difficulty burrowing and may have died or some may have wandered and pupated outside the buried plastic container. I suspect that the lower bulk density and larger pore size of compost is likely to facilitate rapid, successful pupation. Less time on the surface reduces susceptibility to inhospitable abiotic conditions and also reduces the amount of time maggots are exposed to surface foraging predators. Video or visual observations of maggot and predator behaviour and movement could be used to determine the response of both to substrate characteristics and if mortality before pupation contributes significantly to overall survival of blueberry maggot.

The three experiments were conducted at somewhat different densities of blueberry maggot because it is nearly impossible to determine if an intact berry contains a maggot. The higher number of maggots introduced to mesocosms in 2009 than in 2010 may have reduced the apparent effectiveness of the beetles as measured by the proportional reduction in the number of maggots (a 22% non-significant reduction in soil in 2009 versus a significant 37% reduction in 2010) (see also Cameron and Morrison (1977) for predation rates on apple maggot). When calculated as an approximate number of maggots consumed per female beetle (males did not gain weight and were probably not feeding), beetle effects were the same in both years (two maggots per beetle). The lack of statistical differences between treatments in 2009 was likely also due to large variation among replicates in the number of maggots introduced. Using berries from a laboratory culture reduced the variance in 2010. The lower number of pupae introduced in the 2010 mesocosms is likely closer to most field densities (e.g., apple maggot; LeRoux and Mukerji, 1963).

I chose *P. melanarius* for the mesocosm experiments because it was attracted in large numbers to compost mulch, was active at the appropriate time of year, and readily consumed pupae in the laboratory in a preliminary test (J. Renkema, personal observation). However, asynchronous beetle and maggot diel cycles of activity may have contributed to low consumption rates. The behaviour of blueberry maggot has not been studied, but other tephritids maggots exit berries in the morning (Aluja et al., 2005; Boller and Prokopy, 1976). If maggots can burrow rapidly into the soil, they may be relatively safe from *P. melanarius*, which is primarily a nocturnal surface forager. However, modification of habitat or environmental conditions that increase larval wandering or reduce pupation depth might increase encounters and predation rates (Aluja et al., 2005; Alyokhin et al., 2001; Thomas, 1995). There are many other potentially important predators of blueberry maggot in agroecosystems, including crickets, spiders, harvestmen, beetle larvae, centipedes, earwigs, and ants (Allen and Hagley, 1990; Eskafi and Kolbe, 1990; Monteith, 1971; Wong et al., 1984) that could be equally or more effective than *P. melanarius*. Omnivorous crickets may be particularly important if they are attracted to fallen fruit and consume maggots in berries before they exit to pupate (Cameron and Morrison, 1977). Numbers of crickets captured over all trapping periods in this study were similar (38 and 40 in soil and compost, respectively), and they may have contributed to maggot losses in soil in the field. Conservation biological control, when effective, is often due to multiple natural enemies, and knowledge of their life histories, foraging behaviours, and interaction may help optimize conservation techniques and pest control.

My results do suggest that some blueberry maggot mortality can occur on bare soil, and that ground predators are likely contributing to these maggot losses. I have also shown that even though predator densities were much higher on compost than on soil, predators were less effective in reducing maggot numbers in compost. This highlights the important point that sheer numbers of predators in an agroecosystem is not necessarily a good predictor of predator impact against a pest. Manipulations, preferably in the field, are probably the only way to determine conclusively if the predators are actually reducing pest numbers. Many factors influence how effective predators are, including foraging behaviour, prey preference and the biotic and abiotic environment the predator finds itself in. Use of compost mulch in blueberries influences predator numbers, the physical environment and the abundance of alternative prey, which likely affects maggot larval survival as well as predator foraging choices and growth rates. However, the number of potentially important factors, and complexity of the interactions makes the consequences of using mulch nearly impossible to predict without direct experimentation.

The results of this study are particularly important because compost mulch is a very effective way to increase production in highbush blueberries and can be used in both organic and commercial operations (Burkhard, 2007). I have shown that the use of compost mulch is unlikely to promote natural control of maggot by predatory beetles, and that there may even be some reduction in predation rates. Any effect on blueberry maggot numbers is likely to be very small or negligible, however, as predators appear to be responsible for only a small percentage of total mortality throughout the blueberry maggot life cycle (Chapter 7). My results also suggest that it may be worthwhile to investigate modifications to the mulching technique that could lead to higher predator numbers without concomitant increases in non-pest prey (e.g., O'Neal et al., 2005b).

This chapter has been submitted to the journal Biological Control for consideration for publication

5.6 SUMMARY TO CHAPTERS 4 & 5; TRANSITION TO CHAPTERS 6 & 7

Compost mulch in highbush blueberries benefited predatory beetles, but the high level of alternative prey in compost, likely responsible for attracting beetles, appeared to further reduce low predation rates of *P. melanarius* on blueberry maggot. The following chapter examines if mulches and weeding may be attractive to adult flies and result in differing infestation rates in blueberries. In conclusion, I integrate mortality rates measured in different mulches in these chapters in order to formulate practical recommendations.

CHAPTER 6 MULCH, WEED, AND CULTIVAR EFFECTS ON BLUEBERRY MAGGOT (DIPTERA: TEPHRITIDAE) DYNAMICS AND HIGHBUSH BLUEBERRY PRODUCTIVITY

6.1 ABSTRACT

Ground-floor management practices in highbush blueberry fields, such as mulching and weeding, may affect pest numbers in addition to plant growth and productivity. In this chapter the effects of mulching, weeding and cultivars on infestation of berries by blueberry maggot and captures of adult flies, as well as berry size and bush growth were examined. Plots of weeded or unweeded compost mulch, pine needle mulch, and soil were established in 2008. Blueberry maggot infestation rates were higher in compost than in soil in 2008, but not in 2010. The number of flies captured on Pherocon®AM traps was linearly related to infestation rates ($R^2 = 0.30$ and 0.42). Other sources of variability are described below. First, the greatest numbers of flies were captured in unweeded compost possibly due to high weed densities that are attractive to flies. Second, composted bushes may have attracted mature flies not captured on Pherocon®AM traps but resulting in higher infestation levels. Third, increased bush size in 2010 in compost plots likely resulted in greater berry production and thus a reduced proportion of picked berries contained maggots. Cultivar significantly affected fly captures and maggot infestation rates; however, effects may have been confounded by field arrangement. The highest captures and infestation rates occurred in medium-sized, early ripening 'Bluejay' bushes that were closer to hedgerows and a lowbush blueberry field than other cultivars. Greater bush growth in compost plots was due to higher levels of available nitrogen (C:N, ~20:1). Small plots of mulches and weeds affected blueberry maggot fly behaviour in this study. Whether mulch applied to an entire field has similar effects on fly dispersal or fecundity or maggot survival should be investigated as it may significantly affect pest control.

6.2 INTRODUCTION

Blueberry maggot (*Rhagoletis mendax* Curran) can be the most serious pest of blueberries in eastern North America. A quarantined pest in many jurisdictions, stringent controls are necessary to ensure maggot-free fresh berry exports across provincial and national boundaries (CFIA, 2009). Furthermore, customers in general demand high quality produce, and growers supplying local markets must minimize maggot infestation in their fruit.

Insecticides are the most used control tactic for blueberry maggot. They are often applied to entire fields if a single fly is found on a baited sticky trap, with reapplication following additional detection (Delbridge and Rogers, 2010). However, blueberry maggot is not present in all highbush blueberry fields in Nova Scotia, and even when present, fly populations may vary greatly across fields and year-to-year (J. Renkema, personal observation). Multiple factors, such as natural enemies, the soil environment, non-crop hosts and/or habitat, temperature and rainfall, and bush cultivar may cause spatial or temporal variability in populations. Knowledge and prediction of how these factors act and interact will aid in designing management strategies using multiple tools against *R. mendax*.

Ground-floor management in highbush blueberries may include adding thick layers of mulches, including pine bark, wood chips, pulpmill biosolids, composted manures, seafood compost, or pine needles (Burkhard, 2007; Kuepper and Diver, 2010; Odneal and Kaps, 1990,). These materials may increase bush productivity (Burkhard, 2007; Magee and Spiers, 1995), decrease weed growth (Burkhard, 2007), and could have important impacts on blueberry maggot. For example, composted wood waste can alter fly eclosion success and pupation depth, and may reduce fly emergence when applied on pupae in soil (Chapters 2 and 3). On the other hand, this compost may moderate temperature, moisture, and density/compactness extremes compared to soil and create a favourable environment for pupal survival. Compost that encourages high densities of weeds may also attract and harbour high densities of flies as seen in naturally occurring weeds in lowbush blueberry fields (Gaul et al., 1995; Geddes et al., 1992).

Mulches may have opposing effects on different life stages of blueberry maggot; therefore, the net effect of adding mulch needs to be assessed under field conditions. My objectives were to determine the impacts of mulching with and without weeding on: (1) the number of adult blueberry maggot flies, (2) the percent of berries infested with maggots, and (3) crop productivity (blueberry size and bush growth).

6.3 MATERIALS AND METHODS

The study site used was that described in Chapter 4. The north field edge was bordered by two weedy rows of highbush blueberries (Bluejay cultivar) not included in the plots, a 12 m wide hedgerow with small trees, and then a managed lowbush blueberry field (Figure A.1). Predatory beetles were intensively trapped in 2008 and 2009, but not in 2010, thus plots were weeded and row aisles mowed less frequently (three times) than in previous years.

Flies were captured on Pherocon®AM sticky traps (Trécé Inc., CA) from late June to late August 2008-2010. There was one trap per plot placed in the middle of the central plot row. The trap was hung in a V-shape, sticky side out, from a dowel inserted into a wooden post in the ground. The trap hung ~15 cm below the top of the surrounding bush canopy. Bush foliage around the trap (15 cm radius) was removed where necessary. Traps were in the same location every year and replaced every two weeks. Trap height and distances to nearest branches on each side of the trap (> 15 cm, up to 100 cm) were measured. Flies were counted and removed from traps usually every 3-4 days.

Ripe berries were hand picked in mid-August each year from the same two bushes in the central plot row and weighed in 2009 and 2010. Seven hundred and fifty berries were picked and weighed from most plots, but 500 were picked from three plots (two unweeded and one weeded soil) where bushes were small. The number of berries picked in 2010 was visually estimated as a percent (to the nearest 10%) of the total ripe berries on the two bushes. Berries were transferred onto hardware cloth held over boxes of moist sand. In 2009, boxes and berries were placed outside in a screenhouse, but animals disturbed them and data was lost. In 2008 and 2010, boxes were placed indoors and occasionally watered to promote exit of maggots from berries and to maintain moisture levels in sand for pupation. After five weeks berries were removed, and sand was transferred to plastic containers, inundated with water, and repeatedly stirred so that pupae floated and could be easily removed and counted. Berry weight per 100 berries and percent of berries infested with maggots were calculated per plot per year.

Bush canopy volume was approximated as conical. The height and diameter (to nearest 5 cm) of all bushes in the central plot rows was measured in September 2008 and 2010, followed by calculation of volume; $V = 1/3 \pi r^2 h$. The proportional increase in size was determined for each bush as: $(V_{2010} - V_{2008}) / V_{2008}$, and then averaged for each plot.

A bulk sample (~1-2 L) was collected by removing mulch or soil from the top 10 cm in 8-10 randomly chosen locations in each plot in September 2008 and 2010 and stored in a sealed plastic bag at 4ºC. Other samples were collected for pH analysis in 2008 from mulch left-over after application to plots that was piled on the west field edge, and soil was collected from one location in the field. In November 2010, sub-samples from all bulk samples were oven-dried (70ºC for 24 h). The dried pine needles were ground in a ball-mill (MM 301, Retsch® GmbH & Co., Germany) and compost and soil in a roller grinder (Arnold and Schepers, 2004). Nitrogen and carbon levels were determined by dry combustion analysis using a CNS-1000 Elemental Analyzer (LECO® Corp, St. Joseph MI). pH values were obtained from a pH conductivity meter (Model 220, Denver Instrument) on three sub-samples of mulch or soil mixed in slurries with water (USDA-USCC, 2002).

Analysis of variance (ANOVA) was used to determine effects of year, mulch type, weeding, block, and bush cultivar on total numbers of flies captured on traps, percent infestation rates, the percent of ripe berries picked, berry weights, and proportional increase in canopy volume. Distances of traps to nearest foliage were included as continuous covariates in the model evaluating fly captures. Because all traps were placed in the centre of the plot, traps in three plots were approximately 1 m from nearest bush foliage (unweeded soil, weeded soil, weeded compost), and they captured very few flies (0-2) each year. Therefore, they were removed from the analysis on number of flies captured. Differences between means were tested with Tukey's HSD test. Linear regression analysis was used to explore relationships between flies captured on traps and distance from the north field edge and between flies captured and percent infestation rates. One data point was identified as an outlier (maggot infestation = 10%;

Cook's D statistic) and removed from the ANOVA and regression analyses. Analyses were performed using JMP software (SAS, 2009) at $\alpha = 0.05$.

6.4 RESULTS

6.4.1 Effects Of Mulching, Weeding And Cultivar On Blueberry Maggot

Weeds, mulch type, bush cultivar, and year significantly affected the numbers of flies captured on sticky traps (Table 6.1). More flies were captured in unweeded plots than in those that were weeded (24.2±1.9 vs. 16.6±2.1), and this effect appeared to be due in large part to highest fly captures in unweeded compost plots (Fig 6.1). Fewer flies were captured in pine needle mulched than in soil or compost plots. Fly captures were consistently higher in the cultivar Bluejay than in 'Bluetta' or 'Bluecrop', but captures varied year-to-year, being highest in 2010 and lowest in 2009 (Fig. 6.2). Peak captures occurred around Julian day 200 (middle of July) each year in all cultivars (Fig. 6.2). Fly captures also decreased as the distance from the north field edge increased (Fig. 6.3). Even though traps that were at least 1 m from foliage were removed from analysis, trap distances between 0-60 cm influenced fly captures (Table 6.1).

Table 6.1 Results of analysis variance for effects of year, mulch type (compost, pine needles, no mulch), weeding practice (weeded, unweeded), and cultivar on *R. mendax* fly captures, berries infested with maggots.

	Number of flies			Percent infested berries		
	df	*F*	*P*	*df*	*F*	*P*
Year[a]	2	14.35	0.0001	1	1.04	0.3167
Mulch	2	6.10	0.0049	2	1.09	0.3505
Weeds	1	6.12	0.0177	1	1.84	0.1852
Year*Mulch	4	0.45	0.7721	2	2.72	0.0822
Year*Weeds	2	1.76	0.1858	1	2.44	0.1286
Mulch*Weeds	2	2.14	0.1304	2	0.67	0.5187
Year*Mulch*Weeds	4	0.04	0.9964	2	1.17	0.3241
Block	3	2.35	0.0868	3	1.98	0.1385
Bush cultivar	2	47.39	0.0001	2	9.85	0.0005
Distance to foliage[b]	1	9.35	0.0090	-	-	-
Error	40			30		

[a] Flies captured in summers of 2008-2010, infested berries assessed only in 2008 and 2010

[b] Included as covariate in model

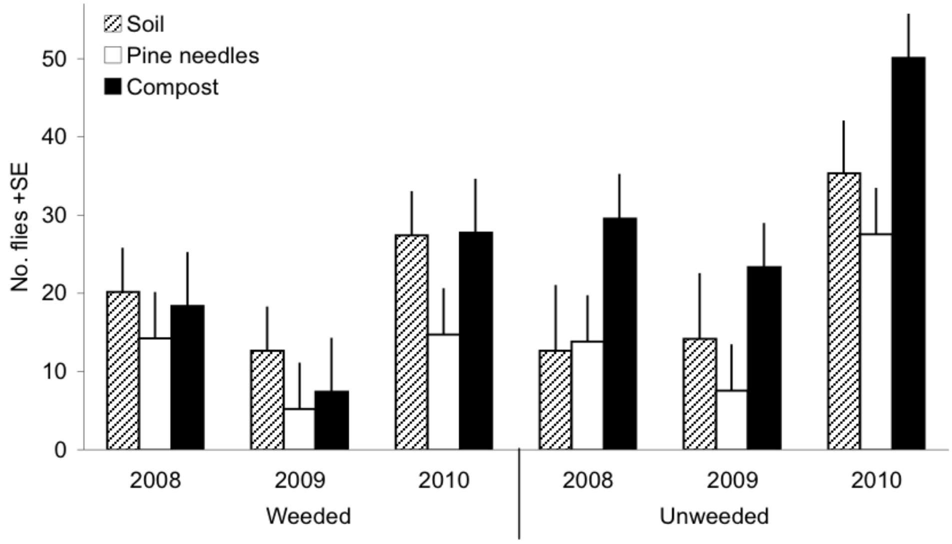

Figure 6.1 Least-square mean *R. mendax* fly captures on a Pherocon®AM sticky trap in weeded or unweeded plots of soil, pine needles, or compost during three years in a highbush blueberry field.

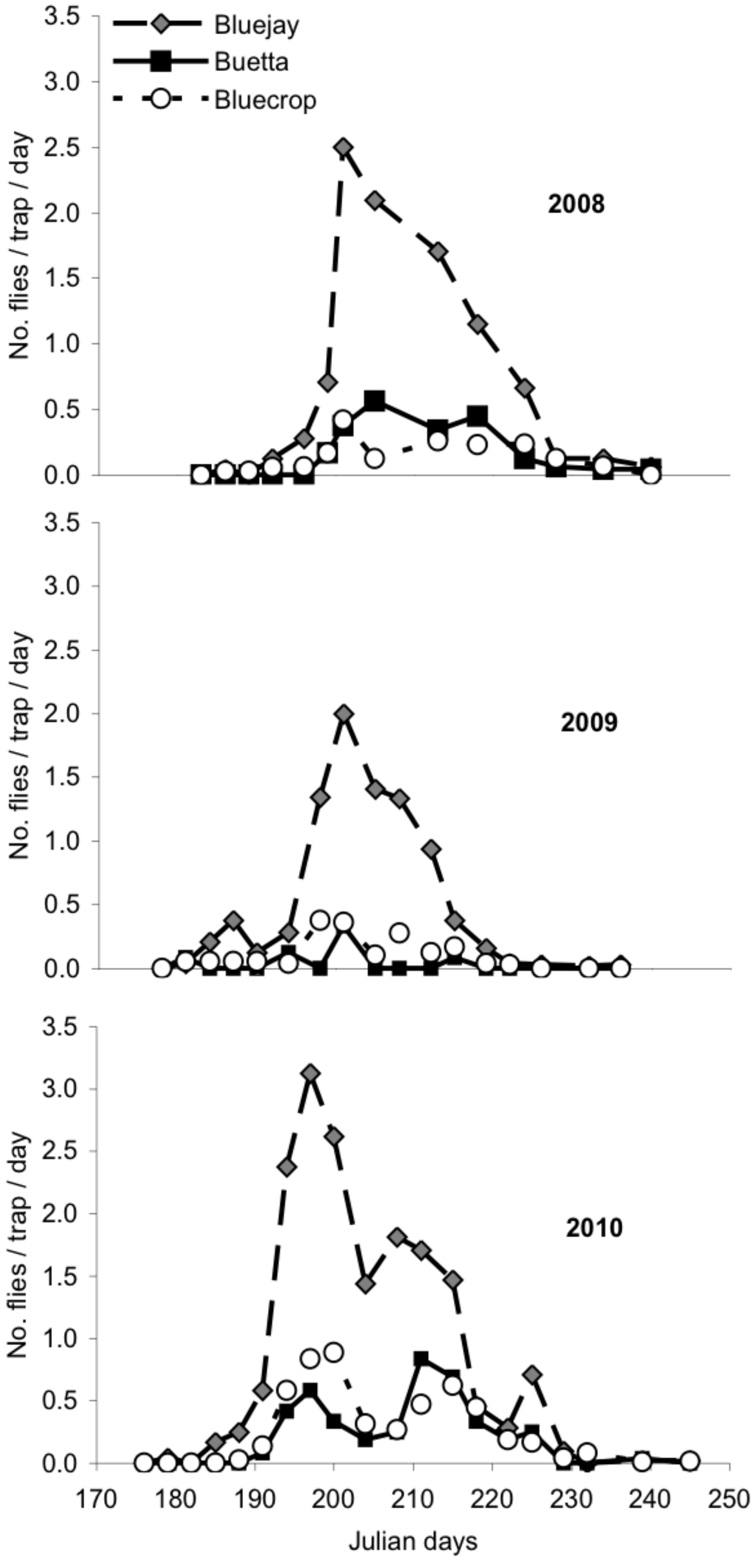

Figure 6.2 Mean *R. mendax* fly captures on a Pherocon®AM trap in three highbush blueberry cultivars, 2008-2010.

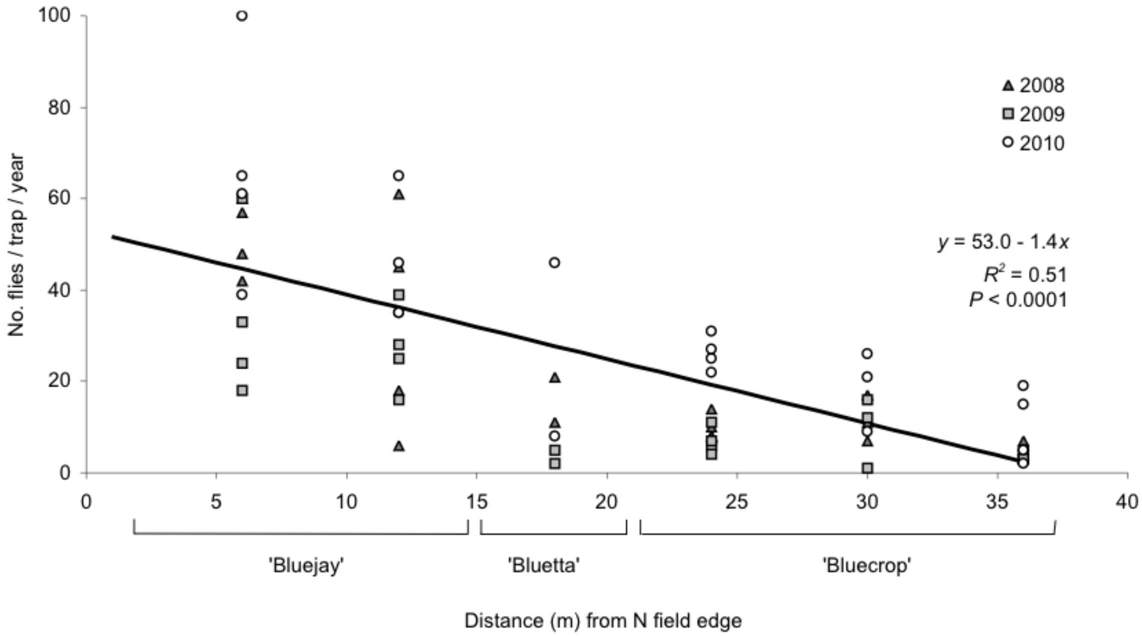

Figure 6.3 Relationship between *R. mendax* fly captures on Pherocon®AM traps in three highbush blueberry cultivars with increasing distance from the field edge that was adjacent to a hedgerow and lowbush blueberry field, 2008-2010.

There was some indication ($P = 0.08$, Table 6.1) of different effects of mulching on berry infestation rates in 2008 versus 2010. In 2008, infestation rates in compost plots were approximately double those in soil plots; however, in 2010 there was no evidence that mulching affected infestation rates (Fig 6.4). Cultivar significantly affected rates of maggot infestation in berries (Table 6.1). The rates were 2.2 ± 0.2% in 'Bluejay' berries compared to 1.3 ± 0.4% in 'Bluetta' and 1.0 ± 0.1% in 'Bluecrop' berries. Weeding did not significantly affect infestation rates (Table 6.1).

Fruit infestation rates were significantly related to fly captures within plots ($F_{1,45} = 19.08$, $P < 0.0001$) (Fig. 6.5). However, the relatively low R^2 (0.30) indicates that much of the variation in fruit infestation could not be explained by fly captures. When four data points were removed (those where the sticky trap was at least 1 m from the bush foliage), the linear fit was somewhat better ($R^2 = 0.42$).

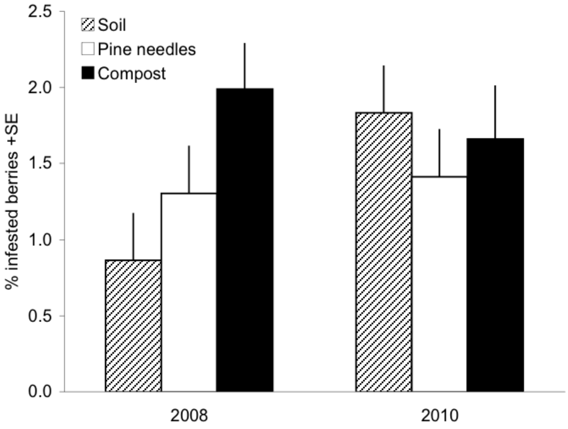

Figure 6.4 Least-square mean *R. mendax* pupae extracted from highbush blueberries in weeded or unweeded plots of soil, pine needles, or compost, 2008 and 2010.

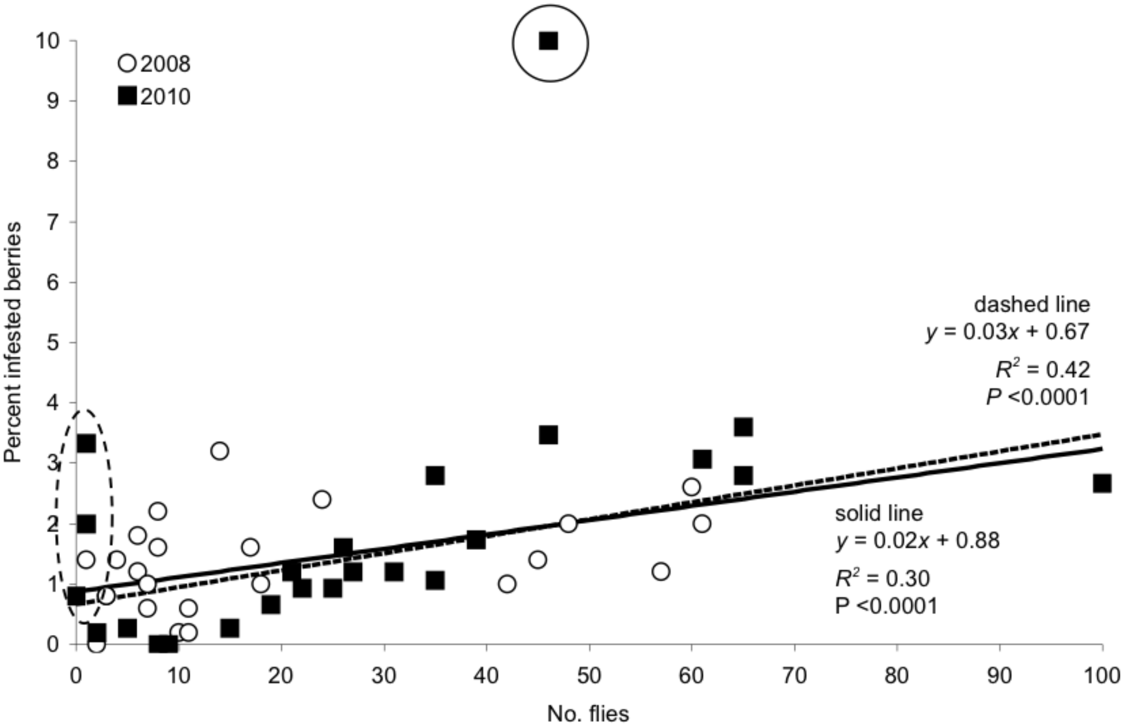

Figure 6.5 Relationship between maggot infestation rates and *R. mendax* fly captures on a Pherocon®AM sticky trap in a highbush blueberry field, 2008 and 2010. Circled data point was removed from both regressions (Cook's D statistic = 5.51), and four points in the dashed oval were removed from the regression shown by the dashed line.

6.4.2 Effects Of Mulching, Weeding And Cultivar On Plant Productivity

While picking 750 ripe berries per plot to estimate maggot infestation rates in 2010, the proportion picked per two bushes provided an approximation of total berries on bushes. Mulch type, but not weeding, significantly affected number of berries per bush (Table 6.2). There were more berries on bushes in compost than on bushes in soil plots, as a lower percent of the ripe berries were picked (Fig. 6.6A). There was a significant negative correlation between the proportion of berries picked and bush canopy volume in 2010 (R = -0.46, P = 0.0273).

Table 6.2 Results of analysis variance for effects of year, mulch type (compost, pine needles, no mulch), weeding practice (weeded, unweeded), and cultivar on estimates of numbers of berries on bushes, berry weight (g), and bush canopy growth.

	Percent of bushes picked			Berry size			Canopy volume		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Year[a]	-	-	-	1	8.91	0.0055	-	-	-
Mulch	2	3.96	0.0479	2	1.83	0.1776	2	10.14	0.0022
Weeds	1	0.13	0.7258	1	0.43	0.5178	1	1.76	0.2080
Year*Mulch	-	-	-	2	1.69	0.2007	-	-	-
Year*Weeds	-	-	-	1	0.02	0.8980	-	-	-
Mulch*Weeds	2	0.04	0.9616	2	2.12	0.1376	2	0.40	0.6781
Year*Mulch*Weeds	-	-	-	2	1.72	0.1951	-	-	-
Block	3	0.48	0.7046	3	1.95	0.1427	3	1.66	0.2240
Bush cultivar	2	1.40	0.2848	2	28.59	0.0001	2	0.06	0.9378
Error	12			31			13		

[a] Number of berries on bushes estimated in 2010, berry size measured in 2009 and 2010, increase in canopy volume calculated from 2008 to 2010

Mulch type or weeds did not significantly affect blueberry size (Table 6.2; Fig. 6.6B), but 'Bluecrop' berries (231.5 ± 5.6g) were significantly larger than 'Bluejay' (188.6 ± 5.7g) or 'Bluetta' (167.1 ± 8.9g) berries. Berries were larger in 2010 (205.3 ± 4.8g) than in 2009 (186.2 ± 4.8g).

Mulch type, but not weeding, significantly affected bush growth (Table 6.2). Canopy volume increase over the period 2008-2010 was greater in compost mulch than in soil, and intermediate in pine needle mulch (Fig. 6.6C). Cultivars grew at similar rates (Table 6.2).

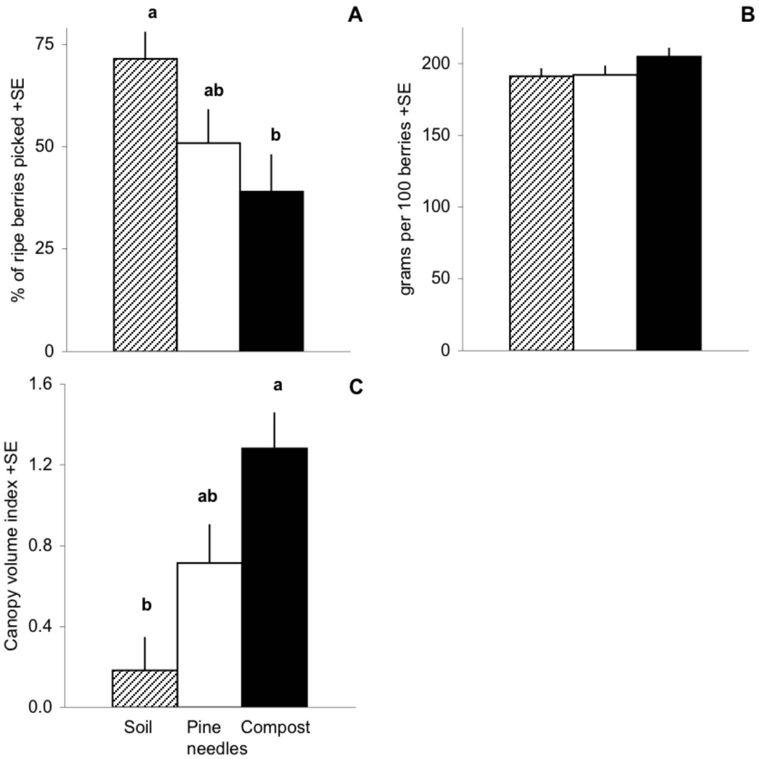

Figure 6.6 Least-square mean percent of ripe berries picked of total ripe berries on bushes (2010) (A), blueberry size (2009 and 2010) (B) and increase in canopy volume of highbush blueberries (2008 to 2010) (C) in weeded and unweeded plots of soil, pine needle or compost mulch.

Mulch quality differed from soil quality as pine needles were entirely organic matter (nearly 50% C), and compost had high nitrogen levels (1 %) but at a lower C:N ratio than pine needles (Table 6.3). Compost was stable between years as the C:N ratio did not change to the extent it did in decomposing pine needles (Table 6.3). The pH was higher in compost than soil or pine needles in both years (Table 6.3).

Table 6.3 Mean (±SEM) carbon, nitrogen, and pH of soil and mulches in weeded or unweeded highbush blueberry plots in September 2008 (3 months after mulch application) and September 2010, Rawdon, NS.

Treatment		Percent C		Percent N		C:N		pH	
		2008	2010	2008	2010	2008	2010	2008	2010
Soil	weeded	1.1±0.3	1.7±0.3	0.06±0.02	0.09±0.02	22.8±4.9	18.7±0.7	4.92±0.02	4.85±0.06
	unweeded	1.4±0.2	2.4±0.3	0.08±0.02	0.14±0.02	17.7±1.4	17.4±0.6	-	4.66±0.02
Pine needles	weeded	47.9±1.1	46.8±1.7	0.88±0.05	0.96±0.06	55.5±4.6	49.5±4.6	4.58±0.06	4.84±0.11
	unweeded	49.3±0.5	46.3±0.9	0.84±0.05	1.04±0.04	59.1±3.6	44.8±2.1	-	4.78±0.05
Compost	weeded	20.6±1.5	20.7±0.5	0.93±0.08	0.99±0.01	22.4±0.5	21.0±0.4	6.45±0.03	5.88±0.02
	unweeded	22.5±0.3	20.9±0.5	1.01±0.02	0.98±0.01	22.4±0.2	21.4±0.3	-	5.94±0.04

6.5 DISCUSSION

Ground-floor management (mulch application and weeding) may influence blueberry maggot survival at various stages of its life cycle, including effects on pupal survival and adult emergence success due to burial depth of pupae (Chapter 2), altered rates of predation on maggots after exiting fruit (Chapter 5), and effects on pupation depth (Chapter 3), which may influence pupal survival. Mulching and weeding may also influence fly behaviour and fecundity within a blueberry field, and thus the proportion of the berry crop that may be infested. The net effect of ground cover management on maggot abundance is difficult to predict from studies of individual life stages, partially because effects are often dependent on environmental conditions and sometimes in opposing directions, and because factors unrelated to ground-floor management (e.g., maggot survival within berries, adult mortality in the field) might have larger impacts on population dynamics. Two measures of blueberry maggot abundance that are most important to growers were examined here: the number of adults detected on sticky traps and percent berries infested by maggots.

In this study, berries from bushes mulched with compost had infestation rates that were more than twice those in bushes in soil in the year the compost was applied. Two years after mulching, infestation rates were similar between mulches and soil. The significance of the regression between fly captures and infestation rates suggests that fly captures were a useful indicator of infestation rates (Geddes et al., 1992, but see Neilsen et al., 1984; Wood et al., 1983). However, the relatively low R^2 values (0.30 or 0.42) suggest that other factors contribute to the spatial variation in infestation rates. Some of these factors may also help explain why infestation rates differed between years.

Despite more flies in unweeded than weeded compost plots, infestation rates were similar. This suggests that more flies than those detected on Pherocon®AM traps were attracted to weeded composted bushes. Fruit on bushes mulched with compost appeared to ripen earlier than on bushes in pine needles or soil (J. Renkema, personal observation). Earlier ripening fruit would have emitted volatiles that were attractive to mature flies (Kwasniewska, 2009; Pelz-Stelinski et al., 2005) that may have been searching for limited mating and oviposition sites early in the season (Smith and Prokopy, 1982).

Furthermore, fruit quality may affect female fly behaviour. If nutrients were elevated or altered in berries on bushes in compost, such as in leaves with high N levels on bushes grown in composts (Burkhard, 2007), and this was detectable by females, then they may have responded by exhibiting a preference for oviposition in these berries. The flies that were attracted to weeded composted bushes for mating and oviposition were not captured because ammonium acetate bait and trap shape and colour may not attract many sexually mature *Rhagoletis* spp. flies (Liburd, 2004; Prokopy, 1968; but also see Teixeira and Polavarapu, 2001a). Therefore, fly captures in weeded compost were lower than expected based on infestation rates because primarily mature flies visited early ripening fruit on these bushes to mate and oviposit and were not as attracted to traps as immature flies.

Bushes in unweeded compost attracted high numbers of flies partially due to weed densities that were higher than in unweeded soil or pine needle mulched plots (see Chapter 4 for 2008 and 2009 weed counts; similar weed densities in plots in 2010, J. Renkema, personal observation). Weedy areas in lowbush blueberries (Gaul et al., 1995; Geddes et al., 1992) and shady areas in highbush blueberries (Pearson and Meyer, 1990) have previously been shown to be associated with more flies probably because flies dispersing into or throughout fields are attracted to areas with shelter, humidity, or food sources (Smith and Prokopy, 1981). Dense weed growth may also attract high numbers of other insects, which in turn attract insectivorous birds. Insect honeydew and bird feces are likely food sources for flies (Boller and Prokopy, 1976) and may be particularly attractive to immature *R. mendax* flies because they contain symbiotic gut bacteria (Lauzon et al., 2000; MacCollom et al., 2009).

While weeds seem to attract *R. mendax* flies, increased infestation rates were not always the result in weedy soil or pine needles. Weeds may be attractive primarily to young flies looking for food and they may respond to other stimuli when mature (e.g., volatiles from early ripening fruit in compost). Therefore, unweeded compost plots attracted many young flies and retained mature flies because ripe fruit was plentiful. Weeds did not appear to affect canopy volume in this study with mature plants, even though Burkhard (2007) reported reductions in growth, leaf tissue N content, and yield in compost mulch in a young planting due to weed pressure. Therefore, it is possible that reduction of weeds would be helpful in limiting *R. mendax* fly habitat, but a more precise analysis will need to be done in fields where flies do not have a choice between plots as they did in this study.

Composted plots attracted more flies than other plots in all years, and these flies likely infested more berries; however, the infestation rate in 2010 was compensated for by a greater load of berries on bushes in compost. Larger bushes in compost should produce more berries (Burkhard, 2007), and the estimates of berries picked from all ripe berries were lower in composted bushes and negatively correlated to bush canopy volume in 2010. Therefore, large numbers of berries on bushes in compost appears to be the best explanation for infestation rates that were not significantly greater than rates in other plots.

Compost mulch increased bush growth primarily due to greater availability of nitrogen, while pine needles had some effect due to moisture retention. Burkhard (2007) reported greater canopy volume and higher leaf tissue N from bushes in seafood compost mulch than from those in pine needles. She also recorded higher moisture levels beneath pine needle, manure and seafood compost mulches than in soil resulting in larger bushes the second year after mulch application (Burkhard, 2007). The high C:N ratio (> 50:1) in pine needles could result in net immobilization of nitrogen due to soil microbes (Yang et al., 2002), whereas high N content with lower and stable C:N ratios in seafood compost (Burkhard, 2007) and the compost in this study (~22:1), result in uptake and storage of N for increased plant productivity. Blueberry bushes preferentially uptake NH_4-N over NO_3-N (Merhaut and Darnell, 1995), and nitrification is typically limited at low pH (Throop and Hanson, 1997). Compost could be too alkaline (pH 6.0-6.5) for plant growth, but it may not change the pH in soil (0-5 cm) below where most bush roots are located (Burkhard, 2007).

Cultivar had a significant impact on fly numbers and infestation rates. More flies were trapped in the Bluejay cultivar probably because of its synchrony of ripening with fly emergence and its size. 'Bluejay' is an early to mid season cultivar with medium to large berries and medium-sized bushes. Small 'Bluetta' bushes have small fruit that ripens very early and 'Bluecrop' is a mid season cultivar with large berries and large bushes. In a study comparing infestation rates (but not fly captures) in multiple highbush cultivars in Rhode Island, Liburd et al. (1998a) reported fewest maggots in early ripening cultivars, such as 'Bluetta', while 'Bluejay' and 'Bluecrop' were grouped as midseason cultivars with similar infestation rates. In this study 'Bluejay' ripened considerably earlier than 'Bluecrop', closer to the ripening time of 'Bluetta' (J. Renkema, personal observation). Therefore, 'Bluejay' bushes attracted more flies than 'Bluecrop' bushes likely because fruit ripened earlier, at the time of greatest fly activity, and attracted more flies than 'Bluetta' bushes likely because bushes were larger and contained more berries.

Differences in fly captures and infestation rates between cultivars may have also been due to their arrangement in the field, as fly captures decreased with increasing distance from the north field edge. 'Bluejay' rows (8 plots) were adjacent to the rows that were not part of the plots, the hedgerow, and the lowbush blueberry field. Highest fly captures have been recorded from highbush blueberry rows adjacent to forest edges or natural vegetation (Zaman et al., 2009). This vegetation may contain non-crop hosts that are sources of immigrating flies or may attract flies because it is a preferred habitat for feeding or shelter. On the south side of the 'Bluecrop' rows was an unused, but mowed field that was not a source of immigrating flies. Poor habitat in the field may have also promoted dispersal of flies towards the north side of the field. Without blocking cultivars, it is difficult to determine the extent that either bush cultivar characteristics or landscape features contributed to the observed differences in fly captures.

Since fly captures varied significantly among years, weather variations were likely important in determining yearly population fluctuations. Drought and low humidity, but also high moisture levels, were associated with lower apple maggot levels (Chen et al., 2002; Neilsen, 1964; Trottier and Townshend, 1979), and blueberry maggot fly emergence tended to be lower in wetter conditions (Chapter 2). Fly captures were highest in 2010 when precipitation was lowest from April to July (312.8 mm), captures were lowest in 2009 when precipitation was greatest (467.7 mm), and intermediary in 2008 when precipitation was also intermediary (342.9 mm) (Environment Canada, 2010). Higher spring moisture may increase pupal mortality in all plots, as year effects were consistent in mulches and soil whether they were weeded or not (no significant year interactions). Rainfall amounts and moisture levels will need to be related to *R. mendax* fly captures over multiple years to validate a relationship.

In summary, mulching highbush blueberries fields with compost rather than with pine needles is a better option for increasing bush growth and improving yields (Burkhard, 2007). *R. mendax* flies appear to be attracted to composted bushes to oviposit in ripe fruit, but this may largely have been the result of an altered distribution in this study where flies were given choices between bushes with different ground cover options. Compost may not affect the year-to-year abundance of *R. mendax* compared to soil unless maggot survivorship is greater in earlier ripening and/or nutrient-rich berries, and this could vary by cultivar. It is known that fertilized plants with higher N levels generally attract and benefit herbivorous insects (Karungi et al., 2006; Mattson, 1980; Yardim and Edwards, 2003). On the other hand, possible increases in maggot survival or female fecundity in composted bushes may be offset by increased production, thereby lowering the proportion of infested berries. Pine needles may be favoured by some growers mainly for weed management. Besides potentially limiting maggot and pupal survival (Chapters 2 and 3), pine needles may also allow growers to use compost mulch as part of an edge-based *R. mendax* management plan. Since areas of high fly captures are associated with field edges (Zaman et al., 2009), a composted outer row may complement other tactics in attracting flies and preventing dispersal further into fields. The combined effects of pine needle or compost mulch on all life stages and behaviours of blueberry maggot will need to be further studied in conjunction with other horticultural considerations and pest management strategies in order to recommend a best practice for highbush blueberry growers.

CHAPTER 7 CONCLUSIONS

Integrated pest management can be defined as a decision system that selects and uses multiple pest control tactics and strategies that are cost effective and ecologically conscious (Kogan, 1998). Integration begins by monitoring pests before using selective pesticides and progresses towards strategies that interface with social, political, or legal concerns and constraints (Prokopy, 1994). Steps along the way include substituting cultural, biological or other controls for pesticides, targeting multiple pests with these substitutes, and integrating pest management into other aspects of crop production (Prokopy, 1994; e.g., Prokopy et al., 1996). IPM succeeds because scientific knowledge and technological developments are available to meet demands and pressures coming from multiple sources. Current blueberry maggot management in highbush blueberries is facing change as customers demand organic produce, countries heighten pesticide regulations and restrict movement of fresh blueberries, and economic factors force growers to try new approaches. New selective insecticides and refined monitoring techniques are available that may help meet these demands. However, a higher level of integration may be achieved if mulch that is being used for production purposes can also be part of successful blueberry maggot management.

In this thesis I examined factors likely to affect the mortality of blueberry maggot from the time maggots exit fruit and contact the ground to the time flies emerge at the surface, a period of 10-11 months in a one year life cycle. I was particularly interested in determining how ground-floor management practices (mulching, weeding) might affect mortality during this part of the life cycle. I considered the effects of mulches both *in situ* and applied onto soil. If mulches create habitats that significantly increase mortality compared to soil, they could be incorporated into a pest management system. Conversely, if particular mulches enhance maggot survival, additional pest management measures might be required.

7.1 MULCHES *IN SITU*

Here I divide the ground portion of the life cycle of blueberry maggot into three stages: wandering and burrowing maggots (August), overwintering and diapausing pupae (August to April), and post-diapause pupae and emerging flies (April to July). I attempt to integrate the results of my experiments by estimating mortality rates for each stage, assuming no mulching (soil), mulching with compost and mulching with pine needles. Mortality during maggot wandering and burrowing was estimated by adding predation rates averaged between field and 2009 mesocosm experiments (Chapter 5) and the percent of pupae that were not found in stacks of cups (Chapter 3). Predation in pine needles was not studied and is not part of the estimate. Mortality rates of pupae during the overwintering stage (August – April) were not measured directly but were estimated from differences between fly emergence from 5 cm in 2010, when pupae were put in the field in April (assuming no mortality due to pupal holding conditions), and 2009, when pupae were put in the field in September (Chapter 2). These estimates should be taken as a first approximation, as they also assume that mortality rates in the spring (April - July) were the same each year. Finally, mortality during the post-diapause period was estimated from averaging fly emergence in growth chamber and field 2010 experiments from 5 cm in pine needles and from 1 cm in compost and soil, the depths at which maggots normally pupate (Chapter 2).

7.1.1 Maggot Mortality

Maggots emerge from fruit in August, drop to the ground below bushes, and spend a short time (minutes to hours) on the surface before burrowing into the soil. Mortality was estimated at 25-30% in soil and pine needles but was less than 10% in compost (Fig.7.1). Low moisture in pine needles appears to be a major deterrent to successful pupation in the laboratory, and under field conditions high temperatures (> 40º C recorded in afternoons) could further increase the mortality rate due to desiccation of maggots. Lower mortality rates in compost than in soil are due to alternative prey that distract or satiate predators and to suitability of compost for pupation. Mortality due to desiccation may be minimal in compost because it has a high water-holding capacity that moderates temperature fluctuations and because it has a low bulk density, compared to soil, that enables maggots to burrow quickly. Mortality could be greater in compost if high rainfall coincides with the time maggots drop from berries or if irrigation is used (Chapter 3) because maggots may be unable to penetrate a saturated material. Furthermore, entomopathogens that may be found at higher levels in compost should benefit from wet conditions, thereby increasing the likelihood that maggots are infected. On the other hand, rainfall, shade, and time of day maggots drop from berries could increase the suitability of pine needles in the field and reduce mortality rates.

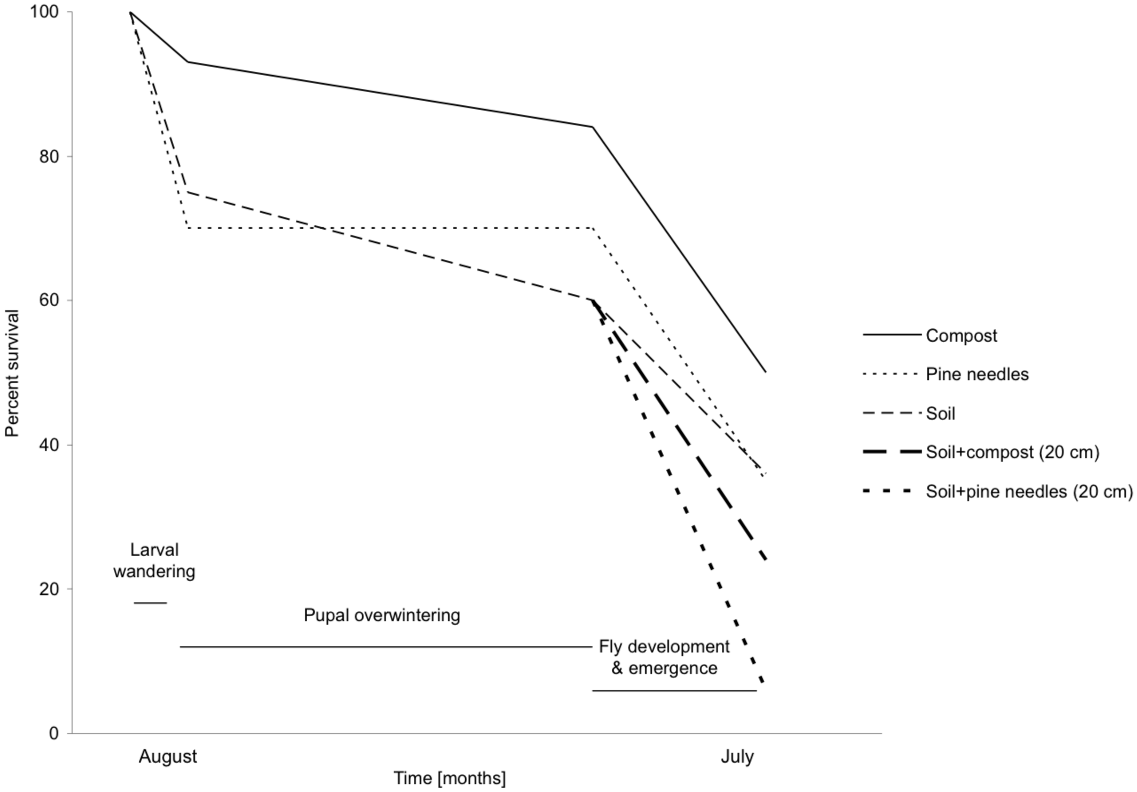

Figure 7.1 Survival of *R. mendax* maggots, pupae, and emerging adults in soil and mulches estimated from mortality inflicted by abiotic and biotic factors studied in this thesis (Chapters 2,3,5).

7.1.2 Pupal Overwintering Mortality

Pupae remain buried 1-5 cm in the ground overwinter to undergo diapause until early spring. Zero overwintering mortality in pine needles may be due to low pathogen levels and deeper burrowing of maggots for pupation where they may largely avoid abiotic stresses. Mortality in compost and soil was 10-15% for pupae buried 5 cm, but maggots typically pupate at 1-2 cm and pupation may be <1 cm in wet conditions (Fig. 7.1). Incidence of desiccation, drowning, freezing, pathogen infection and predation could be highly dependent on pupation depth, and it is expected that shallow pupae would be more susceptible. Therefore mortality may be higher in wet years or where irrigation is used. I suspect that compost mulch may cause some pupal mortality during overwintering compared to none in pine needle mulch.

7.1.3 Fly Mortality During Development And Emergence

Flies begin to develop inside puparia with rising spring temperatures, and emergence occurs in early July in Nova Scotia. Mortality was 40-50% in mulches and soil during this period. High moisture levels in all materials can reduce emergence (laboratory). This may be due to increased pathogen levels, but higher temperatures at near the surface (field) can also cause some emergence failure, probably due to desiccation of pupae. It appears that pupae are quite susceptible to direct and indirect effects of temperature and moisture extremes at this stage, whether in mulch or soil. However, very wet conditions may be more detrimental than hot, dry conditions and could be promoted by irrigation regardless of mulch used.

Overall, the survival rate from wandering maggots to emerging flies (August to July) is nearly identical in soil and pine needles but is higher by ~25% in compost. Comparisons between mulching and soil may change with soil or compost type or if weeds and other management practices are considered (e.g., shallow tillage). Since these are only estimates and not based on a thorough life table analysis, further studies may show that pine needles do provide some control or that compost increases survival by less than 25%. If some maggots can be tolerated in berries for sale locally, mulches *in situ* may be recommended for horticultural purposes without exacerbating blueberry maggot problems. However, a greater level of control through other management practices will be needed to ensure maggot-free fruit.

7.2 MULCHES APPLIED

Addition of a thick layer of compost or pine needles mulch may be the single best method of using mulch to reduce fly emergence. Mortality rates during the post-diapause stage after 20 cm of mulches were added onto soil in the early spring were estimated from average fly emergence levels in 2010 experiments and compared to those from 1 cm in soil (Chapter 2). I used estimates from spring-applied mulch because it is a more common practice than fall-applied mulch.

Both thickly applied pine needle and compost mulch appear to suppress fly emergence below levels in soil, but the net effect of pine needles was greater than that of compost (Fig. 7.1). The major deterrent to successful emergence from 20 cm appears to be the inability of eclosed flies to reach the surface. Flies may lack the energy to navigate through the material, they may become disoriented if they rely on positive phototaxis to move upwards, or they may have difficulty gaining traction to move upwards, especially in low-density pine needles. When both mulches were consistently wet (laboratory) no flies emerged because few eclosed from pupae. High moisture may have created anoxic conditions that drowned pupae and/or increased pathogen levels that may have caused pupae to rot (especially in compost). Yearly weather patterns will likely influence efficacy of 20 cm of compost mulch, as emergence rates were nearer to those from 1 cm of soil under drier spring conditions (2010). Rainfall probably has less effect in pine needles where emergence rates were consistently low at varying moisture levels.

To minimize the survival rate (<10%), pine needles applied at 20 cm may be the best option, but if spring conditions are wet or substantial irrigation is used, 20 cm of compost may lower the survival rate below ~25%. The major limitation to this method is that thick mulch is normally applied infrequently. Reapplication may be possible 3-4 years after mulch has settled and decomposed but would probably need to be motivated by reasons such as increasing fertility for bush productivity or weed management.

Therefore, thick mulching should be recommended as a tool for blueberry maggot management since it can reduce emergence to very low levels in the year it is applied, but mulches *in situ* may not continually provide the required level of control.

7.3 RECOMMENDATIONS FOR FUTURE RESEARCH

Management strategies for blueberry maggot with reduced insecticide use or reduced-risk insecticides are being developed, but further research is needed to determine best practices for growers. There are three areas that future research on mulch use for effective maggot management should address: (1) how can mortality be maximized at each life stage, (2) what is the best mulch application strategy, and (3) how do mulches fit with other strategies in a management program?

7.3.1 Maximized Mortality In Mulch

The highest mortality rate during the post-diapause and fly emergence period occurred with the application of 20 cm of pine needles. If the reasons why flies did not emerge were determined, then future management could focus on a specific tactic. Identification and levels of pathogens occurring naturally in mulches, how they are affected by environmental conditions or irrigation regimes, and how this relates to pupal mortality at varying depths could be important for greater control. This knowledge may also inform how to effectively use soil drenches of biopesticides to increase mortality rates in mulches or soil. In addition, after emergence, flies may remain on the surface for 24 h and whether predation occurs at this time should be explored.

Pine needles may create an inhospitable pupating environment, since in the laboratory 30% of maggots either desiccated or pupated elsewhere. Significant mortality did not occur on compost, probably because maggots spend less time wandering, and are therefore less susceptible to predation and adverse environmental conditions. If the relative contributions of surface temperature, moisture and porosity to maggot wandering time or burrowing ability were known, it might be possible to strategically choose a mulch, time irrigation, or alter weeding schedules to maximize mortality of the descending maggots.

Low mortality rates were estimated during the overwintering stage in all materials. Predation or pathogens may increase rates in compost or soil if shallow pupation (<1 cm) can be induced by high moisture levels. It appears that there is potential for high mortality of maggots in dry pine needles or mortality due to shallow pupation in wet compost. A direct comparison will be needed to predict the mulch and moisture level combination with the highest mortality rate in the field during the maggot burrowing and early overwintering stages.

7.3.2 Mulch Application Strategies

In these experiments, I analyzed the effects of mulch without reapplication on blueberry maggot and some surface-active predators; however, my results and recommendations of Burkhard (2007) suggest that layering mulches may be a good integrative approach to increasing bush productivity, controlling weeds, and reducing maggot levels. A possible scenario may be to apply 10 cm of compost on soil in the fall, followed by 10 cm of pine needles in the early spring. This method should reduce maggot emergence in July and create an unfavourable pupating environment in August if dry pine needles do increase mortality of maggots. The bottom layer of compost would provide fertility and moisture to boost bush growth and pine needles will control most weeds. Reapplication rates and timing will need to be studied, but if less mulch is applied initially (5 cm of each) and still causes significantly reduced emergence, then more frequent reapplication could be attempted. Pine needles will need to be reapplied in the second or third year to maintain control of weeds and some compost added to continue fertility benefits. Pine needles used in this study were obtained at no cost while the compost can be purchased at $5 CDN per cubic yard (Envirem, 2003). In the future, the purchase and application costs of mulching will need to be evaluated against the production and pest managemet benefits to optimize reapplication frequency.

Predacious beetles (Carabidae and Staphylinidae) benefited from compost mulch, likely due to increased food sources. This should also be considered when applying mulch. Generalist predators may not significantly contribute to maggot control when other prey is available, but an abundant and diverse beetle guild always has potential to negatively impact other pests (e.g., Lepidopteran pests, weed seeds) or increase nutrient cycling (e.g., detritivorous staphylinidae), thus providing as of yet unquantified services to highbush blueberry growers. If layering pine needles on compost does not provide the same beneficial habitat as using only compost, or if frequent reapplication disrupts seasonal life cycles or community succession and lessens potential ecosystem services, then recommendations for best practice ground-floor management highbush in blueberries should acknowledge this.

7.3.3 Mulches And Other Management Strategies

For sufficient maggot control, mortality caused below-ground by mulches will likely need to be augmented by above-ground tactics. This will be particularly true in years following thick mulch application where mortality rates appear similar in mulches and soil (Fig. 7.1). New strategies may focus management primarily on field edges as recent results have shown that many fields where conventional insecticides are used have highest infestations on edges that border natural vegetation (Zaman et al., 2009). Strategies could include removing any secondary host plants around fields (Smith et al., 2001), spraying insecticides on edge rows only (Collins and Drummond, 2004), or developing attract-and-kill systems. As for apple maggot (e.g., Prokopy et al., 2005), such a system may be based on using attractive spheres baited with insecticides and placed in the outer crop row to attract-and-kill flies entering the field or those that emerge in the field near the edges (Barry et al., 2004). The attractiveness of this row may be improved if it is mulched with compost and possibly also enhanced by planting cover crops - clover, alfalfa, rye, or other grasses - in the compost or reducing the level of weed control. As an alternative to spheres, insecticides or specifically GF-120 because it contains feeding baits, could be used to kill flies in this row. However, compost would not increase attractiveness in this row if growers choose to mulch entire fields with compost.

Mulches may also increase the effectiveness of emerging biological control strategies for *R. mendax*, including entomopathogenic fungi and nematodes and parasitoids. Efficacy of entomopathogens (e.g., *B. bassiana*) applied as a drench at the time of maggot drop may be improved in irrigated compost where shallow pupation should increase susceptibility, and the moist environment and greater arthropod activity may favour persistence and dispersal of pathogens. Similarly, porosity of pine needles could improve penetration of a drench with fall or spring applications preferable due to lower temperature. Entomopathogens may kill other soil dwelling pests (e.g., weevils and grubs), and thus have an important role in meeting IPM goals. Nematodes are being considered for tephritid control (Sirjani et al., 2009) and could include *R. mendax*. As with pathogens, efficacy could be increased when applied to mulches that are favourably moist or porous. If mulches can contribute to reduced pesticide use, then they may indirectly increase parasitism rates, as the wasp *D. alloeum* is not effective when insecticides are used (Steliniski et al., 2006).

In summary, this research has shown that mulching can be part of an integrative approach to blueberry maggot management, thus complementing its use for greater crop fertility or weed control. Thick mulching, particularly with pine needles, will be effective against blueberry maggot in the year it is applied due to reduced pupal viability and lower emergence success of flies from depth. Mulches *in situ* did not greatly affect maggot survival in these experiments, but testing of hypotheses generated from these results may show that mulches can negatively affect blueberry maggot to a greater extent than estimated so far. Thus, other management strategies to achieve low levels of fruit infestation are necessary in years after mulch application. The value of mulches may be further increased if they complement or improve new management strategies, such as attract-and-kill systems or use of entomopathogens. There may be other aspects of highbush blueberry production that are affected by mulching (e.g., root-feeding pests, crop diseases, or mycorrhizal fungi), and these should be investigated before a final cost/benefit analysis of mulches can be made. In the meantime, blueberry maggot management is improved by mulch when it is applied, and this should benefit highbush blueberry production.

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APPENDIX A HIGHBUSH BLUEBERRY FIELD AND SURROUNDING LANDSCAPE, RAWDON NS

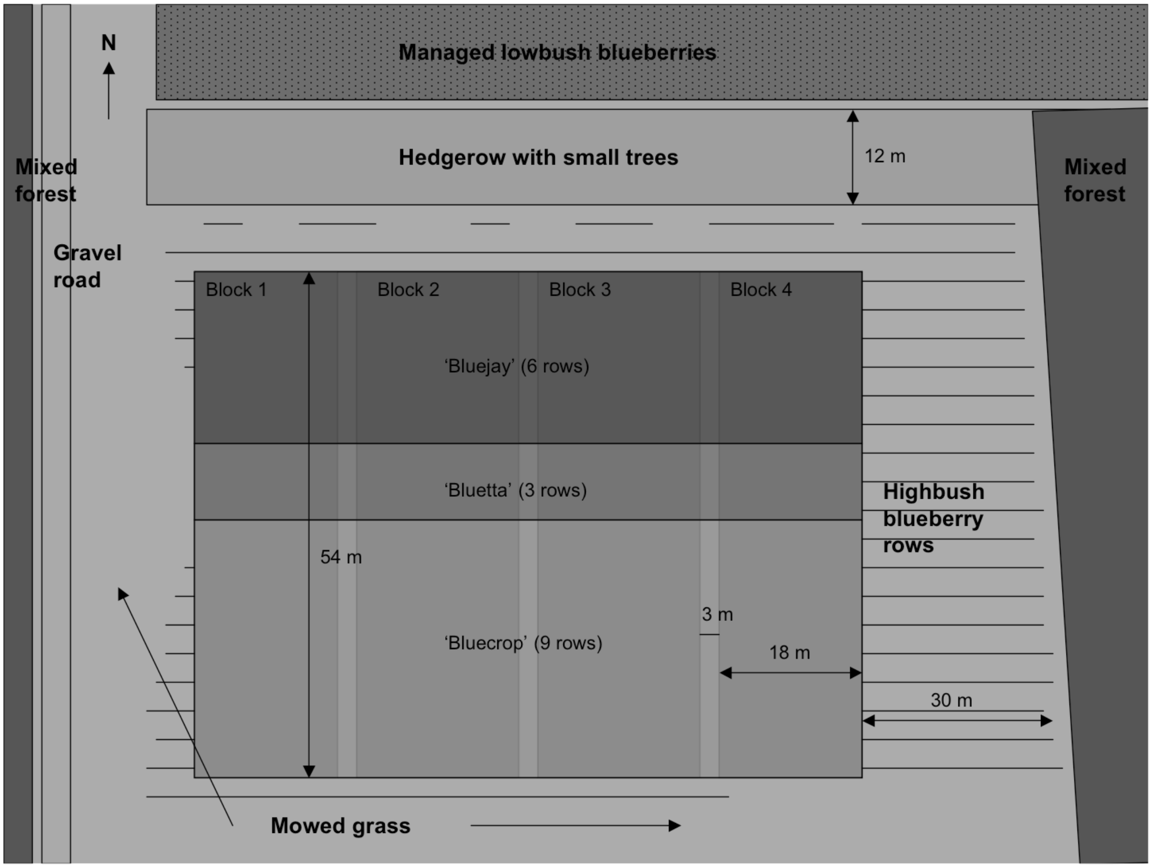

Figure A.1 Portion of a highbush blueberry field with three cultivars that was divided into four blocks of experimental plots as reported in Chapters 2,4,5,6. Highbush blueberry rows not used in experimental plots (black lines) and surrounding landscape also depicted.

APPENDIX B EARTHWORMS AND MILLIPEDES EXTRACTED WITH AITC FROM MULCHES IN A HIGHBUSH BLUEBERRY FIELD

Table B.1 Results of repeated measures analysis of variance on the effect of mulch type (compost, pine needles, no mulch) and vegetation (weeded, unweeded) on macroinvertebrates extracted with AITC (small earthworms, julid millipedes) in a highbush blueberry field. Sampling day was the repeated measure, ‘date’. Data square root transformed for analysis.

	Model Factors	*df*	Small earthworms <30 mm		Julid millipedes	
			F	*P*	*F*	*P*
Between Factors	Mulch	2,9	17.32	0.0001	5.32	0.0299
	Weeds	1,9	0.06	0.8115	1.48	0.2551
	Mulch*Weeds	2,9	4.22	0.0195	0.56	0.5898
Within Factors	Date	4, 6	6.39	0.0003	52.68	0.0001
	Date*Mulch	8, 12	7.99	0.0001	4.37	0.0112
	Date*Weeds	4, 6	1.76	0.1496	5.39	0.0346
	Date*Mulch*Weeds	8, 12	1.47	0.1879	1.97	0.1398

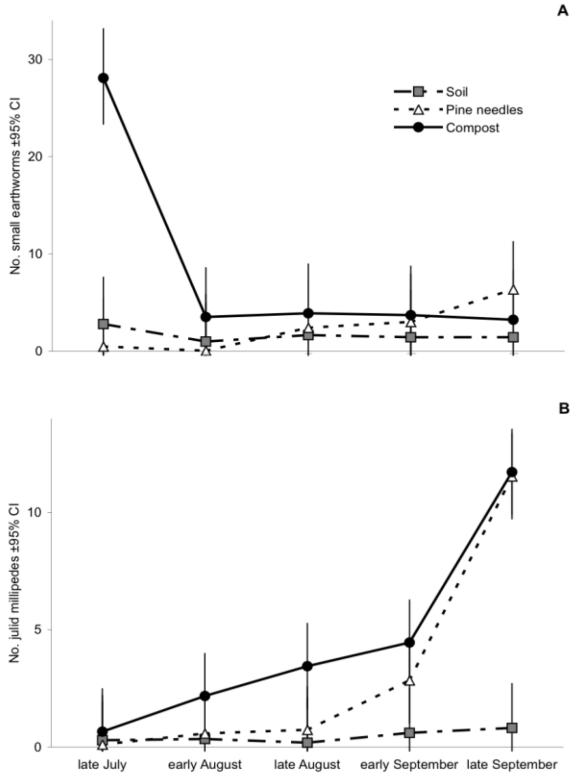

Figure B.1 Effect of ground cover type (compost, pine needles, bare soil) on mean number of small earthworms (A) and julid millipedes (B) extracted with AITC from 880 cm^2 on five days in a highbush blueberry field. Means and confidence intervals are backtransformed from data that was square root transformed for analysis.

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