

LIFE CYCLE OF THE ROVE BEETLE, *ATHETA CORIARIA* (KRAATZ) (COLEOPTERA:
STAPHYLINIDAE) AND SUITABILITY AS A BIOLOGICAL CONTROL AGENT
AGAINST THE FUNGUS GNAT, *BRADYSIA* SP. NR. *COPROPHILA* (LINTNER)

by

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AN ABSTRACT OF A DISSERTATION

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Abstract

The life history of the rove beetle, *Atheta coriaria* (Kraatz) (Coleoptera:Staphylinidae), predation against the fungus gnat *Bradysia* sp. nr. *coprophila* (Lintner) and compatibility with pesticides and plant growth regulators was investigated under laboratory conditions using Sunshine LC1 Professional Growing Mix as a substrate. Duration of life stages was 2.2, 7.1, and 7.8 days for egg, larva and pupa respectively, at 26°C, whereas total development time from egg to adult was 17.0 days. In addition, *A. coriaria* male and female adult longevity was 60.3 and 47.8 days. Average fecundity was 90.2 eggs per female and the number of adults produced per female was 69.1.

There were no significant differences in prey consumption when using second and third instar fungus gnat larvae as prey and starved and non-starved rove beetles. Overall, predation efficacy in Petri dishes was high (70 to 80%) as fungus gnat larval density increased with 3.9, 7.0, 11.1, and 15.3 larvae consumed in 24 hours after exposure of 5, 10, 15 and 20 fungus gnat larvae to one rove beetle adult. However, lower predation rates were found at different predator:prey ratios when using 1 to 5 rove beetles and growing medium as a substrate.

The direct and indirect effects of pesticides and plant growth regulators on *A. coriaria* were investigated under laboratory conditions. Rove beetle survival was consistently higher when adults were released 24 hours after rather than before applying pesticides. Acetamiprid, lambda-cyhalothrin, and cyfluthrin were directly harmful to rove beetle adults, whereas *Beauveria bassiana*, azadirachtin and organic oils were compatible with *A. coriaria*. Similarly, the plant growth regulators acymidol, paclobutrazol and uniconazole were not harmful to rove beetle adults. In addition, *Beauveria bassiana*, azadirachtin, kinoprene, organic oils, and the plant growth regulators did not negatively affect *A. coriaria* development. However, *Beauveria*

bassiana did negatively affect rove beetle prey consumption. This study demonstrated that *A. coriaria* is not compatible with the pesticides acetamiprid, lambda-cyhalothrin and cyfluthrin, whereas there is compatibility with organic oils, *Beauveria bassiana*, azadirachtin, and the plant growth regulators. As such, these compounds may be used in combination with *A. coriaria* in greenhouse production systems.

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Chapter 1 - INTRODUCTION AND LITERATURE REVIEW

Introduction

A variety of insect and mite pests such as whiteflies, aphids, thrips, mealybugs, and fungus gnats are responsible for economic losses associated with damage caused to greenhouse-grown crops including vegetables and ornamentals, with pesticides the primary means used to deal with these pests. The main reason for using pesticides is avoiding pest damage by quickly reducing pest populations below economic or aesthetic injury levels. In addition, other pest management methods may be used to increase pest mortality or reduce overall fitness, including biological, cultural, and physical (Brødsgaard and Albajes, 1999; Parrella et al., 1999; Dreistadt, 2001).

Since the initial use of the parasitoid *Encarsia formosa* (Gahan) against the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Speyer, 1927), many biological control programs have been successfully implemented against major greenhouse pests, with about 5% of the greenhouse area under biological control worldwide (van Lenteren, 2007). Some examples include the use of the predatory mite *Phytoseiulus persimilis* (Athias-Henriot) against the twospotted spider mite *Tetranychus urticae* (Koch), the ladybeetle *Cycloneda sanguinea* (L.) for the cotton aphid *Aphis gossypii* (Glover) in cucumber, and the predatory mite *Amblyseius cucumeris* (Oudemans) for the onion thrips *Thrips tabaci* (Lindeman) in sweet pepper (DeBach and Rosen, 1991). However, the need for producing high quality crops and avoiding aesthetic damage has led greenhouse producers to exclusively use pesticides to reduce the impact of arthropod pests, especially in ornamentals. However, secondary pest outbreaks and resistance are

important problems associated with pesticides (Croft, 1990). Despite this, augmentative biological control is still an important component of pest management programs in greenhouses (Bale et al, 2008) with 38 natural enemy species produced by 22 suppliers in North America (Warner and Getz, 2008). In addition, biorational and reduced-risk pesticides, which are generally less toxic to the environment and natural enemies, have been developed by the pesticide industry; these include insect growth regulators, insecticidal soaps, horticultural oils, and microbials (e.g., fungi and bacteria) (Cloyd, 2006) as well as new compounds associated with specific modes of action such as metaflumizone and the tetramic acid insecticide spirotetramat (Hempel et al., 2007; Marčić et al., 2011).

Fungus gnats (*Bradysia* spp.) are major insect pests of greenhouses worldwide and *Bradysia* sp. nr. *coprophila* has been reported in several greenhouse-grown crops including vegetables, bedding plants and ornamentals (e.g., cyclamen, poinsettia and impatiens) (Harris et al. 1996; Jagdale et al., 2004; Meers and Cloyd, 2005a; Cloyd., 2008). Fungus gnats may cause direct damage as well as indirect damage; indirect damage occurs during feeding when wounds predispose plants to infection by fungal plant pathogens such as *Pythium* and *Fusarium* (Jarvis et al., 1993; Gillespie and Menzies, 1993). Pesticides and natural enemies, including the predatory mite *Stratiolaelaps scimitus* (Womersley) and the beneficial nematode *Steinernema feltiae* (Filipjev), are used against fungus gnats (Cabrera et al., 2004), while the hunter fly *Coenosia attenuata* (Stein) has also been used (Ugine et al., 2010). In the current study, life history parameters and predation of the rove beetle *Atheta coriaria* (Kraatz) on the fungus gnat *Bradysia* sp. nr. *coprophila* were investigated under laboratory conditions to assess potential effectiveness of this predator in commercial greenhouses. In addition, the compatibility of *A. coriaria* with pesticides and plant growth regulators was determined to evaluate the feasibility of using *A.*

coriaria as a biological control agent with commonly used pesticides and plant growth regulators (PGR) without compromising biological control programs designed to manage fungus gnat populations.

Objectives

A more thorough understanding of the use of *A. coriaria* as a biological control agent will be useful in pest management programs designed to regulate fungus gnat populations in greenhouse production systems. As such, the overall goals of this dissertation were to determine the suitability of the rove beetle, *Atheta coriaria*, as a biological control agent against the fungus gnat, *Bradysia* sp. nr. *coprophila*, and ascertain how pest management strategies such as the application of pesticides may affect the rove beetle. Therefore, this dissertation encompasses three specific objectives:

- 1) Quantitatively assess the life history parameters of *A. coriaria* under laboratory conditions, including duration of the life stages from egg to adult, fecundity and longevity.
- 2) Evaluate predation of *A. coriaria* against the fungus gnat *Bradysia* sp. nr. *coprophila* under laboratory conditions.
- 3) Determine direct and indirect effects of pesticides and plant growth regulators on *A. coriaria*.

Despite information associated with the potential of *Atheta coriaria* as a biological control agent, there is no quantitative information available on the predation of *A. coriaria* against the fungus gnat, *Bradysia* sp. nr. *coprophila*. In order to assess potential effectiveness, however, it is important to understand the life history parameters of *A. coriaria*. Furthermore, the

compatibility of *A. coriaria* with pesticides and plant growth regulators used in greenhouse production systems has not been determined.

Objective 1

The life history parameters of *Atheta coriaria* was determined using Sunshine LC1 Mix (Sun Gro Horticulture, Inc.; Bellevue, WA) as a substrate under laboratory conditions [26°C, 50-60% RH, and 12:12 (L:D) hour photoperiod]. Duration of the life stages (egg, larva, pupa and adult), fecundity, longevity and sex ratio were estimated in Petri dishes using oatmeal (The Quaker Oats Company, Chicago, IL) as a supplemental food source (Birken and Cloyd 2007). Previous studies indicated that *A. coriaria* development time was 13 days at 27°C (Miller and Williams 1983). All experiments included 10 replications per treatment and were repeated three times. Results should provide insight on the potential use of *A. coriaria* as a predator against fungus gnats in commercial greenhouse production systems. In addition, these results may serve as a “standard” when assessing the direct and indirect effects of pesticides and plant growth regulators (objective 3).

Objective 2

The predation of *Atheta coriaria* against the fungus gnat *Bradysia* sp. nr. *coprophila* was investigated under laboratory conditions. Experiments were conducted in Petri dishes and 473 mL containers using second instar (six to seven days old) and third instar (eight to nine days old) fungus gnat larvae. In addition, the preferred fungus gnat larval instar fed upon by rove beetle adults was determined. A single rove beetle adult was presented in Petri dishes with 5, 10, 15 and 20 fungus gnat larvae to estimate potential predation rates. Prey consumption was assessed after

24 hours using starved and non-starved rove beetle adults based on the number of fungus gnat larva head capsules present in the Petri dish. Predation in containers was estimated using different predator:prey ratios, based on the number of fungus gnat adults recovered following the procedure described in Cloyd and Dickinson (2008). Rove beetle adults were collected from the main colony by sieving the growing medium and five rove beetle densities (1, 2, 3, 4 and 5 rove beetle adults), and 10, 20, 30 and 40 fungus gnat larvae were used with 10 replications per predator:prey density. In all cases, fungus gnat adult recovery rates were used to indirectly assess prey consumption. Predation using different predator:prey ratios was indirectly assessed based on the number of fungus gnat adults captured on yellow sticky cards attached to the underside of the lid of the deli squat containers. Predation was expected to increase as the number of *A. coriaria* adults increased until a level is reached in which no more prey are consumed.

Objective 3

The direct and indirect effects of designated pesticides and plant growth regulators on *Atheta coriaria* adults was assessed under laboratory conditions using standard application rates. However, in some cases, multiple application rates were also used. The experimental design was a randomized complete block design with days as blocks and treatment as the main effect with 10 replications per treatment. Direct toxic effects were determined using 20 rove beetle adults per 473 mL container. Rove beetle adults were collected from the main colony by sieving the growing medium. Adults were released both, 24 hours before and after pesticide application. Pesticides were applied as a drench to the growing medium. Recovery rates were used to assess survival based on the number of live rove beetle adults recovered from the growing medium ten days and four days after applying the pesticides and the plant growth regulators, respectively.

Based on previous studies, neonicotinoid-based pesticides are directly harmful to *A. coriaria* while the insect growth regulator azadirachtin is non-toxic to rove beetle adults (Cloyd et al., 2009a). A prediction was that *A. coriaria* adults are negatively affected, both directly and indirectly, by neonicotinoid and pyrethroid-based pesticides but not negatively affected by azadirachtin-based pesticides. Indirect effects were assessed based on changes in development time, consumption rate and reproduction. Similarly, the direct and indirect effects of commercially available plant growth regulators applied to the growing medium, on *A. coriaria* was determined based on changes in development time, consumption rate and reproduction. The information obtained from this study will determine which pesticides and PGRs are compatible with rove beetles thus allowing greenhouse producers to select materials that will not compromise biological control programs using *A. coriaria* as a biological control agent against fungus gnat populations.

Literature Review

Greenhouse production and arthropod pests

Greenhouse crops including vegetables and ornamentals represent a large portion of global plant production in terms of both market size and cultivated area. The total area covered by greenhouses during the last decade has increased from 300,000 ha to 2,400,000 ha worldwide (Pilkington et al. 2010). In the USA, the total covered area for floriculture crop production is 6,735 ha with the total value of floriculture crops estimated at \$4 billion, which represents a 100% increase within the last twenty years (USDA-NASS 2010). Bedding plants represent 34% of the wholesale value of floriculture crop sales (NASS 2011). Greenhouse plant production involves multiple horticultural (ornamental and food crop) species. Ornamentals can be divided into several categories including cut flowers (rose, chrysanthemum and transvaal daisy), potted flowering and foliage plants, and bedding plants (begonia, geranium, coleus and impatiens) (NASS 2011). Food crops grown in U.S. greenhouses cover a total area of 660 ha with a cumulative value estimated at \$550 million. Common food crops grown in greenhouses include cucumbers, lettuce, peppers, strawberries, and tomatoes (USDA-NASS 2010).

Protected crops are selected based on rapid growth and high productivity, however, these crops are highly susceptible to attack from a broad range of arthropod (insect and mite) pests (Brødsgaard and Albajes, 1999), which has led to a substantial use and reliance on pesticides (Bethke and Cloyd, 2009). Furthermore, greenhouses provide constant environmental conditions (temperature and relative humidity) throughout the year, which is conducive for pest development and reproduction (van Lenteren, 2000a). In some cases, manipulation of the

environment may induce adverse conditions that inhibit pest development and reproduction (Berlinger et al., 1999; Lindquist and Short, 2004). Moreover, this may enhance the efficacy of biological control by facilitating timing and spacing of natural enemy releases (van Lenteren, 2000a).

Due to the costs associated with crop production and pest management, protected crops are of high economic value. Furthermore, the commercial value of protected crops is associated with high aesthetic standards (Bethke and Cloyd, 2009). Studies suggest using an aesthetic injury level (AIL) to prevent arthropod pest populations from causing aesthetic damage (Raupp et al., 1988; Raupp et al., 1989). However, there are currently no tolerance levels for many insect pests and damage thresholds may be too variable for use (Dik and Albajes, 1999). For example, a leaf damage threshold of 5% was reported for populations of the twospotted spider mite *Tetranychus urticae* (Koch) in impatiens *Impatiens* spp. for different plant ages and infestation levels (Alatawi et al., 2007), whereas damage thresholds of 18 to 30 thrips/trap per week in impatiens and rose (Frey 1993), 20 thrips/trap per week in carnation (Cloyd and Sadof, 2003), as well as 10 to 26 thrips/trap per day and 20 to 50 thrips/trap per day, have been determined for western flower thrips, *Frankliniella occidentalis* (Pergande) in sweet pepper and cucumber, respectively (Shipp et al., 1998; Shipp et al., 1999).

The major arthropod pests associated with greenhouse-grown crops include aphids, fungus gnats, leafminers, mealybugs, mites, scales, shore flies, thrips and whiteflies (Osborne and Oetting, 1989, Kole and Hennekam, 1990). Many cause direct damage to crops as well as indirect damage by vectoring diseases (fungi, bacteria, and viruses), which can substantially affect marketability and salability (Bethke and Cloyd, 2009). Due to problems associated with using broad-spectrum pesticides (insecticides and miticides) to reduce arthropod pest infestations

such as pest resurgence and pesticide resistance (Croft, 1990) there has been an interest in the use of biological control agents or natural enemies (van Lenteren, 2000a, Parrella, 2008). As such, a variety of natural enemies have been successfully used in biological control programs against certain greenhouse pests including the use of parasitoids, predators, entomopathogenic microbials (fungi and bacteria) (van Driesche and Heinz, 2004), and entomopathogenic nematodes such as *Steinernema feltiae* (Filipjev) and *Heterorhabditis bacteriophora* (Poinar) (Gaugler and Kaya, 1990; Jagdale et al., 2007).

Greenhouse arthropod pests are usually polyphagous, and have a high reproductive capacity (Brødsgaard and Albajes 1999). For example, whiteflies (Hemiptera: Aleyrodidae) are of significant economic importance with species such as *Bemisia tabaci* (Gennadius) reported to feed on over 300 host plants including vegetables and bedding plants (Byrne et al., 1990), and the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) is commonly associated with greenhouse and nursery crops (Dreistadt 2001). In addition, whiteflies may vector a number of viral diseases. For example, beet pseudo yellow virus (BPYV) and tomato infectious chlorosis virus (TICV) are transmitted by *T. vaporariorum*, while cucumber yellow stunting disorder virus (CYSDV) and lettuce infectious yellows virus (LIYV) are transmitted by *B. tabaci* (Moriones and Luis-Arteaga, 1999).

Aphids (Hemiptera: Aphididae) are a diverse group of insect pests that can cause severe damage to crops, either directly by feeding on plant vascular tissues or indirectly by transmitting viruses, and producing (excreting) honeydew that may serve as a substrate for the development of black sooty mold fungi, consequently reducing the aesthetic value of crops (Dreistadt, 2001; Chau and Heinz, 2004; Pedigo and Rice, 2009). Several species have been reported feeding on a wide-range of horticultural crops including the green peach aphid *Myzus persicae* (Sulzer) and

the potato aphid *Macrosiphum euphorbiae* (Thomas), while the melon aphid *Aphis gossypii* (Glover) feeds on cucurbits including melon, watermelon and cucumber (van Emden and Harrington, 2007). Examples of viruses transmitted by aphids are cucumber mosaic virus (CMV), lettuce mosaic virus (LMV), and zucchini yellow mosaic virus (ZYMV) (Moriones and Luis-Arteaga, 1999). Thrips (Thysanoptera: Thripidae) are major pests of chrysanthemum, transvaal daisy, sweet pepper, tomato, cucumber and many different types of bedding plants. Similar to aphids, thrips including *Thrips tabaci* (Lindeman) and *Frankliniella occidentalis* have piercing-sucking mouthparts that allow them to not only feed directly on plant fluids but also vector plant viruses such as impatiens necrotic spot virus and tomato spotted wilt virus (Lewis, 1997; Zhang, 2007). Losses caused by *F. occidentalis* and *T. palmi* in sweet peppers in Florida were estimated to be \$10 million in 1993 (Nuessly and Nagata, 1995). In addition, mites including the twospotted spider mite, *T. urticae*; broad mite, *Polyphagotarsonemus latus* (Banks); cyclamen mite, *Phytonemus pallidus* (Banks); and several species of eriophyoid mites including the tomato russet mite, *Aculops lycopersici* (Masse) are important pests of greenhouse-grown crops (Zhang, 2003). Another important insect pest is fungus gnats (*Bradysia* spp.), with *Bradysia impatiens* (Johannsen) and *B. coprophila* (Lintner) the most common species encountered in greenhouses and nurseries (Gillespie, 1986; Gillespie et al., 2001; Cloyd, 2008).

Fungus gnats and their importance in greenhouse production systems

Sciarid flies (Diptera: Sciaridae) are phytophagous insect pests that may cause damage to many greenhouse-grown crops by feeding on plant roots (Hungerford, 1916; Steffan, 1966; Graham and McNeill, 1972; Dennis, 1978). Of the approximately 150 sciarid fly species reported

in North America, 65 are in the genus *Bradysia* including *B. impatiens* and *B. coprophila* (Steffan, 1966). A thorough listing of fungus gnat species and associated plant hosts is provided by Harris et al. (1996). Fungus gnats (*Bradysia* spp.) were not initially considered major pests of greenhouses and nurseries until larvae were reported to be feeding on the roots of greenhouse crops such as peas, lettuce, cucumbers as well as carnations and other flowering plants (Hungerford, 1916; Ellisor, 1934). Studies have demonstrated fungus gnats damaging field crops including soybean (Wilkinson and Daugherty, 1970a), corn, wheat, and legume seedlings (Springer, 1995). In addition, fungus gnats have been reported to be major pests of cultivated mushrooms (Thomas, 1931; Davis, 1941) and forest nurseries associated with pine seedlings (Keates et al., 1989; Hurley et al., 2007). Fungus gnats can cause substantial economic losses in greenhouse production systems, with *B. impatiens* the major species that feeds on vegetable transplants as well as ornamental crops including chrysanthemum, cyclamen, geranium, impatiens, poinsettia, and bedding plants (Jagdale et al., 2004).

The fungus gnat life cycle consists of an egg, four larval instars, pupa and adult with the duration of the life cycle varying depending on the species, genetic variability, food source, and temperature (Harris et al., 1996; Wilkinson and Daugherty, 1970a; Kennedy, 1974). Hungerford (1916) reported that the entire life cycle may be completed in 24 to 32 days. In a separate study, the duration of the larval instars was found to be two to four days for the first instar, two to three days for the second instar, one to three days for the third instar, and three to ten days for the fourth instar. In addition, the duration of the pupal stage was three to five days whereas adult longevity was four to ten days (Steffan, 1966). Development from egg to adult of *B. impatiens* was determined by Wilkinson and Daugherty (1970 a,b) at different temperatures. They found that the total development time was 48, 21 and 20 days at 13, 24 and 32°C, while adult longevity

was 9.5, 6 and 1.5 days, respectively. However, Kennedy (1973, 1974) reported a development time of 15.6 days at 25°C for *B. impatiens* reared on Brewers' yeast, while Steffan (1974) reported a development time of 16.3 days at 20°C. Additionally, a development time of 16 to 20 days at 20°C has been reported (Gillespie et al., 2001). Development was completed in 13 to 15 days at 24°C for *Corynoptera* sp. (Gillespie, 1986) and 19 days at 25°C for *Bradysia tritici* (= *Neosciara ocellaris*) (Coquillett) (Ellisor, 1934). The life cycle of *B. coprophila* was completed in 18 to 23 days at 17 to 21°C (Thomas, 1931) and 11 to 25 days at 25°C (Zanuncio et al., 1996). Apparently, duration of the larval stages is more variable than either the egg or pupa. Several studies indicate that adult females can lay between 100 to 200 eggs (Gardiner et al., 1990; Cloyd, 2008). However, Steffan (1966) reported that individual females lay from 30 to 120 eggs. Kennedy (1974) found that *B. impatiens* female fecundity was 129 and 156 eggs at 20 and 25°C whereas Wilkinson and Daugherty (1970b) reported only 75 eggs per female regardless of temperature.

Although fungus gnat larvae primarily develop on fungi, they may also feed on plant tissue as an alternative food source (Kennedy, 1974; Mead, 1978). As such, direct damage is caused by larval feeding on plant roots, especially those of young plants and seedlings, while adults are primarily a visual nuisance at high densities (Hamlen and Mead, 1979). Larval feeding inhibits the uptake of water and nutrients of infested plants. Furthermore, larval feeding may weaken plants, consequently increasing their susceptibility to infection by plant pathogens. In addition, feeding wounds predispose plants to fungal infection and facilitate the entry of soilborne fungal plant pathogens such as *Fusarium* spp. and *Pythium* spp. (Leath and Newton, 1969; Gardiner et al., 1990). Larvae may also cause indirect damage by disseminating plant pathogenic fungi such as *Pythium*, *Fusarium*, *Phoma* and *Verticillium* (Kalb and Millar, 1986;

Keates et al., 1989; Jarvis et al., 1993; Gillespie and Menzies, 1993) while adults may be involved in aerial dispersal of certain fungal spores such as *Botrytis* (Gillespie and Menzies, 1993; El-Hamalawi and Stanghellini, 2005). However, it has been suggested that larval feeding may inhibit *Pythium* infection of geranium *Pelargonium* spp. seedlings and induce resistance by activating plant defense mechanisms possibly associated with the jasmonate pathway (Braun et al., 2009). Ingestion and excretion of viable spores of *Pythium* and *Thielaviopsis* by the larva have been reported (Gardiner et. al., 1990; El-Hamalawi, 2008); however, aspects of the mechanism involved in disease transmission are still unclear.

Fungus gnat larvae have been shown to negatively affect certain plant pathogenic fungi including *Sclerotinia sclerotiorum* (Lib.) de Bary. For example, Anas and Reeleder (1988a) found that *Bradysia coprophila* (Lintner) larval feeding inhibits infection of *S. sclerotiorum* by degrading sclerotia, and that damage to sclerotia increases with higher content of organic matter in the soil (Anas and Reeleder, 1988b). Furthermore, when exposed to fungus gnat larvae, *S. sclerotiorum* spores are more susceptible to attack by the fungal antagonist, *Trichoderma viride* (Pers.) (Anas and Reeleder, 1988b; Gracia-Garza et al., 1997). It has also been demonstrated that *T. viridae* is not a suitable food source for fungus gnat larvae and the strain *T. harzianum* T-22 does not attract fungus gnat adults to the growing medium, which may allow the use of these fungal antagonists as biological control agents against certain soilborne plant pathogens (Anas and Reeleder, 1988a; Cloyd et al., 2007a).

In addition to food source, fungus gnat survival and development may be influenced by the growing medium. For example, fungus gnats can survive and develop in a variety of growing media; however, this primarily depends on the growing medium type and moisture content. In addition, growing media vary in their attractiveness to fungus gnats (Cloyd et al., 2007b).

Lindquist et al. (1985) suggested that attractiveness may be associated with microbial activity, with less attractive growing media associated with increased plant injury. Survival and feeding behavior may be affiliated with water retention of the growing medium as well as by the particular combination of growing medium constituents (Ellisor, 1934; Lindquist et al., 1985). Taylor et al. (2000), for example, found that a mixture of coir and vermiculite, with 15% perlite may prevent fungus gnat population growth. In addition, Olson et al. (2002) determined that a growing medium moisture content of 52% was suitable for fungus gnat larval survival. Similarly, Cloyd and Dickinson (2008) discovered the highest adult recovery rates at percent moisture contents between 56% and 71%, which involved the application of 50 to 75 mL of water to 300 mL of a soilless growing medium. Growing medium moisture content is important for fungus gnat development and survival; however, there is minimal effect on growing medium attractiveness, which may be associated with particular growing medium volatile constituents. For example, Meers and Cloyd (2005a) reported that Metro-Mix 900 Professional Growing Mix (Sun Gro Horticulture, Inc.; Bellevue, WA), which contains composted bark was more attractive to adult fungus gnats than Sunshine LC1 Mix (Sun Gro Horticulture, Inc.; Bellevue, WA). This suggests that fungus gnats may be more attracted to composted bark. Similar results were reported between fresh growing media and those moistened after pasteurization, by heating growing media in a microwave. As such, organisms present in growing media may be eliminated via pasteurization without compromising attractiveness to fungus gnat adults (Cloyd and Zaborski, 2004; Meers and Cloyd, 2005a; Cloyd et al., 2007a). This is important because fungus gnats and other arthropods including thrips and mites may be present in commercial bagged soilless growing media, which may be a source of infestation in greenhouses (Cloyd and Zaborski, 2004). Composted pine bark mixes are known to suppress soil-borne plant pathogens

such as *Fusarium*, *Pythium*, and *Phytophthora*; however, these mixes are associated with high fungus gnat populations compared to sphagnum peat moss mixes (Lindquist et al., 1992). In addition, it has been shown that fungus gnats will infest and develop in coir-based as well as peat-based growing media (Evans et al., 1998). Also, it appears that females do not exhibit preferences among growing media to lay eggs. Meers and Cloyd (2005a) suggested that females may rely on other factors such as moisture content in deciding where to lay eggs. Several rearing methods for fungus gnats have been reported (Kennedy, 1973), and a laboratory colony of *Bradysia* sp. nr. *coprophila* has been maintained using pasteurized soilless growing medium, potato, and oatmeal as a supplemental food source (Cabrera et al., 2005a).

Fungus gnat assessments under laboratory conditions are important in determining the effect of pesticides on fungus gnat survival as well as the efficacy of biological control agents against fungus gnats. Several sampling techniques have been developed to quantitatively assess the recovery of fungus gnat eggs, larvae and adults from growing media. For example, Meers and Cloyd (2005b) developed a flotation method designed to recover fungus gnat eggs from growing media. Similarly, techniques to recover fungus gnat larvae and adults have been reported (Zaborski and Cloyd, 2004; Cloyd and Dickinson, 2008). Using second and third instar larvae and SB300 Universal Growing Mix growing medium, the highest number of adults recovered was 16 to 17 adults out of 20 after applying 60 to 65 mL of water to the growing medium (Cloyd and Dickinson, 2008).

Fungus gnat management in greenhouses primarily involves the use of pesticides (Harris et al., 1996; Hamlen and Mead, 1979). Broad-spectrum pesticides (organophosphates and carbamates) were used during the 1970's and 1980's to reduce fungus gnat populations (Lindquist, 1977, 1981; Lindquist et al., 1985). For example, Lindquist et al. (1985) reported that

oxamyl and aldicarb provided better control than bendiocarb, diazinon and the insect growth regulator (IGR) methoprene. Furthermore, acephate, aldicarb, diazinon and oxamyl were effective against larvae seven days after application, whereas the IGRs kinoprene and methoprene provided mortality 21 days after application (Hamlen and Mead, 1979). However, the insect growth regulators, cyromazine and diflubenzuron provided control of fungus gnat larvae seven days after treatment (Parrella, 1998). Since many of the older broad-spectrum pesticides have been discontinued or registrations have elapsed, insecticides currently used against fungus gnats include pyrethroids, neonicotinoids, pyrrole, and insect growth regulators. In addition, botanical pesticides such as azadirachtin, plant-derived essential oils obtained from *Chenopodium ambrosioides* (L.), and the microbial pesticide *Bacillus thuringiensis* spp. *israelensis* (Bti) have demonstrated to be effective against fungus gnat larvae (Osborne et al., 1985, Cloyd and Dickinson, 2006a, Cloyd and Chiasson, 2007). Osborne et al. (1985) reported that Bti was toxic to *B. coprophila* larvae with an 8% survival rate; however, second and third instar fungus gnat larvae were found to be less affected by Bti (Cloyd and Dickinson, 2006a). In the first study (Osborne et al., 1985), larval mortality was evaluated after direct exposure to the pesticide, whereas in the second study (Cloyd and Dickinson, 2006a), larval mortality was determined after application of the pesticide as a drench to the growing medium. As such, differences in the experimental procedures as well as application rates may account for the discrepancies between these two studies. The effectiveness of microbial pesticides against fungus gnats may be evaluated using a short-term toxicity test as described by Taylor et al. (2007). In brief, this involves exposing second instar fungus gnat larvae to the pesticide solution using potato wedges as a substrate.

Monitoring for fungus gnats is important in timing the application of pesticides or biological control agents. However, no damage thresholds for either adults or larval populations have been established (Cloyd, 2008). Monitoring for adults involves using yellow sticky cards while potato disks left on the surface of soilless growing media for 48 hours are used to monitor for the presence of larvae (Cabrera et al., 2003). Studies have demonstrated the potential use of fungus gnat pheromones for trapping or mating disruption (Frank and Dettner, 2008). In addition, cultural practices including water management and sanitation are important in avoiding fungus gnat infestations. Yellow sticky traps attached to the underside of refuse containers in greenhouses captured variable numbers of fungus gnat adults indicating the importance of removing debris from inside greenhouses (Hogendorp and Cloyd, 2006). Furthermore, the abundance of moisture and organic material in nurseries is associated with the presence of fungus gnats. Plastic-covered nurseries with soil floors were conducive to the accumulation of algae leading to the establishment of high fungus gnat populations (Keates et al., 1989). Another fungus gnat management practice is the use of biological control agents with enthomopathogenic nematodes and predatory mites the most commonly used for regulating fungus gnat populations.

Biological control in greenhouses

Biological control may provide a safe and cost-effective means of managing arthropod pests (van Lenteren 1993; van Lenteren, 2007; Bale et al., 2008). Biological control has increased in acceptance by the greenhouse industry as more greenhouse area has been devoted to biological control compared to the last forty years (van Lenteren, 2007), with approximately 32,000 ha to 2.4 million ha globally under some type of biological control program (van Driesche and Heinz, 2004; Parrella, 2008). Greenhouses are a relatively isolated environment,

which may prevent immigration of arthropod pests (van Lenteren, 2000a) and minimize emigration of biological control agents.

The first biological control agent used in greenhouses was the parasitoid, *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae) against the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) in 1926 (Speyer, 1927; Bale et al., 2008). Since the early 1960's, biological control has increased in use in greenhouses within the USA. In fact, in some instances biological control has become the standard management practice in greenhouse production systems (van Lenteren, 2007).

There are three distinct strategies associated with biological control including classical, augmentation, and conservation (Gurr et al., 2000; DeBach and Rosen, 1991; Pedigo and Rice, 2009). Augmentation and seasonal inoculative releases of biological control agents is the most commonly used method in greenhouses (van Lenteren, 2000b). Many natural enemies including the whitefly parasitoid *E. formosa* and the predatory mites *Phytoseiulus persimilis* (Athias-Henriot) and *Neoseiulus cucumeris* (Oudemans) have been incorporated into pest management programs in a variety of vegetable and ornamental crop production systems (Pilkington et al., 2010). The success of using biological control agents including parasitoids, predators and microbials to regulate the major arthropod greenhouse pests has been extensively documented (Parrella et al., 1999; van Driesche and Heinz, 2004). However, effectiveness of natural enemies may be influenced by factors such as release strategy and the selection of appropriate biological control agents (Yano, 2006).

In greenhouses, where multiple crops are produced simultaneously, the use of natural enemies may be difficult because of the different production requirements and crop schedules. For example, the predatory mite *Amblyseius swirskii* (Athias-Henriot) is an effective predator

against whiteflies and thrips; however, usually only in combination with other natural enemies (Messelink et al., 2008; Chow et al., 2010; Calvo et al., 2011). In some cases, combining biological control agents represents an important component of pest management in tomato production systems in Europe (van Lenteren, 2007). In addition, Paterno et al. (2004) suggested the use of the minute pirate bug, *Orius insidiosus* (Say) for thrips management in chrysanthemum. Parasitoids, including *Eretmocerus mundus* (Mercet), *E. eremicus* (Rose and Zolnerowich), and *Encarsia* spp. are commercially available for use against certain whitefly species (van Lenteren and Martin, 1999), while *Aphidius colemani* (Viereck), *Lysiphlebus testaceipes* (Cresson), and predators such as *O. insidiosus* and the green lacewing *Chrysoperla carnea* (Stephens) are examples of natural enemies that may be used against certain aphids (Chau and Heinz, 2004). The simultaneous use of the parasitoid *Aphelinus asychis* (Walker) and the coccinellid beetle, *Harmonia axyridis* (Pallas) against the potato aphid *Macrosiphum euphorbiae* (Thomas) in greenhouse-grown roses was successfully demonstrated (Snyder et al., 2004). Biological control of other major insect pests such as mealybugs involves using the parasitoid *Leptomastix dactylopii* (Howard) and the coccinellid *Cryptolaemus montrouzieri* (Mulsant) (Parrella et al., 1999), while *Aphytis* spp. and *Metaphycus* spp. are parasitoids of certain scales (Dreistadt, 2001).

Biological control of fungus gnats is based on using the predatory mites *Stratiolaelaps scimitus* (Womersley) (Cabrera et al., 2005a; Cabrera et al., 2005b), and *Hypoaspis aculeifer* (G. Canestrini), as well as the entomopathogenic nematode *Steinernema feltiae* (Filipjev) (Chambers et al., 1993; Harris et al., 1995; Oetting and Braman, 2004; Jandricic et al., 2006; Warner and Getz, 2008). Effective regulation (80% mortality) using *H. aculeifer* involved releasing 125 individuals per cucumber plant (Gillespie and Quiring, 1990), whereas efficacy rates of 90, 95

and 100% were obtained after releasing 5, 10 and 20 individuals of *H. aculeifer*, respectively, in poinsettia seedlings (Jeon et al., 2007). Furthermore, releasing 665 to 1330 *S. scimitus* per container reduced populations of *Bradysia matogrossensis* (Lane) in mushroom production systems (Prado et al., 2007, Castilho et al., 2009). In addition, application rates of 1.25 and 2.5 x 10⁵ infective juveniles/m² of *S. feltiae* have been determined to reduce populations of *B. impatiens* and *B. difformis* in New Guinea impatiens (*Impatiens hawkeri* W. Bull) and poinsettia (Jagdale et al., 2004; Jagdale et al., 2007). Moreover, *S. feltiae* has been shown to significantly reduce fungus gnats populations, more so, compared to *Bacillus thuringiensis* spp. *israelensis* and the insect growth regulator, kinoprene (Harris et al., 1995). Studies have demonstrated that the parasitoid *Synacra* sp. naturally regulates sciarid fly populations in greenhouses in Sweden (Hellqvist, 1994). In fact, the potential commercial use of this parasitoid has been investigated using *Bradysia paupera* (Tuomikoski) as a host, which may complement the activity of entomopathogenic nematodes in regulating fungus gnat populations in cucumbers and tomatoes (Hellqvist, 1994). Also, the hunter fly, *Coenosia attenuata* Stein has been reported to be a potential predator of fungus gnats (Tellez et al., 2009; Ugine et al., 2010). Additional biological control agents have been evaluated against fungus gnats such as the rove beetle *Atheta coriaria* (Kraatz), which has been promoted as a candidate to regulate fungus gnat populations (Carney et al., 2002, Jandricic et al., 2005a, Jandricic et al., 2005b, Jandricic et al., 2006).

The rove beetle, Atheta coriaria, as a biological control agent

The rove beetle *Atheta coriaria* (Coleoptera: Staphylinidae) is a soil-dwelling polyphagous predator that feeds on a number of greenhouse pests such as shore flies (*Scatella* spp.), fungus gnats (*Bradysia* spp.) and thrips (Gillespie et al., 2001; Carney et al., 2002;

Jandricic et al., 2006; Cox et al., 2006). In addition, *A. coriaria* has been reported to feed on sciarid flies in cultivated mushroom production (Lewandowski et al., 1999; Gorski, 2006). A list of known prey species is presented in Meihls and Hibbard (2009).

There is some controversy regarding the taxonomy of *A. coriaria*. Muona (1979) indicated that *Taxicera academica* (Sawada) is synonymous with *A. coriaria*. Later, however, Gusarov (2003) revised the type species of the genus *Dalotia* and concluded that *Dalotia*, instead of *Atheta*, is the valid genus name. However, there is a consensus in the scientific literature and among commercial suppliers in using *Atheta coriaria* (Kraatz) instead of *Dalotia coriaria* (Birken and Cloyd, 2007; Cloyd et al., 2009a).

Basic life history information on *A. coriaria* and its role as a biological control agent in greenhouse production systems has been reported (Miller and Williams, 1983; Carney et al., 2002; Helyer et al., 2003). Although there is minimal scientific literature available, most studies have focused on the use of *A. coriaria* as a biological control agent based on feeding behavior and compatibility with pesticides (Birken and Cloyd, 2007; Jandricic et al., 2005b; Jandricic et al., 2006; Cloyd et al., 2009b). In addition, it has been reported that *A. coriaria* may serve as a bioindicator and model insect to determine potential direct and indirect toxic effects of transgenic *Bt*-maize on soil predators by evaluating mortality and life history parameters of *A. coriaria* adults that were allowed to feed on prey exposed to the *Bt*-toxin Cry1Ab (Büchs et al., 2007; Porcar et al., 2010; Garcia et al., 2010).

The life cycle of *A. coriaria* consists of an egg, three larval instars, and adult (Miller and Williams, 1983; Ashe and Watrous, 1984). Egg hatch occurs after an incubation period of 2.3 days at 27°C, 90% relative humidity (RH) and a photoperiod of 16:8 hours (L:D) (Miller and Williams, 1983). The duration of the larval and pupal stages is 4.8 and 5.5 days after feeding on

the eggs of *Carpophilus hemipterus* (Linnaeus) (Miller and Williams, 1983). In addition, the estimated development time from egg to adult is 13 days at 27°C. Furthermore, there is a negative relationship between duration of larva and pupa, and food availability (eggs of *C. hemipterus*). Helyer et al. (2003) demonstrated that *A. coriaria* development time is 21 to 22 days at 25°C and 11 to 12 days at 32°C. It also appears that cannibalism may occur during the larval stage, and that the sex ratio (female:male) is 1:1 (Helyer et al., 2003). In a separate study, development time was approximately 3 weeks at 25°C; population increase was 15.6 rove beetles per female in the first generation and adult longevity was approximately 21 days (Carney et al., 2002). Studies have been conducted to assess other rove beetle species as biological control agents. For example, the life history of *Aleochara bilineata* (Gyllenhal), a predator of the cabbage root fly *Delia radicum* (L.), has been documented (Read, 1962; Colhoun, 1953; Whistlecraft et al., 1985). The duration of the larva and pupa was 20.5 and 14 days at 24°C. Adults emerged within 20 to 30 days when the soil temperature was 22 to 23°C, whereas soil temperatures less than 10°C inhibited development. Average fecundity has been shown to be 400 eggs per female, with 6 eggs laid per female per day (Hertveldt et al., 1984; Whistlecraft et al., 1985).

In order to conduct life history studies, it is important to maintain laboratory-reared colonies. *Atheta coriaria* can survive in a variety of growing media including peat-based mixes, coconut fiber and rockwool (Carney et al., 2002; Helyer et al., 2003). Although *A. coriaria* primarily feeds on fungus gnat larvae, a laboratory-reared colony can be successfully maintained using moist SB300 Universal Professional Growing Mix growing medium (Sun Gro Horticulture, Inc.; Bellevue, WA) containing 50% bark, Canadian sphagnum peat moss, perlite, dolomitic limestone, gypsum and a wetting agent supplemented with raw oatmeal (The Quaker

Oats Company, Chicago, IL) (Birken and Cloyd, 2007; Cloyd et al., 2009b). Furthermore, results from laboratory choice tests indicate that *A. coriaria* adults and larvae prefer to feed on second instar larvae of the fungus gnat, *Bradysia* sp. nr. *coprophila* (Lintner) (Sciaridae: Diptera) more so than oatmeal, although oatmeal may serve as a supplemental food source without influencing predator effectiveness (Birken and Cloyd, 2007). However, rearing procedures as well as the effect of rearing conditions including growing medium type and moisture content, food quality, and population density on the biology and reproduction have not been thoroughly demonstrated. In addition, determining an appropriate colony size may be important in the commercial production of *A. coriaria* as this may be influenced by factors including cannibalism and intraspecific competition (Safranyik and Linton, 1985).

Atheta coriaria is commercially available and is primarily used against greenhouse insect pests including fungus gnats (Warner and Getz, 2008). Overall effectiveness of *A. coriaria* against different prey species has been evaluated under both laboratory and greenhouse conditions. The number of second instar fungus gnat larvae consumed by first, second and third instar rove beetle larva, and the adult is 21, 30, 10 and 46, respectively (Carney et al., 2002). In addition, Jandricic et al. (2006) determined that more fungus gnat first instar larvae (4.2) than shore fly larvae (2.0) were consumed by one rove beetle adult in 24 hours. In a separate study, however, no effective control was reported against fungus gnat larvae when using five rove beetle adults and 20 fungus gnat larvae (Cloyd and Chiasson, 2007). Gillespie et al., (2001) found that a single *A. coriaria* adult consumes over 120 fungus gnat eggs in 24 hours, while over 80 thrips pupae may be consumed over a similar time period. Predation on sciarid flies using five and ten rove beetle adults per container reduces populations 75 and 85% in potted parsley while in cultivated mushroom, population decreases are approximately 77% when using 12 rove beetle

adults per container (Gorski, 2006; Bennison et al., 2008). *Atheta coriaria* may also reduce the number of eggs and first instar larvae of the European pepper moth, *Duponchelia fovealis* (Zeller), which damages ornamental plants including cyclamen and begonia, by 87 and 64% (Messelink and van Wensveen, 2003). Predation on western flower thrips, *F. occidentalis* (all life stages) in impatiens caused a population decrease of 82% (Bennison et al., 2008) whereas predation on first instar larvae of the western corn rootworm *Diabrotica virgifera virgifera* (LeConte) using five and ten rove beetle adults was 100% after four hours (Meihls and Hibbard, 2009). The potential use of *A. coriaria* against the cabbage root fly *D. radicum* has been reported (Bennison et al., 2011a; Bennison et al., 2011b). Studies suggest that *A. coriaria* mimics a Type I functional response (Miller and Williams, 1982). Based on these studies, 7, 12, 21 and 13 eggs of *Stelidota germinata* (Say) were consumed (on average) after 24 hours by first, second, and third larval instar, and adult *A. coriaria*, respectively. In addition, prey consumption was temperature-dependent, with more eggs consumed as temperature increased (Miller and Williams, 1982).

Several biological control agents are commercially available for use against fungus gnats including the entomopathogenic nematode, *Steinernema feltiae* and the predatory mites *S. scimitus* and *H. aculeifer* (Oetting and Braman, 2004). Studies have determined the potential of using *A. coriaria* in combination with *S. feltiae* under controlled conditions; however, compatibility with predatory mites is less likely because *H. aculeifer* reportedly feeds on *A. coriaria* larvae, whereas rove beetle adults feed on *H. aculeifer* eggs (Jandricic et al., 2006). Recently, the advent of reduced-risk pesticides or biopesticides with more selective activity has provided an opportunity to integrate pesticides with natural enemies in greenhouse production systems (Weinzierl, 2009).

Compatibility of natural enemies with pesticides

The negative effects associated with broad-spectrum pesticides (insecticides and acaricides) such as resistance, pest resurgence and secondary pest outbreaks, has elicited potential use of alternatives including natural products and biological control (van Lenteren, 2007). As such, the use of biological control has increased in greenhouse production systems, which has led to an overall decline in the use of pesticides in protected environments (Parrella, 2008; Pilkington et al., 2010). This may be due to health risks and environmental considerations associated with pesticides (Bennison, 2004). In the USA, many biological control agents have been successfully incorporated into integrated pest management (IPM) programs and are currently used in many greenhouse production systems. For example, the adoption of biological control agents for whitefly management in poinsettia has increased dramatically (van Driesche and Lyon, 2003). However, despite this, pesticide use still remains an important component of greenhouse pest management (Bethke and Cloyd, 2009; Gradish et al., 2011). The development of novel compounds has led to the use of reduced-risk pesticides or biopesticides, which are more selective and therefore less harmful to the environment and potentially natural enemies. Examples include insect growth regulators, insecticidal soaps, horticultural oils, antifeedants, selective-feeding blockers, plant-derived essential oils, and microbial-based insecticides (the entomopathogenic bacterium, *Bacillus thuringiensis* and fungus, *Beauveria bassiana*) (Cloyd, 2005; Cloyd, 2006; Parrella, 2008). Although low to moderately toxic, these pesticides may directly or indirectly negatively affect natural enemy populations. Pesticide effects include direct mortality via exposure of natural enemies to pesticides or pesticide residues and indirect effects such as reduced reproduction, prey consumption, longevity, egg viability, delayed development, change in sex ratio or fitness (Moriarty, 1969; Greathead, 1995; Blümel et al., 1999). In addition,

natural enemy populations may be indirectly affected through host elimination (van Driesche and Bellows, 1996). Croft (1990) suggested using a life table analysis to assess any indirect effects on fecundity, longevity, development rate, and sex ratio. Martinez et al. (2005) used a life table study to evaluate the indirect effects of azadirachtin on the twospotted spider mite, *T. urticae*. In addition, the reproductive capacity, measured as the number of individuals (offspring) in the next generation, has been proposed as a means to evaluate the indirect effects of pesticides on soil-dwelling predators (Grimm et al., 2000). In fact, reduced fecundity is the most common indirect effect observed in regards to pesticides and natural enemies (Theiling and Croft, 1989). However, Burn (1989) indicated that indirect effects on reproductive capacity may only become apparent in the long term.

Susceptibility to pesticides may be influenced by the development life stage. Based on different studies and a scale from 1 (most tolerant) to 5 (most susceptible), Croft (1990) indicated that relative toxicity of the egg, larva, pupa and adult stage in coccinellids is 1.3, 2.3, 1.5 and 3.3, respectively, which suggests that tolerance of a particular life stage may be associated with physiological, behavioral and chemical factors. In addition, Bartlett (1964) concluded that *Chrysoperla carnea* adults are, in general, more susceptible to pesticides than larvae.

The direct and indirect effects of conventional pesticides including those in the chemical classes organophosphate, carbamate, and pyrethroid on insect pests and their natural enemies have been extensively documented (Moriarty, 1969; Croft and Brown, 1975; Elzen, 1989; Croft, 1990). Broad-spectrum pesticides such as organophosphates (malathion, parathion, dimethoate, and diazinon) and carbamates (carbaryl, pirimicarb, methomyl, and carbofuran) are harmful to most natural enemies (Elzen, 1989; Croft, 1990). Furthermore, several pyrethroid-based insecticides including lambda-cyhalothrin and cyfluthrin, which are commonly used in

greenhouses, are relatively toxic to natural enemies (Croft and Whalon, 1982; Elzen, 1989; Prabhaker et al., 2007). Studies have demonstrated that both pyrethroids are directly harmful to *O. insidiosus* adults whereas lambda-cyhalothrin is highly toxic to *Bracon mellitor* (Say), *Cardiochiles nigriceps* (Viereck), *Coleomegilla maculata* (De Geer), *Geocoris punctipes* (Say) adults but less toxic to *Cotesia marginiventris* (Cresson) and *Hippodamia convergens* (Guérin-Méneville) (Studebaker and Kring, 2003; Tillman and Mulrooney, 2000; Al-Deeb et al., 2001). The pirate bug, *O. insidiosus* and the big-eyed bug *G. punctipes* are both egg predators of the tomato fruitworm, *Helicoverpa zea* (Boddie) in cotton. There is low to moderate mortality associated with cyfluthrin on the males and females of *O. insidiosus*, while for *G. punctipes* male and female mortality is moderate (44%) to low (22%), respectively. In addition, cyfluthrin indirectly affects *O. insidiosus* by reducing fecundity (7%) and egg consumption (13%), while no effects on *G. punctipes* egg consumption have been reported (Elzen 2001).

Insect growth regulators (IGRs) are a group of compounds that disrupt insect development, and are used against a variety of greenhouse insect pests including whiteflies, aphids, fungus gnats and mealybugs (Parrella and Murphy, 1998; Rothwangl et al., 2004; Cloyd and Dickinson, 2006b). Although IGRs are generally considered non-toxic to natural enemies, some negative effects have been reported (Blümel et al., 1999). The IGRs pyriproxyfen and kinoprene are juvenile hormone mimics, whereas azadirachtin and tebufenozide interfere with ecdysone metabolism and the molting process (Medina et al., 2003a; Cloyd, 2006; Yu, 2008). Pyriproxyfen and kinoprene, for example, have been shown to be compatible with the whitefly parasitoid *Encarsia luteola* (Howard) (Parrella, 2002). Furthermore, kinoprene was demonstrated to negatively affect survival and parasitism of *Leptomastix dactylopii* (Howard), whereas both azadirachtin and pyriproxyfen were not directly harmful to the parasitoid (Rothwangl et al.,

2004). Azadirachtin, is extracted from the seeds of the neem tree, *Azadirachta indica* (A. Juss.), is a known feeding deterrent and repellent, and may also reduce overall insect reproduction (Mordue and Blackwell, 1993; Williams and Mansingh, 1996). In fact, azadirachtin comprises a mixture of several biologically active materials (EFSA, 2011). Although azadirachtin is generally not harmful to natural enemies, negative effects on certain natural enemy species have been reported under laboratory conditions (Lowery and Isman, 1995; Tedeschi et al., 2001; Saber et al., 2004). For example, the green lacewing *C. carnea* is compatible with tebufenozide and pyriproxyfen even at high application rates, but not compatible with azadirachtin and diflubenzuron (Medina et al., 2003a). Similarly, tebufenozide and pyriproxyfen were non-toxic to eggs; however, azadirachtin negatively affected *C. carnea* female oviposition. Based on laboratory studies, pyriproxyfen is non-toxic to the protonymphs of the predatory mite *S. scimitus*, which is used against fungus gnats. However, an increase in the duration of this life stage has been reported (Cabrera et al., 2004). In a separate study, pyriproxyfen did not negatively affect the parasitoid *L. dactylopii* and the mealybug destroyer, *C. montrouzieri*, which are both natural enemies of the citrus mealybug, *Planococcus citri* (Risso) (Cloyd and Dickinson, 2006b).

Tebufenozide displays minimal direct toxic effects on *O. insidiosus* adults while increasing prey consumption on the eggs of *H. zea*. Similarly, no negative direct or indirect effects on *G. punctipes* adults and the predatory mites *Typhlodromus pyri* (Scheuten) and *T. occidentalis* (Nesbitt) have been reported (Valentine et al., 1996; Elzen, 2001). Pyriproxyfen is also non-toxic to *Orius* sp. when used against *Thrips palmi* (Karny) (Nagai, 1990). In addition, there were no negative direct or indirect effects on the parasitoid *Eretmocerus mundus* (Mercet) and the predatory mite *Amblyseius swirskii* (Athias-Henriot) when pyriproxyfen was applied

against the sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Calvo et al., 2009). Studies determining the effects of diflubenzuron and pyriproxyfen on the predatory bug, *Podisus maculivestris* (Say) indicated high direct mortality on fifth-instar nymphs via ingestion of 1 µg of active ingredient with water; however, no indirect effects were reported (De Clercq et al., 1995). The IGR novaluron was found to be non-toxic to the fungus gnat predatory mite *Stratiolaelaps scimitus* (Cabrera et al., 2005b). The chitin synthesis inhibitor, cyromazine was reported to be compatible with the parasitoid *Chrysocharis parksi* (Crawford) when used against the American serpentine leafminer, *Liriomyza trifolii* (Burgess) (Parrella et al., 1983).

Tedeschi et al. (2001) found direct mortality and reduced female fecundity associated with the mirid predator *Macrolophus caliginosus* (Wagner) when exposed to three azadirachtin-based products. However, all three products (Neem-Amin, EC Biochim, Italy; Stardoor, Intrachem Bio Italia, Italy; and B.P. 20/S ARVI, Italy) displayed short residual toxicity, which suggests the natural enemy can be released just after pesticide application. In general, IGRs are toxic to some coccinellid species. For example, buprofezin is harmful to the larvae of *Harmonia axyridis* (Pallas) and *Stethorus punctum picipes* (Casey). However, buprofezin is non-toxic to *C. montrouzieri* adults (Smith and Papacek, 1990). In addition, buprofezin displays high nymphal mortality to the non-coccinellid predators *G. punctipes* and *G. pallens* (James, 2004).

Plant-derived essential oils and microbial insecticides are commercially available and used in greenhouses with variable toxic effects on arthropod pests (Cloyd et al., 2009b); however, they may also exhibit direct and indirect toxic effects on natural enemies. For example, peppermint and rosemary oil are directly harmful to adult *Amblyseius swirskii* females, while rosemary and sweet marjoram oil negatively affect the predacious mites *A. barkeri* and *A. zaheri* (Momen and Amer, 1999; Amer and Momen, 2002). Indirect effects, including reduced

fecundity and food consumption, have also been reported. Miresmailli and Isman (2006) suggested using rosemary oil in combination with the predatory mite *Phytoseiulus persimilis* to regulate populations of the twospotted spider mite, *T. urticae*. This study reported a significant decrease associated with the toxic effects of rosemary oil 24 hours after application. In addition, soybean and rosemary oil were directly harmful to the citrus mealybug *P. citri* (Cloyd et al., 2009b).

Microbial insecticides may vary in their effects on biological control agents. For example, the entomopathogenic fungus *Beauveria bassiana* (Balsamo) is not harmful to the parasitoids *Lysiphlebus testaceipes* (Cresson) and *Aphidius colemani* (Viereck) when used against *Aphis gossypii* (Glover) (Murphy et al., 1999). In addition, BotaniGard WP (Bioworks, Inc.; Victor, NY), a pesticide based on *B. bassiana*, was non-toxic to the predatory mite *Amblyseius cucumeris* (Oudemans) (Jacobson et al., 2001). However, direct toxicity to the green lacewing, *C. carnea* (adult and larva) has been reported, with variable susceptibility across different stress treatments (temperature, starvation, and nutrition) (Donegan and Lighthart, 1989). *Beauveria bassiana* is highly toxic, with infection rates >80%, to *Orius insidiosus*, *P. persimilis*, *E. formosa* and *Aphidius colemani* under laboratory conditions, while toxicity under greenhouse conditions is low with infection rates <60% (Ludwig and Oetting, 2001). In two separate studies, direct toxicity to the coccinellid *H. convergens* was determined, with the highest toxicity obtained at low temperatures ($\leq 20^{\circ}\text{C}$) and high relative humidity (>96%) (James and Lighthart, 1994; James et al., 1998). However, the *B. bassiana* isolate 731 has been shown to be non-toxic to the coccinellid, *C. maculata* but harmful to *Eriopis connexa* (Mulsant) after exposure to leaf surface residues (Magalhaes et al., 1988).

Studies have evaluated the compatibility of natural enemies with another group of pesticides, the neonicotinoids including acetamiprid and imidacloprid, and other pesticides such as spinosad and metaflumizone. Neonicotinoid pesticides such as acetamiprid, clothianidin, dinotefuran, imidacloprid and thiamethoxam have been shown to directly affect several species of natural enemies under laboratory conditions (Cloyd and Bethke, 2011). For example, clothianidin, dinotefuran and thiamethoxam are directly toxic to adult *A. coriaria* (Cloyd et al., 2009a). In a separate study, acetamiprid was found to be harmful to the predators *G. punctipes*, *Orius tristicolor* (White), *Chrysoperla carnea* (Stephens) and *Hippodamia convergens* (Guérin-Méneville) (Naranjo and Akey, 2005). Acetamiprid has also been shown to be highly toxic to the coccinellid beetle, *Harmonia axyridis* (Youn et al., 2003). In addition, acetamiprid is directly harmful to the predator, *Deraeocoris brevis* (Uhler) (Kim et al., 2006); the parasitoid, *E. formosa*; and the predators *Orius laevigatus* (Fieber), *Macrolophus caliginosus* (Wagner) and *Amblyseius californicus* (McGregor) (van de Veire and Tirry, 2003). However, spinosad exhibits no direct toxicity to the predatory mite, *P. persimilis* (Holt et al., 2006). Metaflumizone is a relatively new pesticide with minimal risk to non-target organisms including beneficial insects and pollinators (Hempel et al., 2007). For example, no negative effects have been reported associated with adults of *O. insidiosus*, *A. swirskii* and the parasitoid *Eretmocerus eremicus* (Rose and Zolnerowich) (Gradish et al., 2011). Similarly, the pesticides flonicamid and pymetrozine, and two formulations based on natural pyrethins and insecticidal soaps (potassium salts of fatty acids) were not directly toxic to the rove beetle, *Aleochara bilineata* (Gyllenhal) (Jansen et al., 2010; Jansen et al., 2011).

A number of greenhouse pesticides have been evaluated to determine compatibility with *A. coriaria* under both laboratory and greenhouse conditions. Studies have demonstrated that

certain pesticides such as insecticides and fungicides, when applied to the growing medium, are not directly harmful to *A. coriaria*. Pesticides based on *Bacillus thuringiensis* spp. *israelensis* and *Metarhizium anisopliae*, spinosad, and azadirachtin were compatible with *A. coriaria* (Cloyd et al., 2009b). Conversely, Indoor Pharm (Pharm Solutions, Inc.; Port Townsend, WA), a botanical insecticide based on soybean and rosemary oil negatively affected *A. coriaria* adults (Cloyd et al., 2009a).

In pesticide compatibility bioassays, the IGRs cyromazine and diflubenzuron were less harmful than bendiocarb and imidacloprid to *A. coriaria* adults. However, cyromazine was toxic to second instar larvae. Furthermore, the insect growth regulators were not directly harmful to adults (Jandricic et al., 2006). In two separate studies, diflubenzuron did not reduce egg-hatch and urea-based herbicides did not decrease the survival rate of the rove beetle, *A. bilineata* when applied at the recommended label rates. However, methabenzthiazuron, bromoxynil, and carbaryl significantly reduced survival rate and egg production (Gordon and Cornect, 1986, Samsøe-Petersen, 1995). The negative effect of most herbicides tested on egg hatch or egg production, with low direct rove beetle adult mortality, demonstrates the importance of evaluating the indirect effects of pesticides on natural enemies under laboratory conditions.

Pesticide persistence in the soil or growing medium may vary depending on the active ingredient. For example, the half-life (amount of time required for degradation of 50% of the pesticide after application) of cyhalothrin is 7 to 21 days while the half-life of acetamiprid is 1 to 2 days (Yu, 2008). As such, pesticide persistence in the soil or growing medium may influence the effect of pesticides on natural enemies. Furthermore, dosage (rate), soil or growing medium type, and depth of placement may influence pesticide degradation (Kirknel, 1985). Grimm et al.

(2000) proposed an exposure time of four weeks before evaluating the effect of pesticides applied to the soil or growing medium on *A. bilineata*.

Plant growth regulators (PGRs) are synthetic or natural products that modify the rate of response of a crop to internal and external factors involved in plant development (Nickell, 1994). There is evidence of indirect toxic effects associated with PGRs on insect pests (Dreyer et al., 1984). Furthermore, changes in the biochemical composition of the host plant may have an influence on insect survival (Hedin and McCarty Jr., 1994). Currently, minimal research has been conducted to determine whether PGRs are compatible with natural enemies. One study demonstrated that the plant growth regulator, daminozide was harmful (67% mortality) to the thrips predator *Neoseiulus cucumeris* 48 hours after application; however, this involved using four times the standard application rate (Oetting and Latimer, 1995).

Chapter 2 - LIFE HISTORY, FECUNDITY AND LONGEVITY OF THE ROVE BEETLE, *ATHETA CORIARIA* (KRAATZ), UNDER LABORATORY CONDITIONS

Introduction

Rove beetles (Coleoptera: Staphylinidae) comprise one of the largest and most diverse families of beetles (Ashe, 1981), which are found in a variety of terrestrial habitats, including leaf litter and decomposing organic matter (Booth et al. 1991; Hagen et al., 1999). Some species are active predators and studies suggest they are responsible for regulating populations of soil-dwelling insect pests; however, there is minimal basic information on this insect group (Ashe, 1981). The rove beetle, *Atheta coriaria* (Kraatz) is a well-established, introduced European species (Klimaszewski and Peck, 1998). Both adults and larvae are predators of fungus gnat (*Bradysia* spp.) larvae, while adults may also feed on shore flies (*Scatella* spp.) and thrips nymphs and pupae; all of which are major insect pests of greenhouse-grown crops (Carney et al. 2002; Helyer et al., 2003; Meihls and Hibbard, 2009). Studies have documented the potential use of rove beetles as biological control agents (Fournet et al., 2000, Jandricic et al., 2006; Padmavathi et al., 2008).

The life history of *A. coriaria* has been investigated under laboratory conditions using eggs of *Carpophilus hemipterus* L. and *Musca domestica* L., as a food source. Duration of the life cycle from egg to adult was 13, and 11 to 12 days at 27 and 32°C, respectively (Miller and Williams, 1983); however, there is no information on the life cycle of *A. coriaria* using growing medium as a substrate. *Atheta coriaria* is polyphagous (Miller and Williams, 1983), apparently exhibiting more activity under dark conditions and at dusk (17:00 to 20:00 hours) (Echegaray,

personal observation). In addition, *A. coriaria* development from egg to adult was found to be 21 to 22 days at 25°C (Carney et al, 2002; Helyer et al., 2003; Meihls and Hibbard, 2009). Overall, minimal information on *A. coriaria* or any related species is available associated with fecundity and adult longevity. However, adult longevity is approximately 21 days while the number of individuals (offspring) in the F₁ generation is 15 rove beetles per female, although this may be variable (Carney et al. 2002). In addition, previous studies emphasize the importance of understanding the biology of *A. coriaria* (Carney et al., 2002; Birken and Cloyd, 2007), which is marketed in the USA by several biological control suppliers (Warner and Getz, 2008). Furthermore, as the use of natural enemies in greenhouses increases, there is a need to determine natural enemy life history, which will be imperative in evaluating the performance of natural enemies prior to their introduction (van Lenteren and Manzaroli, 1999).

Knowing the biology of a natural enemy is important for the success of any biological control program (DeBach and Rosen, 1991; Gurr et al., 2000). For example, basic studies on natural enemy attributes including development, fecundity, longevity and sex ratio are critical in selecting natural enemies for augmentative biological control (King, 1993; van Driesche and Bellows, 1996; van Driesche and Hoddle, 2000). At the population level, these parameters are associated with the intrinsic rate of increase as described by Birch (1948), which is a criterion for natural enemy performance (Bigler, 1989). In addition, predation capacity and host specificity may also be important for selection prior to release (Perumalsamy et al., 2009; Hokkanen and Pimentel, 1984).

Basic research may be useful in predicting natural enemy effectiveness. As such, studies on several rove beetle species have demonstrated their potential use as biological control agents. For example, the rove beetles *Aleochara bilineata* (Gyllenhal) and *A. bipustulata* (L.) have been

shown to be effective predators of the cabbage root fly, *Delia radicum* (L.) based on their life history as well as other parameters including the intrinsic rate of increase, host specificity, and host acceptance (Fournet et al., 2000). In addition, predator effectiveness may be associated with synchrony with the prey life cycle (Mackauer, 1976; Fournet et al., 2000). As such, development time of *A. bilineata* and *D. radicum* was found to be similar, which enhanced predation. In a separate study, effectiveness of the rove beetle *Oligota pygmaea* (Solier), a predator of the red spider mite, *Oligonychus coffeae* (Nietner), was affiliated with high predation capacity, with one *O. pygmaea* adult consuming 5 to 8 adult red spider mites per day, and a high intrinsic rate of increase and extended life cycle (Perumalsamy et al., 2009). This study also found the duration of the egg, larval and pupal stages of *O. pygmaea* was 3.2, 5.7, and 12.5 days respectively, with a total development time of 23 days from egg to adult. Life history studies may determine differences in consumption rates, for example, between life stages or larval instars. In a field study using spider mites as a prey, third instar larvae of the rove beetle *Oligota kashmirica benefica* (Naomi) displayed higher consumption rates than first instar larvae (Shimoda et al., 1997). A separate study showed *Oligota flavicornis* (Boisduval and Lacordaire) development time and adult longevity was 16 and 48 days at 28°C, while fecundity was 186 eggs per female, with 5 eggs produced per female per day (Chen and Ho, 1993). In addition, development time of the rove beetle, *Paederus fuscipes* (Curtis) was 16 days, with duration of egg, larva and pupa stages 5, 7 and 4 days. Male and female longevity was 54 and 72 days while fecundity was 21 eggs per female, and the sex ratio was 0.9:1 (female:male) (Devi et al., 2002).

In order to minimize the impact of pesticides on natural enemies, pesticides may be applied in ways that will avoid natural enemy susceptible life stages (ecological selectivity). As such, this strategy requires knowledge of natural enemy life cycles (van Driesche and Bellows,

1996). In addition, studies have shown that longevity, development time, fecundity and sex ratio may be influenced by pesticides (Croft, 1990; Greathead, 1995). In fact, the reproductive capacity of the rove beetle *A. bilineata*, based on live offspring, has been proposed as a way to assess the indirect effect of pesticides on soil-inhabiting natural enemies under laboratory conditions (Grimm et al., 2000). Therefore, information on life history attributes may be required before assessing any pesticide indirect effects on natural enemies. Plewka et al. (1975), for example, demonstrated that longevity and fecundity of the egg parasitoid *Trichogramma evanescens* (Westwood) were negatively correlated with increasing insecticide concentrations. In addition, basic studies are essential in assessing the impact of external factors such as growing medium, environmental conditions, food source, food availability, and pesticides on *A. coriaria* survival and development. Moreover, life history studies are useful to develop efficient mass-rearing techniques and quality control, which may be important in terms of natural enemy production as well as economic benefits. In addition, rearing conditions and diet may influence natural enemy quality and productivity. Based on life history parameters, *Chrysopodes (Chrysopodes) lineafrons* (Adams and Penny) was found to be a suitable predator for mass-rearing and release under the climatic conditions of the target area (Silva et al., 2007). Furthermore, manipulation of rearing conditions and diet improved production and storage of adult chrysopids (Chang, 1995; Lopez-Arroyo, 1999).

The life cycle and host egg preference of *A. coriaria* has been studied previously under laboratory conditions using eggs of eight species of Nitidulidae as a food source (Miller and Williams, 1983). In addition, Miller and Williams (1983) assessed the effect of different temperatures and food supply levels on *A. coriaria* development time (egg, three larval instars, and pupa) in a Petri dish. In contrast, the current study investigated *A. coriaria* life history

parameters including fecundity as well as male and female longevity, which has not been evaluated previously, using growing medium as a substrate and oatmeal as a supplemental food source. Therefore, the goal of this study was to determine *A. coriaria* life history parameters as well as fecundity and longevity, which may be useful for augmentative biological control programs as well as improving mass-rearing efficiency.

Materials and Methods

A rove beetle, *Atheta coriaria* and a fungus gnat, *Bradysia* sp. nr. *coprophila* colony were maintained in a laboratory in the Department of Entomology (Kansas State University, Manhattan, KS) using the following rearing procedures.

Fungus gnat colony

A laboratory colony of the fungus gnat, *Bradysia* sp. nr. *coprophila* was initiated using Sunshine Metro-Mix 900 Professional Growing Mix growing medium (GM) (Sun Gro Horticulture, Inc.; Bellevue, WA) as a substrate and raw oatmeal as a supplemental food source following the procedure described by Cabrera et al. (2005a). Before preparation of the substrate, the GM was pasteurized as described by Cloyd and Chiasson (2007) in order to avoid the presence of microorganisms, insects, or any other contaminants (Cloyd and Zaborski 2004). A 6.0 L Rubbermaid® container was filled with GM and then heated in a microwave at the highest power (1,200 W) for 10 minutes. After heating, 3.0 L of GM was transferred to a 7.8 L Rubbermaid® container using a 600 mL beaker, which was allowed to cool for 30 minutes. Afterward, 500 mL of water was added to the GM and mixed thoroughly.

A 2.0 L beaker filled with 1.2 L of water was placed in the microwave for 10 minutes. Subsequently, three to four potatoes (approximately 200 g) ≈12 cm in size were placed into the

water for 10 minutes. Potatoes were peeled and then cut into small pieces (6.5 to 7.5 g) before being placed into a FreshChop food chopper (Hamilton Beach[®]/Proctor-Silex, Inc.; Southern Pines, NC). After operating the processor at high power for 2 to 3 minutes, the potato mixture was poured into the 7.8 L Rubbermaid[®] container and thoroughly mixed with the GM.

Approximately 5 to 6 g of raw oatmeal was then placed onto the surface of the GM. About 40 to 50 fungus gnat adults were randomly collected into a 9-dram vial, from existing colonies, using an aspirator, and then the vial was placed into the container in order to initiate a new colony. A new vial containing fungus gnat adults was placed daily into the container for 10 days after initial inoculation. The aspirator was inserted into the containers with modified lids and screened ventilation holes to collect adults. Approximately 15 mL of water was sprayed every 2 to 3 days onto the GM surface using a 946 mL spray bottle in order to maintain adequate moisture, which has been shown to encourage oviposition (Gillespie et al., 2001).

Rove beetle colony

A laboratory-reared colony of *A. coriaria* was maintained initially using Sunshine SB 300 Universal Professional Growing Mix growing medium (Sun Gro Horticulture, Inc.; Bellevue, WA) consisting of bark, Canadian sphagnum peat moss, perlite and a wetting agent, and oatmeal as a supplemental food source. However, the growing medium type was changed after the Sunshine SB300 Universal Professional Growing Mix supply was discontinued in May 2010. Instead, Sunshine LC1 Professional Growing Mix containing Canadian sphagnum peat moss (75%), perlite (25%), dolomitic lime, gypsum and wetting agent was used, with the main difference between the two consisting of the absence of composted bark in the LC1 Mix (Cloyd et al, 2007b). Rove beetles were originally obtained from an established colony maintained at the University of Illinois, Urbana, IL (Birken and Cloyd, 2007). It is possible that continuous rearing

of rove beetles for over five years may have negatively affected their overall fitness by genetic decay or inbreeding (Mackauer, 1976; Bigler, 1989; Kuriwada et al., 2011); however, the detrimental effect of loss of fitness on the population is unknown.

Colonies were maintained in 7.8 L Rubbermaid® containers and 1.89 L Gladware® plastic containers. Substrate preparation was as follows: 1.5 L (approximately 500 g) of GM was mixed with 350 mL of water (70 mL water per 300 mL GM) in a 1.89 L Gladware plastic container. Supplemental food was provided by placing approximately 1 tsp of raw oatmeal (≈ 2 g) (The Quaker Oats Company; Chicago, IL) onto the surface of the GM every 4 to 5 days. In order to maintain constant moisture, approximately 15 mL of water was applied or sprayed every 1 to 2 days on the surface of the substrate using a 946 mL plastic spray bottle. Rove beetle colonies were maintained under ambient laboratory conditions of 22 to 24°C and 30 to 50% relative humidity (RH), under 0:24 (L:D) hour photoperiod.

Rove beetle adults were collected by sieving the GM using a sieve screen kit (Hubbard Scientific, Co.; Northbrook, IL), with mesh size #5 and #10. Rove beetles were subsequently collected into a 9 dram plastic vial using an aspirator. Mites (species unknown) were present in the colonies and appeared to attack the rove beetles throughout the year, especially from late fall until the end of winter. Mites were observed feeding on different rove beetle life stages (eggs, larva and adults). Mite populations increased rapidly and severely compromised the viability of the rove beetle colony eventually completely depleting the rove beetle population (Echegaray, personal observation), which coincides with the observations of Carney et al. (2002) under laboratory conditions. As such, commercially available *A. coriaria* were purchased from a biological control supplier (IPM Laboratories Inc.; Locke, NY) in order to ensure *A. coriaria* availability for the study. Voucher specimens are deposited as accession number 220 in the

Kansas State University Museum of Entomological and Prairie Arthropod Research (Manhattan, KS).

Duration of life stages and development time from egg to adult on oatmeal

Atheta coriaria life history parameters were determined under two temperature regimes (controlled and ambient environmental conditions). In order to obtain viable eggs, rove beetle (RB) adults, males and females, randomly collected from the main colony, were placed in a 90 x 10 mm Petri dish with approximately 2 tsp (\approx 3 g) of moistened Sunshine LC1 Professional Growing Mix growing medium (GM) containing Canadian sphagnum peat moss (75%), perlite (25%), dolomitic lime, gypsum and a wetting agent, and 1 to 2 pieces of raw oatmeal as a supplemental food source. Rove beetles were maintained in an illuminated incubator (Thermo Electron Corporation; Marietta, OH) at 26°C and 50 to 60% RH under 12:12 (L:D) hour photoperiod. In a separate experiment, duration of the life stages was determined under ambient laboratory conditions [22 to 24°C and 40 to 60% RH, and 0:24 (L:D) hour photoperiod].

Every 24 hours, the GM was carefully examined under a dissecting microscope for the presence of RB eggs. Rove beetle eggs were collected using a moistened, soft fine camel-hair paintbrush. Eggs were then placed individually on a filter paper disk lining the bottom of a 90 x 10 mm Petri dish with a piece of moistened cotton to enhance survival. Petri dishes were placed separately into a 740 mL plastic container. A total of 10 Petri dishes were examined every 12 hours for 2 to 3 min, and the time from oviposition to egg hatch was recorded.

Immediately after egg eclosion, first instar larvae were collected using a moistened soft fine camel-hair paintbrush and transferred to a 90 x 10 mm Petri dish with a filter paper disk lining the bottom and approximately 2 tsp (\approx 3 g) of GM. Approximately 1 to 2 pieces of raw oatmeal were placed in the Petri dish near the GM. The GM was examined daily to prevent the

growth of mold and fungi. A small piece of moistened cotton was placed inside the Petri dish and replaced every 1 to 2 days to maintain constant moisture. Duration of the larval stage was determined by examining larvae every 24 hours from egg hatch until pupation using a dissecting microscope (10 x 22). Likewise, duration of the pupal stage was assessed by evaluating pupae from formation of the pupal chamber until adult emergence. Development time from egg to adult was also determined. Life cycle duration was evaluated under both a constant temperature of 26°C and ambient laboratory temperature of 22 to 24°C. Temperature (°C) and relative humidity (RH) were recorded using HOBO data loggers (Onset, MicroDaq; Contoocook, NH) at 30-minute intervals. The mean \pm SEM temperature and relative humidity in the laboratory from April to December 2009 to 2011 are presented in Appendix A (Tables 1 and 2).

Fecundity

In order to determine female RB fecundity, recently emerged RB adult males and females (< 24 hours old) were processed using the following procedure. Rove beetle adults, males and females, were collected from the main colony into 9 dram vials using an aspirator, assuming the sex ratio was 1:1, and then placed into a 473 mL polypropylene deli container (Fabri-Kal Corp., Kalamazoo, MI) filled with 300 mL of moistened Sunshine LC1 Professional Growing Mix growing medium. Approximately ten days after inoculating the GM, third instar RB larvae were collected by sieving the GM using #5 and #10 mesh size sieves. Third instar larvae (head capsule width of 0.34 mm) were then recovered in 9 dram vials using an aspirator and placed separately into a 90 x 10 mm Petri dish with 2 tsp (\approx 3 g) of moistened GM and 1 to 2 pieces of raw oatmeal in order to allow the larvae to pupate. A small piece of moistened cotton was placed in the Petri dish to maintain constant moisture. After one week, the GM was examined for the presence of rove RB adults.

After emergence, adults were immobilized by collecting them into a 9 dram vial using an aspirator, and then they were transferred into a 12 x 75 mm disposable culture tube (Fisherbrand[®]; Fisher Scientific, Pittsburgh, PA), which was placed into a 473 mL deli squat container half-filled with crushed ice, for 3 to 4 minutes, before examination under a dissecting microscope to determine gender (male or female). Sex of adults was determined by the shape of the eighth abdominal tergite as described by Klimaszewski and Peck (1998). Subsequently, male and female adults were grouped together to form mated pairs. Males and females were allowed to mate for 24 hours, and then mated pairs were placed separately into a 90 x 10 mm Petri dish containing approximately 2 tsp (\approx 3 g) of moistened GM. One to two pieces of raw oatmeal were placed near the GM. Adults were transferred every three days to a new Petri dish containing moistened GM and raw oatmeal in order to provide enough moisture for survival but prevent growth of mold or fungi. The number of eggs laid per day by each female was determined by examining the GM every 24 hours. In addition, the total number of eggs laid per female was recorded.

Longevity

Rove beetle adult longevity was determined using newly emerged adults (< 24 hours old). Longevity was defined as the period (days) from emergence until death of RB adults. In order to determine longevity, individual adults were placed in a 90 x 10 mm Petri dish containing approximately 2 tsp (\approx 3 g) of moistened GM and 1 to 2 pieces of raw oatmeal. Rove beetles were transferred every three days into a new Petri dish with moistened GM. The GM was examined daily for the presence of RB and longevity was recorded.

Number of rove beetle adults produced per female (F1 generation) and sex ratio

Ten mated RB adult pairs were placed separately into a 473 mL deli squat container with moistened GM. To prepare the substrate, 300 mL of GM was measured using a 600 mL beaker and placed into the container. Subsequently, 70 mL of water was added to the container and mixed thoroughly using a spatula. Approximately 2 tsp (\approx 3 g) of raw oatmeal was added to the surface of the GM.

To prevent the RB parental generation (P_1) from mating with their progeny (F_1 adults), every 10 days each RB pair was collected into a 9 dram vial, using an aspirator, and transferred to a new 473 mL deli squat container with 300 mL of moistened GM, as described previously. Approximately 15 mL of water was sprayed every 1 to 2 days onto the surface of the substrate using a 946 mL plastic spray bottle in order to maintain constant moisture. In addition, 2 tsp (\approx 3 g) of raw oatmeal was added every 4 to 5 days onto the surface of the GM assuming this was sufficient food to avoid competition that could potentially affect survival. The GM in the deli squat containers was examined three times—one, two and three weeks after removing the RB pairs, for the presence of adults (F_1 generation). The number of adults was counted and recorded. Each RB pair was transferred three to four times, each time using a new 473 mL deli squat container until cessation of the oviposition period (approximately 40 days after initiation of the experiment). The total number of adults produced per female (F_1 generation) obtained as the sum of all adults recovered from the deli squat containers was recorded. In addition, gender (male or female) was determined in order to obtain the sex ratio. For a given pair, if only the female was found to be alive, the missing male was immediately replaced by a new male collected from the main colony. Females that lived less than 10 days or failed to lay eggs were discarded.

Statistical analysis

Data associated with *A. coriaria* life history parameters were analyzed using a statistical analysis software program, SAS for Windows, version 9.2 (SAS Institute, 2002). Differences in development time at a constant temperature (26°C) versus ambient laboratory conditions, as well as longevity (males and females), and fecundity and number of RB per female (F₁ generation) were assessed using a paired t-test procedure (SAS Institute, 2002).

Results

Duration of the incubation period (from egg-laying to egg hatch), larval stage (egg hatch to pupation), and pupal stage (pupation to adult emergence) under a constant temperature of 26°C, and 50 to 60% RH, and ambient laboratory temperature of 22 to 24°C, and 40 to 60% RH, are presented in Table 1. At 26°C, the mean duration of the incubation period was 2.2 ± 0.1 (mean \pm SEM) days, while duration of the larval and pupal stages was 7.1 ± 0.5 and 7.8 ± 0.1 days, respectively. In addition, duration of the incubation period, and larval and pupal stages at 22 to 24°C and 40 to 60% RH was 2.8 ± 0.2 , 8.9 ± 0.3 and 6.7 ± 0.2 days, respectively. Differences in the duration of *A. coriaria* development time from egg to adult under both constant (26°C) and ambient temperature (22 to 24°C) conditions as determined by the t-test procedure are presented in Table 2.

Based on visual observations conducted in the laboratory, female adult RB initiate egg-laying 1 to 2 days after emergence. Development time from egg to adult was 16.9 ± 0.6 days at 26°C while development time at 22 to 24°C and 40 to 60% RH was 18.4 ± 0.5 days. There were no significant differences ($t = -1.99$; $df = 1, 19$; $P = 0.062$) in the duration (days) of RB development time from egg to adult at both 26°C (17.0 ± 0.53) and 22 to 24°C (18.4 ± 0.45).

There were two generations per month. The shortest development time from egg to adult observed was 15 days while the longest was 21 days. The generation time (time required to complete one generation) from a particular stage to the same stage in the next generation including the preoviposition period was 18 to 19 days at 26°C.

Female fecundity, adult longevity, and reproductive potential (number of RB adults produced per female) are presented in Table 1. The mean number of adults per female in the F₁ generation was 69.1 ± 6.0 . The lowest number of adults per female was 39 whereas the highest number was 104. The mean number of males produced was 32.6 ± 3.1 and the mean number of females was 36.5 ± 3.6 . Rove beetle females laid 90.2 ± 8.9 eggs with the lowest number of eggs laid 50 and the highest number of eggs laid 138. Rove beetle females laid from 0 to 8 eggs per day throughout their lifespan, although two females laid 10 and 14 eggs in one day (Figure 1). However, in a separate experiment with 10 replications, the mean number of eggs per female was found to be 15.0, 21.4, 54.7, 79.2 and 94.1 after 5, 10, 15, 20 and 25 days (Figure 2), with the highest number of eggs laid 114. The sex ratio was approximately 1:1 (females:males) based on the total number of RB adults produced per female. Male longevity was 60.3 ± 4.3 with the shortest longevity 47 days and the longest 86 days, female longevity was 47.8 ± 4.7 days with the shortest longevity 31 days and the longest 81 days. Based on a paired t-test procedure, there were no significant differences ($t = 2.05$; $df = 1, 19$; $P = 0.055$) between male (60.3 ± 3.81) and female (47.80 ± 4.73) longevity. In addition, there were no significant differences ($t = 1.95$; $df = 1, 19$; $P = 0.068$) between the number eggs (90.20 ± 8.93) and the number of adults produced per female (69.10 ± 6.01). Five males remained alive for 94, 80, 86, 30 and 85 days while two females remained alive for 66 and 80 days.

Discussion

The effect of food source and temperature on *A. coriaria* development has been investigated (Miller and Williams 1983). However, there is no information available associated with using growing medium as a substrate and adult production efficiency under different rearing densities is unknown. Furthermore, *A. coriaria* fecundity and longevity has not been previously documented. In the current study, the life cycle was approximately four days longer than Miller and Williams (1983) with development time from egg to adult taking 12.5 days at 26.7°C, which may be due to the use of a different food source [oatmeal vs. live diet (eggs of Nitidulidae)] and growing medium as a substrate. Moreover, duration of the larval and pupal stages also differed from Miller and Williams (1983); in the current study larval and pupal stage durations were longer. However, duration of the egg stage (2.3 days) was similar to Miller and Williams (1983). Apparently, duration of the larval stage may be influenced by adverse conditions such as low growing medium moisture content or inadequate food source, with the larval stages taking 10 to 11 days before transitioning into the pupa stage.

First and second instar larvae are extremely active and larvae tend to escape from the Petri dish within one to five days after egg hatching. In addition, minimal mortality was detected when larvae escaped from the Petri dish to the plastic container. Attempts to seal the Petri dish using laboratory film were unsuccessful apparently because high humidity and lack of ventilation inside the Petri dish resulted in larval death. In order to deal with this, 1 tsp of moist GM was placed into the 740 mL plastic container every 1 to 2 days. Larvae were then recovered from the GM into a 9 dram plastic vial using an aspirator and immediately returned to the Petri dish. Larval mortality was associated with excessive mold and fungi inside the Petri dish and the mites (described previously). In addition, cannibalism was observed, especially among young larvae

and at high larval densities (Echegaray, personal observation). Despite this, the overall impact of larval mortality was low.

Ambient air temperature has been documented to influence *A. coriaria* development (Miller and Williams 1983), while soil temperature affected the development time of *Aleochara bilineata* (Read 1962). On average, females produced 2 to 4 eggs per day during their lifespan and egg deposition was fairly constant throughout the oviposition period, which ended 2, 3, and up to 10 days before termination of the female lifespan. In a separate experiment, females laid 3.7 eggs per day on average after 25 days, which is similar to *Oligota flavicornis* (4.6 eggs per female) (Chen and Ho, 1993). However, results associated with female fecundity as well the number of RB produced per female (F₁ generation) were highly variable, which corroborates the findings of Carney et al (2002). Therefore, in order to obtain a more consistent mean value (and lower standard error), a higher number of replicates may be necessary.

Female fecundity was obtained by counting the number of eggs in the GM; however, due to their small size, eggs were not easily detected. To deal with this, GM in the Petri dish was examined for three days, which allowed all viable eggs to hatch. After three days, not only the number of eggs but also the number of first instar larvae was counted. Rove beetle females that did not lay eggs or exhibited a short longevity (less than 10 days) were discarded. The number of RB adults per female was lower than the number of eggs per female (69.1 and 90.2, respectively) indicating that mortality in the immature stages (egg, larva and pupa) was about 22%. However, mortality during development of *A. bilineata* was 60% (all life stages) (Fournet et al. 2000). It also appears that the use of pasteurized GM substantially reduces survival and reproduction (Echegaray, personal observation), which may be associated with pasteurized GM being less suitable for *A. coriaria* oviposition because of a lack of fissures in the GM, which may reduce

the probability of laying eggs. Differences in GM moisture content, as well as the presence of predaceous mites in the GM, may have influenced RB survival in the deli squat containers. Moreover, differences between number of eggs and number of adults produced per female may also be explained by females having a short longevity, and therefore, having a lower oviposition period or fecundity.

Adult longevity and number of RB produced per female differed from Carney et al. (2002) in which adult longevity was < 21 days with RB producing 15.6 ± 6.4 adults per female although adult production was assessed assuming female longevity was about three weeks. In the current study, RB adults remained alive for 50 to 60 days, suggesting adults may live longer than three weeks and adult production was 69.1 ± 6.0 per female. Adult longevity was similar to *Aleochara bilineata* at 49 days (Read, 1962). In addition, Colhoun (1953) reported 47 and 49 days for females and males. These findings, however, differed from the results obtained with *A. coriaria*, in which male longevity was more extended compared to the female (60 and 48 days). In the current study, maximum adult longevity was 80 to 90 days, which was lower than both *A. bilineata* and *A. bipustulata* at 126 and 169 days, respectively (Fournet et al. 2000). In addition, Fournet et al (2000) reported a higher number of eggs per day (637) and total fecundity (1139 eggs per female) for both *A. bilineata* and *A. bipustulata*. Differences in food source (oatmeal vs. live diet) and reproductive potential may account for variations between species.

Atheta coriaria development time and female preoviposition period were comparable to the rove beetle *Oligota pygmaea* (Solier), about 23 and 2.9 days. However, fecundity was higher (400 eggs per female) (Perumalsamy et al. 2009). In the same study, duration of the egg and larval stage were similar (3 and 6 days) while the pupal stage was longer for *O. pygmaea* (13 days). In addition, *O. pygmaea* adult longevity (54 days) was similar to *A. coriaria*. Compared to

A. coriaria, adult *O. pygmaea* longevity has been reported to be longer for females than males (59 and 49 days) (Perumalsamy et al. 2009). However, as with *A. coriaria* adults, *Paederus fuscipes* (Curtis) female longevity was more extended than the males (72 and 54 days) with high variability (40 to 50 days between the shortest and the longest duration) (Devi et al., 2002).

Fecundity is an important measure of reproductive potential in natural enemies; serving as an indicator of the quality of biological control agents (Hohmann et al., 1988). Based on the results from the current study, it appears that *A. coriaria* has a lower reproductive potential and fecundity compared to the fungus gnat *Bradysia* sp. nr. *coprophila* (80 to 90 eggs per female for *A. coriaria* compared to 100 to 200 eggs per fungus gnat female) (Gardiner et al., 1990; Cloyd, 2008). This may limit the effectiveness of *A. coriaria* as a biological control agent unless higher numbers of predators are released. Fecundity was also lower compared to the shore fly, *Scatella tenuicosta* (710 eggs per female) (Ugine et al., 2007) and *S. stagnalis* (315 eggs per female) (Vänninen, 2001). However, duration of the life cycle was similar for *A. coriaria* and *B. coprophila* (development time from egg to adult of approximately 18 days), which corroborates the findings of Carney et al. (2002) based on visual observations under greenhouse conditions. In addition, *A. coriaria* adult longevity (50 to 60 days) may positively impact effectiveness as adults may prey and lay eggs for extended periods of time thus enhancing their ability to regulate fungus gnat larval populations in greenhouses.

In fact, both adult longevity and fecundity have been reported by Syme (1977) as important factors in determining the effectiveness of any natural enemy. Additionally, parameters such as predation capacity (Chapter 3) and searching ability may warrant further investigation, as well as the effectiveness of the RB larval stage. As such, knowing the *A. coriaria* life history parameters, fecundity and longevity will be useful in terms of augmentative

biological control by providing information that can be used to improve mass-rearing productivity, quality control, and overall effectiveness.

Figures and Tables

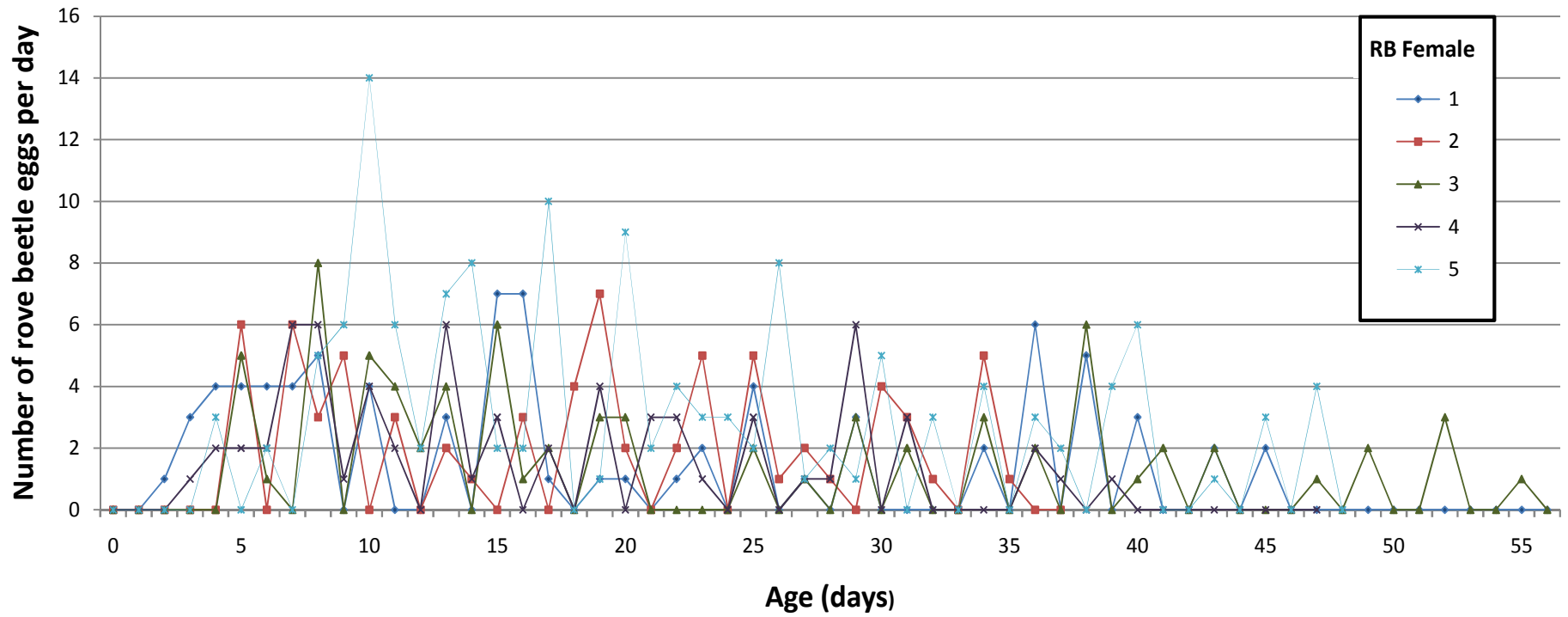


Figure 2-1 Number of rove beetle, *Atheta coriaria* eggs per day associated with five rove beetle females at 26°C and 50-60% relative humidity, using oatmeal as a supplemental food source.

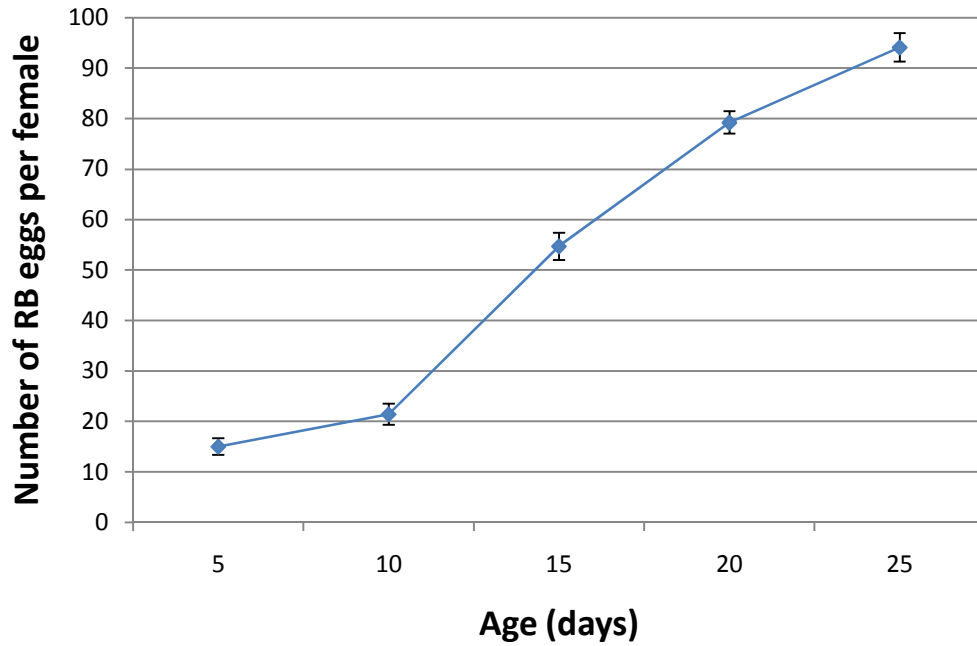


Figure 2-2 Total number of rove beetle (RB), *Atheta coriaria* eggs per female at 26°C and 50-60% relative humidity using oatmeal as a supplemental food source. There were ten females per age.

Table 2-1 Summary of descriptive statistics of the rove beetle, *Atheta coriaria* life history parameters, including mean, standard deviation, standard error, variance, and minimum and maximum observations (n = number of replications).

Parameters	n	Mean	Std. Dev	Std. Error	Variance	Minimum	Maximum
Egg ¹	10	2.20	0.42	0.13	0.17	2	3
Larva ¹	10	7.10	1.66	0.52	2.76	5	11
Pupa ¹	10	7.80	0.42	0.13	0.17	7	8
Egg to adult ¹	10	17.0	1.69	0.53	2.88	15	21
Egg ²	10	2.80	0.63	0.20	0.40	2	4
Larva ²	10	8.90	1.10	0.34	1.21	7	10
Pupa ²	10	6.70	0.48	0.15	0.23	6	7
Egg to adult ²	10	18.40	1.42	0.45	2.04	16	20
Male longevity (days)	10	60.30	12.05	3.81	145.34	47	86
Female longevity (days)	10	47.80	14.95	4.73	223.73	31	81
Fecundity (eggs per female)	10	90.20	28.26	8.93	799.06	50	138
Rove beetle adults per female (F ₁)	10	69.10	19.02	6.01	361.87	39	104

¹Duration (days) of egg, larva, pupa and development from egg to adult at constant temperature (26°C; 50 to 60% relative humidity).

²Duration (days) of egg, larva, pupa and development from egg to adult under ambient laboratory conditions (22 to 24°C; 40-60% relative humidity).

Table 2-2 Mean (\pm SEM) differences in development time in days from egg to adult at 26°C and ambient laboratory conditions (22-24°C; 40-60% relative humidity) (variable 1), adult longevity in days of rove beetle (RB) *Atheta coriaria* males and females (variable 2), and live offspring (F₁ generation) measured as number of RB eggs (fecundity) or number of RB adults per female (variable 3).

Variable 1	Constant temperature	Ambient temperature
RB development time from egg to adult	17.0 \pm 0.53a ^z	18.40 \pm 0.45a
Variable 2	Males	Females
Adult RB longevity	60.30 \pm 3.81a ^z	47.80 \pm 4.73a
Variable 3	Eggs (fecundity)	Adults
RB per female	90.20 \pm 8.93a ^z	69.10 \pm 6.01a

^zMeans in a row with the same letter are not significantly different from each other (Student's t-test, $P \leq 0.05$).

Chapter 3 - ROVE BEETLE *ATHETA CORIARIA* PREDATION ON THE FUNGUS GNAT *BRADYSIA* SP. NR. *COPROPHILA*

Introduction

Natural enemy effectiveness is associated with several attributes such as host specificity, searching efficiency, reproductive potential and seasonal synchronization (Bigler, 1989; Van Lenteren and Manzaroli, 1999). In the greenhouse environment, natural enemies should respond rapidly to an increase in prey density, have high dispersal capacity and survive at low prey densities (Albajes and Alomar 1999). Natural enemy assessments usually involve the use of life table analysis to ascertain demographic parameters, although studies on both predation rate and life history may also be important in selecting suitable biological control agents for pest management (Farhadi et al., 2011). In addition, preliminary assessments may help to detect potential interactions with other natural enemies before release into the environment. For example, Fournet et al (2000) concluded, based on life history parameters, that the rove beetles *Aleochara bilineata* (Gyllenhal) and *A. bipustulata* are effective natural enemies against the cabbage root fly, *Delia radicum* L. Similarly, Perumalsamy et al. (2009) reported that *Oligota pygmaea* (Solier) is an effective predator against red spider mites based on a life table analysis, which also included predation capacity, host detection ability and adult longevity.

Despite the importance of conducting preliminary assessments, in some cases, natural enemies have been introduced and commercially available before information on their efficacy has been established (Van Driesche and Heinz, 2004). Furthermore, although there is a consensus regarding the importance of rove beetles as soil predators, minimal information is available on their efficacy including *Atheta coriaria* (Kraatz) and related species. However,

several species have been reported as predators of animal and human disease vectors and of pests in mushroom production, field crops and greenhouse production systems, including species in the genera *Aleochara*, *Oligota*, *Paederus*, *Atheta*, *Staphylinus* and *Philonthus* (Hagen et al., 1999).

In this study, the number of fungus gnat *Bradysia* sp. nr. *coprophila* larvae consumed after exposure of one rove beetle (RB) adult to different larval instars was investigated in order to avoid any confounding effect of larval age on predation rate. In fact, a number of factors may influence efficacy in terms of quantity of prey consumed including prey size and age. Moreover, based on nutritional requirements, predators may exhibit variation in food source throughout their lifespan. In addition, predation on fungus gnats at different predator-prey ratios was assessed. This is important, especially in greenhouses, where efficacy is associated with the appropriate number of individual predators that should be released per plant based on a particular prey density in order to achieve effective pest regulation. Furthermore, prey consumption at increasing prey densities is determined by the predator-prey functional response. Gaudchau (1982) found that predator:prey ratios of 1:5 and 1:2.5 reduced populations of the pea aphid, *Acyrtosiphon pisum* up to 45% and 24%, respectively, due to predation by syrphid larvae. In a separate study, releasing *Aphidoletes aphidimyza* in greenhouses at a predation rate of 1:10 resulted in effective control of the green peach aphid, *Myzus persicae* on green pepper (Gilkeson and Hill, 1987). Based on laboratory studies, Cheng et al. (2012) suggested that the predator *Mallada basalis* (Walker) be released at a predator:prey ratio of 1:10 or higher in order to suppress populations of *Tetranychus kanzawai* (Kishida) and *Panonychus citri* (McGregor) on papaya. Similarly, a predator:prey ratio of 1:4 was recommended to regulate populations of *Tetranychus urticae* (Koch) on geranium (Opit et al., 2004).

Another component of the current study was associated with *A. coriaria* predation capacity. Studies have determined predation rates for several RB species. For example, in both laboratory and greenhouses the predation capacity of *Aleochara bilineata* on the cabbage maggot, *Hylemya brassicae* (Bouché) was determined with *A. bilineata* adults consuming 23 eggs or early instar larva per day (Read, 1962). In addition, *A. bilineata* may consume three third instar larva of *H. brassicae* per day whereas one *A. bilineata* pair may parasitize 1,210 eggs and 128 third instar instar larvae throughout their lifespan. Furthermore, Colhoun (1953) reported that *A. bilineata* prefers larvae over pupa, and may consume five *H. brassicae* larvae per day. Based on these findings, it was concluded that this species may be an important biological control agent against the cabbage maggot. In a separate study, high predation capacity was reported for the rove beetle, *Paederus fuscipes* (Curtis), a predator of rice planthoppers (Padmavathi et al., 2008). The predation capacity of *P. fuscipes* was 10 brown planthopper (BPH), *Nilaparvata lugens* (Stål) nymphs and seven white backed planthopper (WBPH), *Sogatella furcifera* (Horvath) nymphs per day, whereas consumption of adults of the green leafhopper, *Nephotettix virescens* (Distant), BPH and WBPH was lower (1 to 5 per day). *Paederus fuscipes* was reported to consume on average 39 larvae of the corn earworm, *Heliothis armigera* (Hübner), 41 spotted bollworm, *Earias vitella* (Fabricius) larvae, 59 rice moth, *Corcyra cephalonica* (Stainton) eggs, or 181 *A. gossypii* during their lifespan (Devi et al., 2003).

Rove beetle egg consumption may be substantial during their lifespan. For example, *Oligota flavicornis* (Boisduval and Lacordaire) has been reported to feed on spider mite eggs (Hagen et al. 1999), with 4,656 and 2,125 eggs of *Tetranychus kanzawai* (Kishida) consumed by one adult female and male, respectively, whereas 333 eggs are consumed during the larval stage (Chen and Ho, 1993). In addition, *O. pygmaea* larva may consume 31 to 133 eggs and 3 to 11

adults of the red spider mite, *Oligonychus coffeae* (Nietner) per day (Perumalsamy et al, 2009). Similarly, *O. pygmaea* adults consume 31 to 50 eggs and 5 to 8 red spider mite adults per day. In a separate study; first, second and third instar larva of *O. kashmirica benefica* were reported to consume 4, 6 and 19 twospotted spider mites, *Tetranychus urticae*, while *O. kashmirica benefica* adults consumed 20 red spider mites after 150 minutes (Shimoda et al., 1997).

Based on previous studies, 5 to 10 *A. coriaria* adults per container significantly reduced *Bradysia difformis* (Frey) populations (Bennison et al., 2008). In addition, 5 to 10 *A. coriaria* adults consumed 10 western corn rootworm, *Diabrotica virgifera virgifera* (LeConte) first instar larvae in 4 hours (Meihls and Hibbard, 2009). Carney et al. (2002) reported that one *A. coriaria* adult may consume 154 eggs, 46 second instar larvae or 10 late instar fungus gnat *Bradysia impatiens* (Johannsen) larvae in a 24 hour time period. Furthermore, 134 eggs of the shore fly *Scatella stagnalis* (Fallen) or 95 thrips may be consumed by one *A. coriaria* adult in the same time period. In addition, 8 first instar larvae of the European pepper moth, *Duponchelia fovealis* (Zeller) were consumed after releasing 10 rove beetle adults into a jar containing kalanchoe leaves infested with prey for 10 days (Messelink and van Wensveen, 2003). In a separate experiment, approximately 30 adult shore flies were consumed after releasing 12 rove beetle adults per container in cultivated mushrooms (Gorski et al., 2006). However, there were no differences compared to the control when releasing 10 *A. coriaria* adults against either 20 second or third instar fungus gnat larvae per container (Cloyd and Chiasson 2007). The predation capacity of other fungus gnat natural enemies has been investigated. For example, daily predation rates of the predatory mite, *Hypoaspis aculeifer* (Canestrini) were found to be 2 to 5 fungus gnat larvae (Jeon et al., 2007) whereas larvae of the hunter fly *Coenosia attenuata* (Stein) consumed up to 463 fungus gnat larvae during their 12 day development time (Ugine et al.,

2010). *Atheta coriaria* predation has been assessed in terms of percent reduction of thrips, the cabbage root fly (*D. radicum*), and sciarid fly populations (Bennison et al., 2008; Bennison et al., 2011a,b) or based on the number of fungus gnat larvae consumed (Carney et al., 2002) with minimal information associated with the procedures involved. Therefore, the objectives of this study were to 1) determine the effect of fungus gnat (FG) larval age using second and third instar FG larvae on predation by one RB adult, and 2) ascertain *A. coriaria* prey consumption at different predator-prey densities. The results obtained in this study will provide a more comprehensive assessment of *A. coriaria* effectiveness as a biological control agent.

Materials and Methods

Atheta coriaria predation on the fungus gnat *B. coprophila* was investigated in the laboratory using different prey densities in Petri dishes and predator:prey ratios in 473 mL deli squat containers (Fabri-Kal Corp., Kalamazoo, MI) using Sunshine LC1 Professional Growing Mix growing medium (Sun Gro Horticulture, Inc.; Bellevue, WA) as a substrate. In order to assess FG and RB survival in 473 mL deli squat containers, recovery rates were determined under experimental conditions [22 to 24°C; 40 to 60% relative humidity, 0:24 (L:D) hour photoperiod]. Temperature (°C) and percent relative humidity (RH) during the experiments was recorded using HOBO data loggers (Onset, MicroDaq; Contoocook, NH) at 30-minute intervals. The mean \pm SEM temperature and RH in the laboratory from April to December 2009 to 2011 are presented in Appendix A (Tables 1 and 2).

Number of fungus gnat larvae consumed using second and third instar larvae, and one rove beetle adult

Before determining *A. coriaria* predation rates on FG larvae in Petri dishes, the number of FG larvae consumed by RB adults was investigated in a no-choice 2-by-2 factorial design

experiment using second and third instar FG larvae with 10 replications per treatment. Additionally, in order to avoid the effect of satiation, both starved and non-starved RB adults were used, with starvation consisting of RB deprived of food for 24 hours. In total, there were four treatment combinations using second and third instar FG larvae and, starved and non starved RB adults. Second instar (6 to 7 days old) and third instar (8 to 9 days old) FG larvae were obtained based on the procedure described by Cloyd et al (2009a). A total of 10 FG larvae were collected from a FG-inoculated Sunshine LC1 Professional Growing Mix growing medium sample using a 150 mm disposable flint glass non-sterile Pasteur pipet (Fisher Scientific[®]; Pittsburgh, PA), which was then placed into a 50 x 15 mm glass Petri dish filled with 10 to 15 mL of water. Subsequently, 1.6 mL of water was poured into a 100 x 15-mm glass Petri dish using a 10 mL capacity plastic graduated cylinder. The bottom of the Petri dish was lined with 90 mm diameter qualitative filter paper (Whatman Int. Ltd.; Maidstone, UK). Larvae were collected individually using the Pasteur pipet, transferred to the glass Petri dish and counted. After this procedure, the total amount of water added to the Petri dish was 2.0 to 2.2 mL. A single RB (male or female) adult (< 24 hour old) was placed into the Petri dish and the number of FG larvae consumed was determined after 24 hours by counting the number of head capsules (which was a visual assessment of larvae consumed) and live FG larvae (larvae not consumed) in the Petri dish for each treatment combination (starved and non-starved RB with second instar FG larvae, and starved and non-starved RB with third instar FG larvae) under a dissecting microscope (10 x 22). The number of head capsules and FG larvae was recorded.

Predation in Petri dishes using different prey densities

Atheta coriaria functional response was investigated using five treatments with 10 replications per treatment, consisting of five different prey densities –0 (control), 5, 10, 15, 20

and one RB adult. In addition, one treatment with 20 FG larvae and no RB adult was used as a check. The filter paper in each Petri dish was inoculated with FG larvae following the procedure described previously. After adult emergence, a single RB (< 24 hour old) was collected into a 9 dram plastic vial using an aspirator and placed into a 100 x 15 mm glass Petri dish. The number of larvae consumed was determined based on the number of head capsules present on the filter paper after 24 hours, and recorded.

Fungus gnat and rove beetle adult recovery rates

After having determined the number of prey consumed using second and third FG larval instars, the predation rates of *A. coriaria* on FG larvae at different predator-prey densities was investigated using 473 mL deli squat containers as the experimental units and oatmeal as a supplemental food source. In order to quantify survival, RB and FG adult recovery rates were obtained separately based on the following procedure.

Rove beetle adults were randomly collected from the main colony by sieving the growing medium (GM) using #5 and #10 sieves, aspirated into a 9 dram plastic vial and subsequently transferred into 473 mL deli squat containers filled with 300 mL of moistened Sunshine LC1 Professional Growing Mix growing medium. Recovery rates were determined 96 hours after inoculating the GM with RB adults at different initial densities. Adults were collected and the number of live RB adults recovered was recorded. In addition, FG adult recovery rates were obtained after inoculating the GM with second or third instar FG larvae at different initial densities and using different GM types. Fungus gnat adults were captured, after emergence, on yellow sticky card (Whitmire Micro-Gen Research Laboratories, Inc.; St. Louis, MO) sections (2.5 x 2.5 cm in size) attached to the inside of the container lid (Cloyd et al., 2007a). The number of FG adults captured per container was recorded.

Fungus gnat adult production under varying densities of predators and prey

A total of five experiments were set-up in a randomized complete block design with two blocks (days), and following the procedures described by Cloyd and Chiasson (2007). Predation was assessed based on five different RB densities: 1, 2, 3, 4 and 5 RB adults. There were 4 treatments consisting of four FG larval densities: 10, 20, 30 and 40, with 10 replications per treatment.

The substrate was prepared based on the following procedure. A 6.0 L plastic container (Rubbermaid Home Products[®]; Wooster, OH) was filled with Sunshine LC1 Growing Mix growing medium consisting of bark, Canadian sphagnum peat moss and perlite, and then heated for 10 minutes at full-power (1,200 W output) in a microwave. After cooling, 300 mL of GM were measured using a 600-mL beaker and placed into a 473 mL deli squat container. Ventilation was provided by a modified lid with insect screening. Subsequently, 70 mL of water and approximately 2 tsp of raw oatmeal (≈ 3 g) was added to the GM, and then thoroughly mixed using a spatula. Deli squat containers were labeled accordingly and maintained in an environmental growth chamber (EGC) (CONVIRON[®] Controlled Environments Inc.; Pembina, ND) at a temperature of $25 \pm 2^{\circ}\text{C}$ and 0:24 (L:D) hour photoperiod for 48 hours prior to artificial inoculation with FG larvae.

Fungus gnat adults were collected from an established laboratory colony in 9 dram vials using an aspirator before being placed into a 739 mL Rubbermaid[®] container, which served as an oviposition chamber. The oviposition chamber consisted of a 100 x 15 mm glass Petri dish lined with 90 mm qualitative filter paper, approximately 20 mL of moistened GM, and 1 tsp of raw oatmeal (≈ 1 to 2 g). The oviposition chamber was placed into the EGC for 6 to 7 days in order to obtain a sufficient number of same-aged FG larvae.

A FG-inoculated GM sample (≈ 10 g) was placed into a 50 x 15 mm glass Petri dish using a spatula, and water was added to loosen the GM. A total of 10, 20, 30 and 40 FG larvae were collected using a 150-mm disposable flint glass non-sterile Pasteur pipet, and then placed into a 50 x 15 mm glass Petri dish filled with 8 to 10 mL of water. Larvae were counted and then the entire contents were poured into the deli squat container with 300 mL of GM. After inoculating all the deli squat containers with FG larvae, the containers were placed in the EGC for 24 hours prior to introducing RB adults.

One to five RB adults (various ages) were collected from the main colony into 9-dram vials using an aspirator, and then placed into the deli squat containers. Rove beetle adults from the main colony were maintained at a temperature of 26°C, 50 to 60% RH, and 12:12 (L:D) hour photoperiod. After inoculating the GM with RB adults, all the containers were maintained under ambient laboratory conditions [22 to 24°C; 40 to 60% RH, and 0:24 (L:D) hour photoperiod]. Moisture was provided by placing each deli squat container into a 14 cm diameter dish filled with 60 mL of water 48 hours after inoculating the GM. Small holes, which had been perforated in the base of the containers, allowed the water to migrate up, via capillary action, and moisten the GM. After 10 days, yellow sticky card sections (2.5 x 2.5 cm in size) were glued to the inside of the deli squat container lids. After seven days, data were collected by counting the number of FG adults captured on the yellow sticky cards (RB adults were also captured on the yellow sticky cards).

In a separate experiment, predation was assessed after inoculating the GM with one RB larva and 10, 20, 30 and 40 FG larvae following the procedure described above. In addition, *A. coriaria* prey consumption was investigated using 20 FG larvae per container with five

treatments consisting of five RB adult densities (0, 1, 5, 10 and 15), with 10 replications per treatment.

Statistical analysis

Data from all experiments were analyzed using a statistical analysis software program SAS Systems for Windows, version 9.2 (SAS Institute, 2002). Data for experiment 1 (assessment of prey consumption using second and third FG larval instars) were subjected to a general linear model (GLM) 2-by-2 factorial design analysis of variance (ANOVA) using the PROC GLM procedure, with the number of second and third instar FG larvae consumed as the dependent variable and FG larval instar and RB nutritional status (starved or non-starved) the main effects. Significance was determined for the two variables and the two-way interaction. In addition, data for experiment 2 (assessment of predation in Petri dishes at different prey densities) were subjected to a linear regression analysis using the PROC REG procedure (SAS Institute, 2002). Percent prey consumption at different FG larval densities was transformed using an arcsin transformation to normalize the data, which were then subjected to ANOVA using the PROC ANOVA procedure (SAS Institute, 2002). Significant differences among the means was determined using a Tukey's mean separation test.

Data associated with predation in the 473 mL deli squat containers when using 1 to 5 RB adults and 10, 20, 30 and 40 FG larvae (experiment 3) were subjected to a linear regression analysis using the PROC REG procedure (SAS Institute, 2002). In addition, data affiliated with predation using 20 FG larvae and 0, 1, 5, 10 and 15 RB adults were subjected to a mixed model analysis using the PROC MIXED procedure (SAS Institute, 2002), and pair-wise comparisons among treatments were performed using a Tukey's mean separation test with a significance level of $P \leq 0.05$.

Results

Number of fungus gnat larvae consumed using second and third instar larvae and one rove beetle adult

The number of second and third instar FG larvae consumed by starved RB was 7.4 ± 1.2 and 7.6 ± 0.7 , respectively, which did not differ statistically. In addition, there were no significant differences between second instar (5.7 ± 0.3) and third instar (6.6 ± 0.7) FG larvae consumed by non-starved RB. No significant effects were observed associated with the FG larval instar ($F = 1.42$; $df = 1$; $P = 0.24$), nutritional status of RB ($F = 3.25$; $df = 1$; $P = 0.08$), and the two-way interaction ($F = 0.23$; $df = 1$; $P = 0.63$).

Predation in Petri dishes using different prey densities

There were significant differences ($F = 78.25$; $df = 3, 39$; $P \leq 0.0001$) in prey consumption among the treatments with higher numbers of FG larvae consumed as prey density increased (Figure 1). There was a strong linear relationship ($R^2 = 87\%$) between the initial number of FG larvae and the number of larvae consumed, with 3.9 ± 0.4 , 6.8 ± 0.6 , 10.4 ± 0.5 , and 14.6 ± 0.9 FG larvae consumed when using 5, 10, 15 and 20 FG larvae as initial densities (Figure 1). The highest number of FG larvae consumed by one RB adult associated with 5, 10, 15 and 20 FG larvae was 5, 10, 12 and 19, and the lowest was 2, 4, 7, and 10. No prey consumption was affiliated with the control (no FG larvae) and the untreated check (20 FG larvae and no RB adult). There were no significant differences ($F = 1.31$, $df = 3, 39$; $P = 0.286$) in regards to percent prey consumption when using 5 (78%), 10 (68%), 15 (69%) and 20 (73%) FG larvae (Figure 2).

Fungus gnat and rove beetle adult recovery rates

Results associated with RB and FG recovery rates are presented in Tables 1 and 2. Rove beetle adult recovery rates obtained in the three experiments was > 90%, 96 hours after inoculating the GM with four different RB adult densities (experiment 1: 5, 10, 15, 20; experiment 2: 10, 20, 25, 30; and experiment 3: 10, 20, 30, 40 RB adults per container) (Table 1). There were significant differences for experiment 1 ($F = 1398.27$; $df = 3, 39$; $P \leq 0.0001$), experiment 2 ($F = 686.61$; $df = 3, 39$; $P \leq 0.0001$), and experiment 3 ($F = 2681.90$; $df = 3, 39$; $P \leq 0.0001$) regarding the number of RB adults recovered. Similarly, FG adult recovery rates were obtained in four experiments with four different GM types (SB300 Universal Professional Growing Mix, Sunshine LC1 Professional Growing Mix, Sphagnum peat moss, and Metro-Mix 360 Growing Mix) (Table 2). Recovery rates were >80% approximately four weeks after inoculating the GM with four different initial FG larval densities (10, 20, 30, and 40) and two GM types: SB300 Universal Professional Growing Mix and Sunshine LC1 Professional Growing Mix in two experiments with 10 replications per treatment (Table 2). There were significant differences among FG larval densities for experiment 1 ($F = 121.89$; $df = 3, 39$; $P \leq 0.0001$) and experiment 2 ($F = 151.38$; $df = 3, 39$; $P \leq 0.0001$). Recovery rates were >80% when using Sphagnum peat moss (Premier Horticulture, Inc.; Quakertown, PA) and <35% when using Metro-Mix 360 Growing Mix (Sun Gro Horticulture, Inc.; Bellevue, WA) as a substrate in the two experiments with 5 replications per treatment (Table 2). Recovery rates were significantly different among FG larval densities for experiment 3 ($F = 51.09$; $df = 3, 19$; $P \leq 0.0001$) and experiment 4 ($F = 15.43$; $df = 3, 19$; $P \leq 0.0001$).

Fungus gnat adult production under varying densities of predators and prey

Results for the five experiments associated with increased RB adult densities (1 through 5) and four treatments consisting of four FG larval densities (10, 20, 30 and 40) are presented in

Table 3. In addition, the relationship between different initial numbers of RB adults, initial FG larval densities, and prey consumption is presented in Figures 3 and 4.

Experiment 1: Fungus gnat adult production when using one rove beetle adult and 10, 20, 30 and 40 fungus gnat larvae

The number of FG adults recovered when using one RB adult was higher as FG larval density increased, with significant differences ($F = 33.86$; $df = 3, 39$; $P \leq 0.0001$) among the treatments (10, 20, 30 and 40 FG larvae). The mean (\pm SEM) number of FG adults captured on the yellow sticky cards was 6.4 ± 0.6 , 13.2 ± 1.2 , 18.4 ± 1.3 , and 21.6 ± 1.3 when using 10, 20, 30 and 40 FG larvae. The number of FG adults recovered was similar for treatments 3 and 4 (18.4 ± 1.3 and 21.6 ± 1.3), which were significantly higher than treatments 1 and 2 (6.4 ± 0.6 and 13.2 ± 1.2) (Table 3).

Experiment 2: Fungus gnat adult production when using two rove beetle adults and 10, 20, 30, and 40 fungus gnat larvae

Fungus gnat adults recovered after exposure to two rove beetle adults was higher as the number of initial FG larvae increased. In addition, there were significant differences ($F = 67.76$; $df = 3, 39$; $P \leq 0.0001$) among the treatments. The number of FG adults captured on the yellow sticky cards was 5.9 ± 0.4 , 11.0 ± 1.3 , 20.7 ± 1.1 , and 21.6 ± 0.7 after using 10, 20, 30 and 40 FG larvae. The number of FG adults recovered was similar for treatments 3 and 4 (20.7 ± 1.1 and 21.6 ± 0.7), and both treatments were significantly higher than treatments 1 and 2 (5.9 ± 0.4 and 11.0 ± 1.3) (Table 3).

Experiment 3: Fungus gnat adult production when using three rove beetle adults and 10, 20, 30 and 40 fungus gnat larvae

The number of FG adults recovered was significantly higher as FG larval density increased when using three RB adults ($F = 32.16$; $df = 3, 39$; $P \leq 0.0001$). The number of FG adults captured on the yellow sticky cards was 5.7 ± 0.4 , 9.0 ± 1.1 , 16.8 ± 1.2 , and 18.8 ± 1.5 when using 10, 20, 30 and 40 FG larvae. The number of FG adults recovered was significantly higher for treatments 3 and 4 (16.8 ± 1.2 and 18.8 ± 1.5) than treatments 1 and 2 (5.7 ± 0.4 and 9.0 ± 1.1) (Table 5). Although the number of FG adults captured was numerically higher for treatment 4, this was not significantly different than treatment 3 (Table 3).

Experiment 4: Fungus gnat adult production when using four rove beetle adults and 10, 20, 30 and 40 fungus gnat larvae

Fungus gnat adults recovered was higher as FG larval density increased when using four RB adults ($F = 22.88$; $df = 3, 39$; $P \leq 0.0001$). The number of FG adults captured on the yellow sticky cards was 4.9 ± 0.5 , 7.5 ± 1.1 , 13.8 ± 1.8 , and 17.6 ± 1.0 when using 10, 20, 30 and 40 FG larvae. The number of FG adults recovered was similar for treatments 3 and 4 (13.8 ± 1.8 and 17.6 ± 1.0) and both treatments were significantly higher than treatments 1 and 2 (4.9 ± 0.5 and 7.5 ± 1.1) (Table 3).

Experiment 5: Fungus gnat adult production when using five rove beetle adults and 10, 20, 30 and 40 fungus gnat larvae

Fungus gnat adults recovered was significantly higher as FG larval density increased when using five RB adults ($F = 71.59$; $df = 3, 39$; $P \leq 0.0001$). The number of FG adults captured on the yellow sticky cards was 9.4 ± 0.6 , 9.8 ± 1.0 , 22.7 ± 1.2 , and 28.1 ± 1.5 when using 10, 20, 30 and 40 FG larvae. The number of FG adults recovered was significantly higher for treatment 4 (28.1 ± 1.5) than treatment 3 (22.7 ± 1.2). However, there were no significant differences between treatments 1 and 2 (9.4 ± 0.6 and 9.8 ± 1.0) (Table 3).

Adult fungus gnat production after exposure of one rove beetle larva to different fungus gnat larval densities

The number of FG adults recovered was higher as FG larval density increased when using one RB larva and 10, 20, 30 and 40 FG larvae ($F = 32.77$; $df = 3, 27$; $P \leq 0.0001$) (Figure 5). The number of FG adults captured on the yellow sticky cards was 8.1 ± 0.6 , 14.4 ± 0.8 , 24.1 ± 1.7 , and 28.9 ± 2.4 when using 10, 20, 30 and 40 FG larvae. The number of FG adults recovered was similar for treatments 3 and 4 (24.1 ± 1.1 and 28.9 ± 2.4); whereas the number of FG adults recovered in treatment 2 (14.4 ± 0.8) was significantly higher than treatment 1 (8.1 ± 0.6).

Adult fungus gnat production when using 20 fungus gnat larvae and 0, 1, 5, 10 and 15 rove beetle adults

Fungus gnat adults recovered when using 0, 1, 5, 10 and 15 RB adults four weeks after inoculating the GM with 20 FG larvae is presented in Figure 6. There were significant differences among the treatments compared to the control ($F = 4.85$; $df = 4, 39$; $P \leq 0.0032$). The number of FG adults captured on the yellow sticky cards for treatments 1 through 5 (RB0, RB1, RB5, RB10, and RB15) was 13.9 ± 0.4 , 12.6 ± 0.9 , 11.0 ± 0.7 , 12.8 ± 0.8 and 9.1 ± 1.2 . Although the number of FG adults captured was numerically higher for RB0 (control), this was not significantly different than RB1, RB5 and RB10; however, the number of FG adults recovered for RB15 was lower than treatments RB1, RB10, and the control.

Discussion

This study has shown that RB adults exhibit similar predation rates whether feeding on either second or third instar FG larvae. Also, the number of larvae consumed by starved RB was not significantly different than non-starved RB. These results suggest that, regardless of FG larval instar or nutritional status of the RB, prey consumption is similar. However, predators were starved for only 24 h. As such, it is possible that a longer period of starvation may have resulted in a different prey consumption.

At different FG larval densities, and 1 to 5 RB adults, the per capita prey consumption was similar among RB densities with the highest total predation when using 4 and the lowest when using 5 adults. Therefore, the release of more than four RB adults regardless of prey density should be avoided since this may increase costs associated with purchasing RB and there is no enhanced efficacy. Predator:prey ratios, when using 5 RB adults, did not result in higher predation rates per RB adult than 1 RB adult for any initial FG larval density. Subsequent examination of the GM indicates that FG larvae were able to avoid predation by hiding inside the potato granules soon after inoculation (Echegaray, personal observation), which may partly explain the low predation by RB adults. However, the extent to which this may have biased the results was not determined.

Low variation was observed among RB densities for each level of prey density when using GM as a substrate and oatmeal as a supplemental food source, which may be due to mutual interference as the number of RB adults increased. Mutual interference is a common phenomenon affiliated with low prey availability or high predator density (Hassell et al., 1976). For example, mutual interference has been demonstrated with the rove beetle *Paederus fuscipes* (Curtis) at high densities when feeding on eggs of the rice leaf roller, *Cnaphalocrocis medinalis*

(Guenée) (Shen and Pang, 1989) as well as *Coccinella septempunctata* L. larvae when feeding on *Lipaphis erysimi* (Kalt.) (Pandey et al., 1984).

The number of FG adults produced associated with 1 and 5 RB adults and 20 FG larvae was consistent indicating that 5 RB adults may consume approximately three more FG larvae than one adult. However, it should be noted that an increase in RB adult numbers may not result in enhanced effectiveness. For example, per capita prey consumption at a ratio of 1:10 did not change regardless of predator and prey numbers, which designates that adjusting the predator:prey ratio is important in terms of a cost-effective FG management strategy when increasing the number of RB adults. Similar differences in prey consumption were found when using 10 and 15 RB adults and 20 FG larvae (predator:prey ratio of 3:4). Nonetheless, based on the number of FG adults produced when using 1 and 5 RB adults, predation was no different than the control.

Prey consumption associated with RB larva was also similar to the adult at low FG larval densities (10 and 20 FG larvae), and lower at higher densities (30 and 40 FG larvae), which differs from the findings of Miller and Williams (1982) with third instar larva consuming more prey (eggs of *Stelidota germinate* Say) than the adult. This may possibly be due to the use of different food sources (larvae vs. eggs).

There was a positive density dependent response in terms of predation on FG larvae with no decline in prey consumption when using 5, 10, 15 and 20 FG larvae. Miller and Williams (1982) reported that the functional response of *A. coriaria* in relation to increased levels of prey (eggs of *Stelidota germinate* Say) density may resemble a Type I functional response as described by Holling (1959), which predicts that higher FG larval densities are consumed by RB adults until the predation curve associated with FG larva prey consumption reaches a plateau

(saturation level). However, the maximum number of larvae exposed to predation was not high enough to reach the saturation level although higher FG larval densities per RB adult may be more common in greenhouses. These results seem to indicate that although the number of prey consumed significantly increased with increasing prey density, the percentage of prey consumed remained constant at about 70-80%. As such, predation efficiency neither increased nor decreased over the range of prey densities (5-20 FG larvae) tested. Therefore, additional releases of RB adults are not needed when the predator:prey ratio increases from 1:5 to 1:20, which will avoid additional expenses to greenhouse producers associated with augmentative releases of RB.

In Petri dishes, predation was high at a predator:prey ratio of 1:20 (75%) indicating that RB adults can potentially consume between 750 and 900 FG larvae during their 50 to 60 day lifespan although further research is needed in order to investigate predation at high (> 20 FG larvae) densities and whether or not prey consumption by adult RB is age-dependent. In addition, predation in Petri dishes was consistent with the findings of Birken and Cloyd (2007) after 4 to 6 hours when using 5 to 10 FG larvae per Petri dish, and predation efficiency when using 5 FG larvae was high (78%), which also supports the results of Cloyd et al (2009a).

There were issues, however, associated with the technique used to determine predation in Petri dishes. For example, after the inoculation process, FG larvae started to migrate towards the edge of the filter paper. During the evaluation, some larvae were found either on the inside of the Petri dish lid or beneath the filter paper therefore avoiding predation. In order to prevent this from occurring, it was necessary to secure the edges of the filter paper firmly against the Petri dish before placing the larvae onto the filter paper. Additionally, observations from the current study indicated that 2.0 to 2.2 mL of water added to the Petri dish was enough to keep FG larvae alive and prevent RB adults from drowning (Echegaray, personal observation) which differs

from Cloyd et al. (2009a). As such, findings from the current study will help enhance the accuracy of the technique used to assess predation in Petri dishes. In addition, providing more than 20 FG larvae may not be practical when utilizing this technique because higher numbers of larval head capsules may result in double-counting due to their small size. Furthermore, live and dead FG larvae were found in the Petri dish during the evaluation period, indicating that RB may kill more larvae than they actually consume although some FG larvae may also have drowned, which supports the findings of Carney et al. (2002). Overall, only FG larval head capsules were present in the Petri dish, while larvae not consumed (live or dead) were detected beneath the filter paper. Low prey consumption was also associated with RB escaping from the Petri dish; however, only if the number of FG larvae consumed was low (1 or 2 FG larvae consumed) or no consumption was detected was this data omitted from the analysis.

Obtaining high (> 80%) FG adult recovery rates in the absence of RB adults is important when assessing predation in deli squat containers because this indicates that FG larval mortality associated with factors unrelated to prey consumption is low. As such, choosing the appropriate GM type is important. For example, low FG adult recovery rates were obtained when using Metro-Mix 360 Growing Mix as a substrate, which may have been unsuitable for both oviposition and FG larval survival due to the structure of the GM. In addition, based on the findings of Birken and Cloyd (2007) it was assumed that the use of oatmeal as a supplemental food source helped to keep the RB adults alive but did not negatively influence predation.

Due to their reproductive potential, more FG are produced within a generation than RB; however, RB prey consumption intensifies as FG populations increase, which may help to alleviate plant damage and losses associated with crop production. Nonetheless, it is difficult to correlate RB numbers with potential FG damage as economic thresholds have not been

determined. Therefore, further research under greenhouse conditions is warranted. Although a certain level of plant damage may occur when using RB as the only management strategy, overall, RB adults will be a useful component in regulating FG populations, and may reduce the need for insecticide applications.

The current study has demonstrated that the per capita RB adult prey consumption is high (70 to 80%) as the number of prey increases from 5 to 20 FG larvae. However, predation was lower when using 1 to 5 RB adults and a mixture of GM and potato as a substrate most likely due to prey avoiding predation. Based on the duration of FG larval instars, the findings from the current study indicate that RB adults can be released from three to six days, and up to approximately twelve days after the first FG adults have been captured on yellow sticky cards with no effect on RB efficacy. Overall, predation increased when using 1 to 4 RB adults and decreased when using 5 adults with no changes associated with per capita prey consumption among different predator:prey ratios. Therefore, this study has quantitatively shown that RB are in fact a viable biological control agent that when used appropriately can regulate FG populations.

Figures and Tables

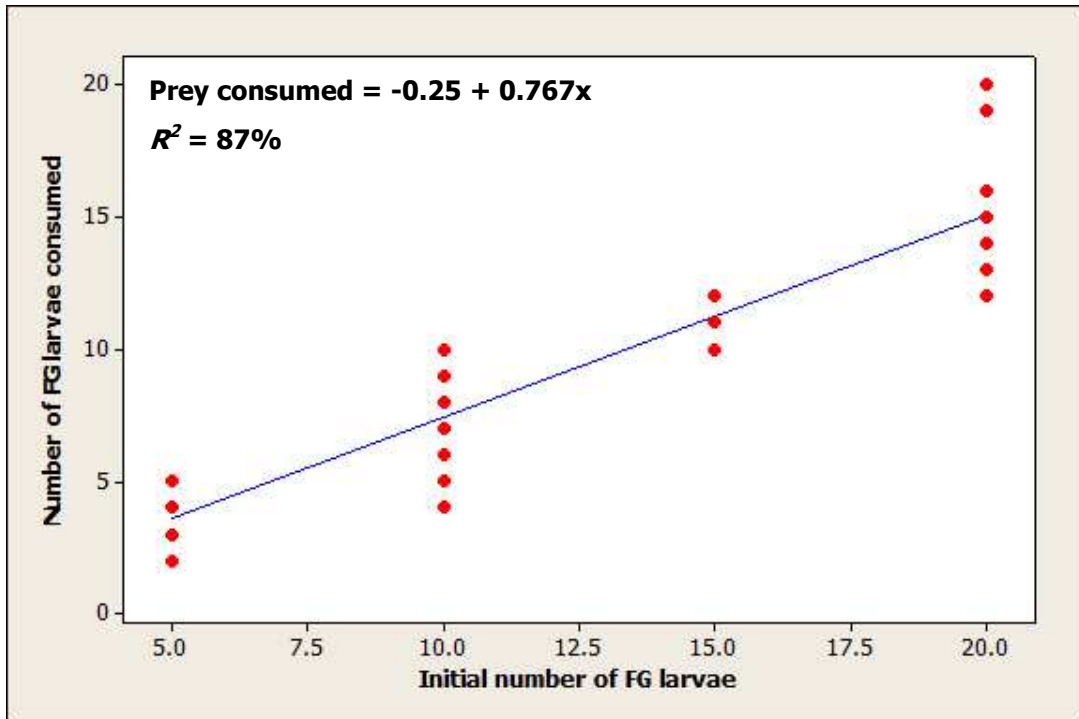


Figure 3-1 Relationship between number of fungus gnat (FG) larvae consumed by one rove beetle, *Atheta coriaria* adult at different initial larval densities. The line equation and coefficient of determination (R^2) is shown.

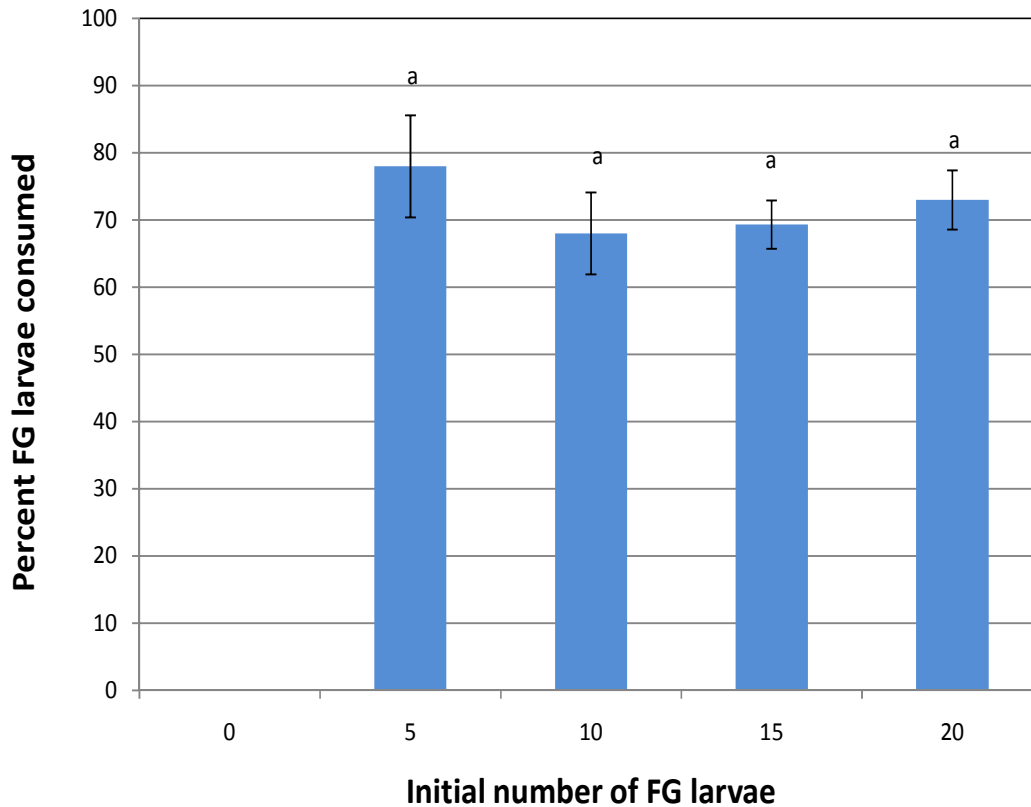


Figure 3-2 Percent rove beetle, *Atheta coriaria* prey consumption on fungus gnat (FG) *Bradysia* sp. nr. *coprophila* larvae at prey densities of 0 (control), 5, 10, 15 and 20 FG larvae in Petri dishes. There were 10 replications per treatment. Vertical bars represent standard errors of the mean (SEM).

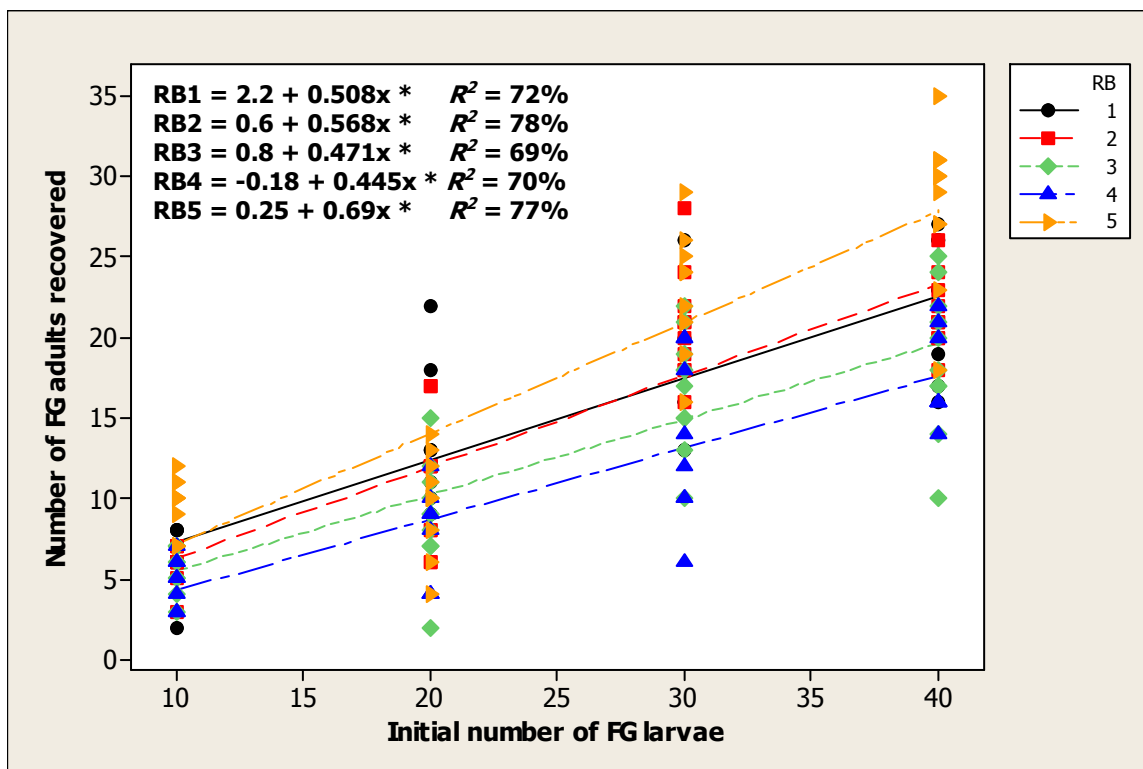


Figure 3-3 Relationship between number of fungus gnat (FG) adults recovered and initial number of FG larvae using 1, 2, 3, 4, 5 rove beetle (RB), *Atheta coriaria* adults. The line equations and coefficients of determination (R^2) are shown. Equations marked with an asterisk are significant at $P \leq 0.05$.

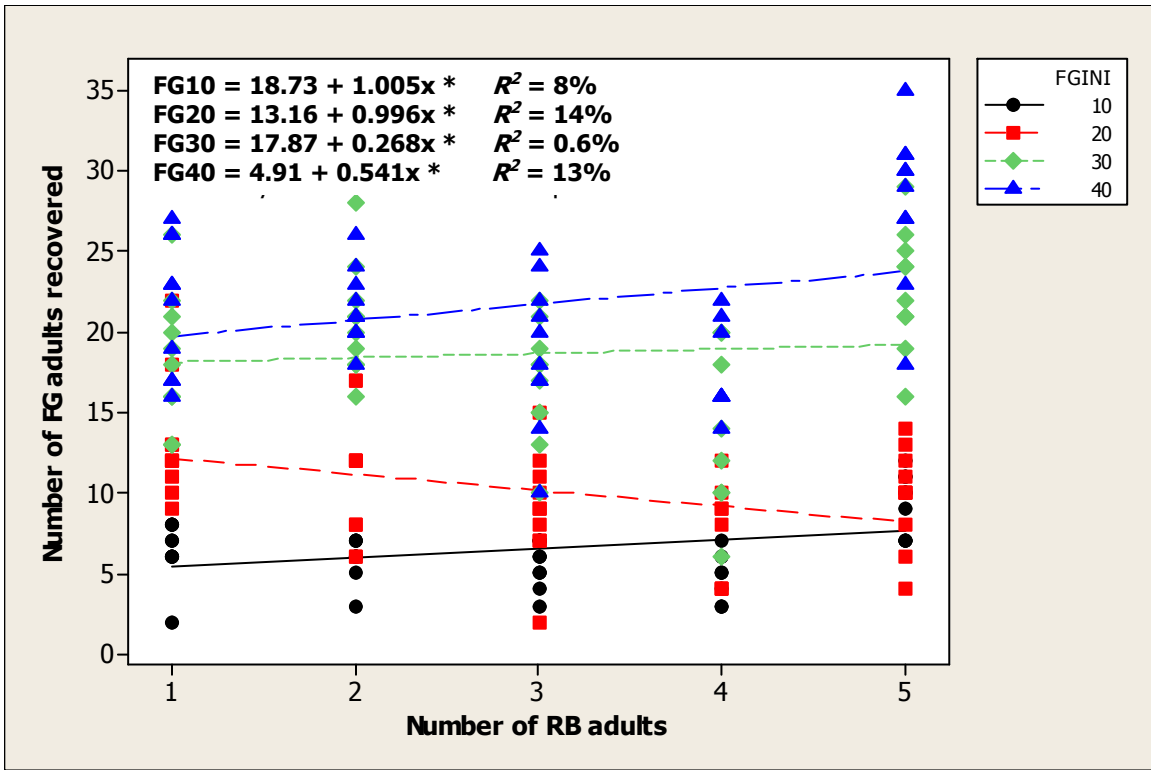


Figure 3-4 Relationship between number of fungus gnat (FG) adults recovered and number of rove beetle, *Atheta coriaria* (RB) adults using initial densities of 10, 20, 30, 40 fungus gnat larvae. The line equations and coefficients of determination (R^2) are shown. Equations marked with an asterisk are significant at $P \leq 0.05$.

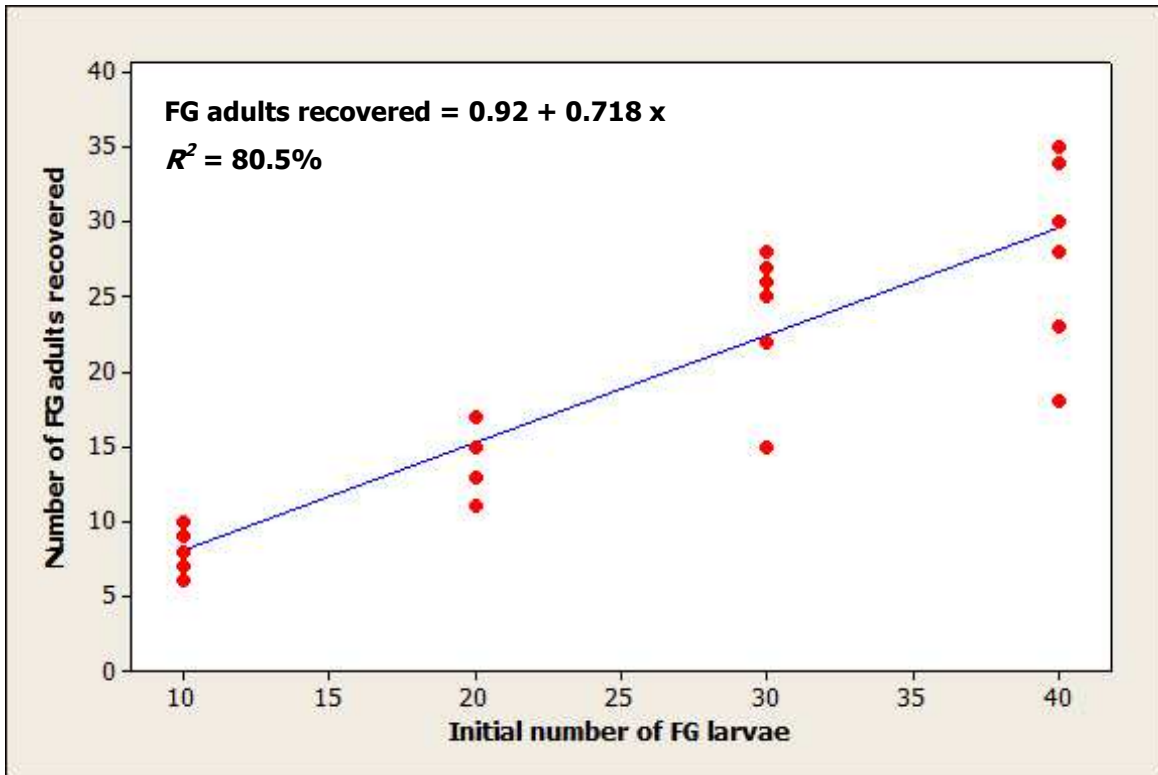


Figure 3-5 Relationship between number of fungus gnat (FG) adults recovered and initial number of FG larvae using one rove beetle, *Atheta coriaria* larva. The line equation and coefficient of determination (R^2) is shown.

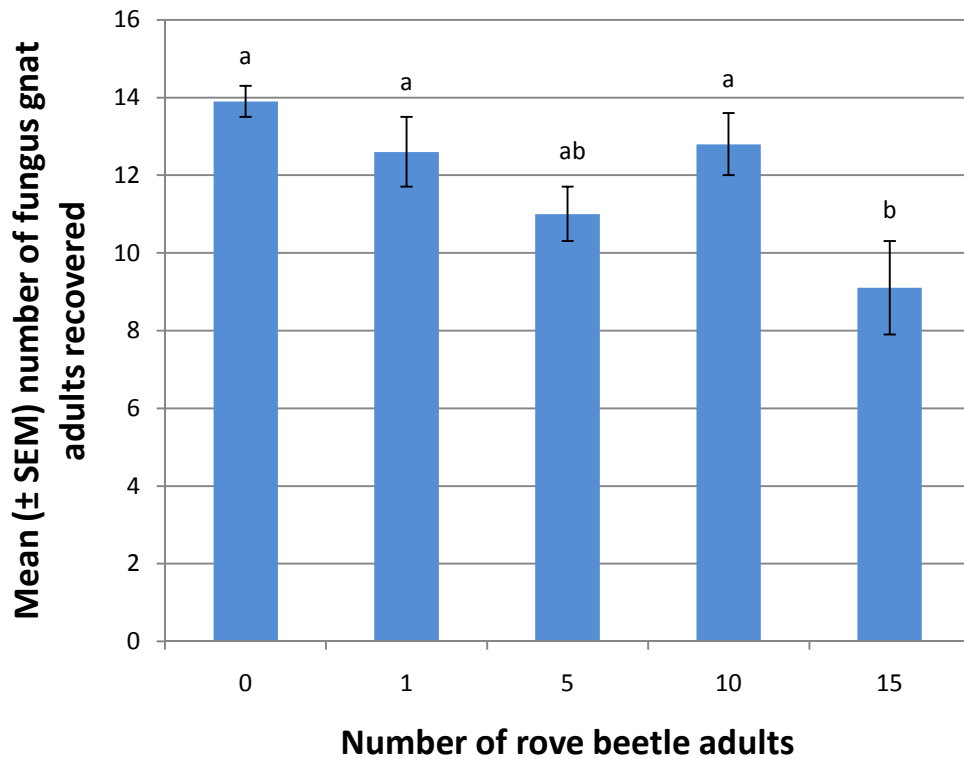


Figure 3-6 Fungus gnat, *Bradysia* sp. nr. *coprophila* adults recovered after inoculating the growing medium (Sunshine LC1 Professional Growing Mix; Sun Gro Horticulture, Inc.; Bellevue, WA) with 20 fungus gnat larvae, and 0, 1, 5, 10 and 15 rove beetle, *Atheta coriaria* adults. There were 8 replications per treatment. Means followed by a common lower case letter among columns are not significantly different ($P \geq 0.05$) as determined by Tukey's mean separation test. Vertical bars represent standard errors of the mean (SEM).

Table 3-1 Mean (\pm SEM) rove beetle (RB), *Atheta coriaria* adult recovery rates associated with different densities, 96 hours after inoculating 300 mL of growing medium (SB300 Universal Professional Growing Mix; Sun Gro Horticulture, Inc.; Bellevue, WA). There were 10 replications per treatment.

Initial RB adult density	Number of RB adults recovered		
	Experiment 1	Experiment 2	Experiment 3
5	4.8 \pm 0.1d ^z	*	*
10	9.5 \pm 0.2c	9.2 \pm 0.2d ^z	9.3 \pm 0.2d ^z
15	14.2 \pm 0.1b	*	*
20	19.4 \pm 0.2a	19.0 \pm 0.3c	19.3 \pm 0.2c
25	*	23.8 \pm 0.4b	*
30	*	28.5 \pm 0.4a	28.5 \pm 0.2b
40	*	*	38.6 \pm 0.3a

^zMeans followed by the same letter within a column are not significantly different ($P \geq 0.05$) as determined by a Tukey's means separation test.

*Asteriks indicate densities that were not evaluated within the experiment.

Table 3-2 Mean (\pm SEM) fungus gnat (FG), *Bradysia* sp. nr. *coprophila* adult recovery rates associated with different prey densities and four different growing medium types: SB300 Universal Professional Growing Mix (Sun Gro Horticulture, Inc.; Bellevue, WA), Sunshine LC1 Professional Growing Mix (Sun Gro Horticulture, Inc.; Bellevue, WA), Sphagnum peat moss (Premier Horticulture, Inc.; Quakertown, PA), and Metro-Mix 360 Growing Mix (Sun Gro Horticulture, Inc.; Bellevue, WA); n = number of replications.

Initial number of FG larvae	FG adults recovered (Mean \pm SEM)			
	Growing medium type			
	SB300 Universal Professional Growing Mix (n = 10)	Sunshine LC1 Professional Growing Mix (n = 10)	Sphagnum peat moss (n = 5)	Metro-Mix 360 Growing Mix (n = 5)
10	11.3 \pm 0.7d ^z	10.3 \pm 0.5d ^z	10.2 \pm 0.4c ^z	3.2 \pm 0.8c ^z
20	20.3 \pm 0.8c	20.7 \pm 0.8c	17.2 \pm 1.0b	6.4 \pm 1.3bc
30	28.4 \pm 0.9b	28.2 \pm 1.1b	29.0 \pm 1.3a	9.0 \pm 0.4b
40	36.5 \pm 1.3a	39.2 \pm 1.3a	32.6 \pm 2.3a	14.0 \pm 1.6a

^zMeans followed by the same letter within a column are not significantly different ($P \geq 0.05$) as determined by a Tukey's means separation test.

Table 3-3 Number of fungus gnat (FG), *Bradysia* sp. nr. *coprophila* adults recovered after inoculating the growing medium (Sunshine LC1 Professional Growing Mix; Sun Gro Horticulture, Inc.; Bellevue, WA) with 10, 20, 30, 40 fungus gnat larvae and 1, 2, 3, 4, 5 rove beetle (RB), *Atheta coriaria* adults; n = number of replications.

Initial number of FG larvae	FG adults recovered (Mean ± SEM)				
	Number of RB adults per 473 mL deli squat container				
	1 (n = 10)	2 (n = 10)	3 (n = 10)	4 (n = 8)	5 (n = 10)
10	6.4 ± 0.6c ^z	5.9 ± 0.4c ^z	5.7 ± 0.4b ^z	4.9 ± 0.5b ^z	9.4 ± 0.6c ^z
20	13.2 ± 1.2b	11.0 ± 1.3b	9.0 ± 1.1b	7.5 ± 1.1b	9.8 ± 1.0c
30	18.4 ± 1.3a	20.7 ± 1.1a	16.8 ± 1.2a	13.8 ± 1.8a	22.7 ± 1.2b
40	21.6 ± 1.3a	21.6 ± 0.7a	18.8 ± 1.5a	17.6 ± 1.0a	28.1 ± 1.5a

^zMeans followed by the same letter within a column are not significantly different ($P \geq 0.05$) as determined by a Tukey's means separation test.

Chapter 4 - EFFECTS OF PESTICIDES AND PLANT GROWTH REGULATORS ON THE ROVE BEETLE, *ATHETA CORIARIA*

Introduction

The continual reliance on conventional broad-spectrum pesticides to manage arthropod pests, in this case insecticides and miticides, has led to problems associated with negative effects to the environment and human health, which has resulted in changes in the pesticide industry, with less toxic and more selective pesticides being introduced particularly for use against greenhouse arthropod pests (Croft, 1990; van Lenteren, 2000a; Pilkington et al., 2010; Bethke and Cloyd, 2009; Gradish et al., 2011). Furthermore, since new pesticides with more selective modes of action are commercially available (biorational, reduced-risk pesticides, and biopesticides) the potential of using biological control agents in combination with pesticides has increased. Consequently, extensive research has investigated the compatibility of biological control agents with pesticides in response to the demand by producers for more effective ways to manage arthropod pest populations in greenhouse production systems (Greathead, 1995; Hassan and van de Veire, 2004; Cloyd, 2005, 2006).

Although conventional pesticides such as those in the chemical classes organophosphate, carbamate, pyrethroid and neonicotinoid are widely used on horticultural crops, pesticide use regulations have restricted their use while promoting pesticides that exhibit lower toxicity and are less of a risk to human health. These include insect growth regulators, microbial pesticides, horticultural oils, insecticidal soaps, and plant-derived essential oil products (Croft, 1990; Cloyd, 2006; Gradish et al., 2011). Most conventional pesticides, in general, are toxic to many natural enemies (Croft and Whalon, 1982). For example, the pyrethroid-based insecticides, lambda-

cyhalothrin and cyfluthrin have been shown to be directly toxic to *Orius insidiosus* (Say), *Hippodamia convergens* (Guérin-Méneville), *Coleomegilla maculata* (De Geer) and *Epicauta pestifera* (Werner) 24 hours after exposure (Al-Deeb et al., 2001), and cyfluthrin is harmful to *Aphytis melinus* (DeBach) based on toxic residues on citrus (Prabhaker et al., 2007) and *Orius insidiosus* (Say) and *Geocoris punctipes* (Say) on cotton (Elzen, 2001). Pyrethroids tend to be harmful to parasitoids, predatory mites and Hemipteran species although they are less toxic to Chrysopids (Blümel et al., 1999). However, Croft and Whalon (1982) noted moderate to high toxicity to certain Chrysopa. The rove beetle, *Aleochara bilineata* (Gyllenhal) is an indicator species for determining the effects of pesticides on soil-dwelling predators (Grimm et al., 2000); however, no information is available on the direct toxic effects of pyrethroid insecticides on other rove beetle species including *Atheta coriaria*.

Neonicotinoid pesticides including acetamiprid, imidacloprid, thiamethoxam, dinotefuran and clothianidin are generally not compatible with many parasitoids and predators (Cloyd and Bethke, 2011). For example, clothianidin and thiamethoxam are directly toxic to *A. coriaria* (Cloyd et al., 2009b) whereas acetamiprid is directly harmful to *Deraeocoris brevis* (Uhler) (Kim et al., 2006), *Encarsia formosa* (Gahan), *Orius laevigatus* (Fieber), *Macrolophus caliginosus* (Wagner) and *Amblyseius californicus* (McGregor) nymphs and adults (van de Veire and Tirry, 2003). In addition, imidacloprid is directly harmful to *A. coriaria* adults and larvae (Jandricic et al., 2006). Many other natural enemy species are sensitive to acetamiprid including *Geocoris punctipes* (Say), *Orius tristicolor* (White), *Chrysoperla carnea* (Stephens) and *Hippodamia convergens* (Guérin-Méneville) (Naranjo and Akey, 2005). Therefore, based on the scientific literature, acetamiprid will likely be directly toxic to *A. coriaria*. In addition, other pesticides have been evaluated to determine compatibility with natural enemies including

metaflumizone, pymetrozine, flonicamid and spirotetramat. Metaflumizone has been reported to be less harmful to beneficial insects than conventional pesticides (Hempel et al., 2007) and slightly toxic to *O. insidiosus*, *Amblyseius swirskii* (Athias-Henriot) and *Eretmocerus eremicus* (Rose and Zolnerowich) (Gradish et al., 2011). Flonicamid and pymetrozine have been shown to be compatible with the rove beetle, *Aleochara bilineata* (Gyllenhal) (Jansen et al., 2011). Spirotetramat is compatible with *Aphidius rhopalosiphi* (De Stefani-Perez), *C. carnea* and *Coccinella septempunctata* (L.), whereas it was harmless to moderately harmful to *Macrolophus caliginosus* (Wagner), *Orius* spp., *Anthocoris nemoralis* (Fabricius), *Chilocorus nigritus* (Fabricius), *C. septempunctata* (L.), *Forficula auricularia* (L.) and *Episyrphus balteatus* (De Geer) (Marčić et al., 2011).

The direct and indirect effects of insect growth regulators (IGRs) including kinoprene, pyriproxyfen, azadirachtin, diflubenzuron and cyromazine on natural enemies has been extensively documented (Hassan and van de Veire, 2004; Cloyd, 2005, 2006). However, there is minimal information available on the effects of IGRs on rove beetles. Insect growth regulators are pesticides that interfere with molting (diflubenzuron, cyromazine) or mimic juvenile hormones (kinoprene, pyriproxyfen), and are widely used against a variety of insect pests of greenhouse-grown crops including whiteflies, aphids, mealybugs, and fungus gnats (Parrella and Murphy, 1998; Cloyd, 2003; Yu, 2008). For example, diflubenzuron was found to be compatible with first instar larvae and adults of *A. bilineata* (Gordon and Cornect, 1986). In addition, diflubenzuron and cyromazine were determined to be harmless to *A. coriaria* adults although high (>80%) mortality was reported for second instar larvae and moderately high (>60%) mortality for third instar larvae (Jandricic et al., 2006). In fact, IGRs that interfere with the

production of chitin such as cyromazine and diflubenzuron may not affect adults and therefore should be compatible with *A. coriaria*.

Pyriproxyfen was compatible whereas diflubenzuron and azadirachtin were harmful to *C. carnea* (Stephens) (Medina et al., 2003a). In two separate studies, kinoprene was harmful to *L. dactylopii* (Rothwangl et al., 2004) and *E. eremicus* (Hoddle et al., 2001). Natural enemy compatibility with azadirachtin is highly variable (Hassan and van de Veire, 2004). For example, azadirachtin was directly toxic to *Trichogramma cacoeciae* (Marchal) although longevity and reproduction were not affected (Saber et al., 2004). In addition, Cloyd et al (2009a) demonstrated compatibility of azadirachtin with *A. coriaria* adults when they were applied to the growing medium after applying the pesticide; however, azadirachtin was directly toxic to adults when applied after release. Furthermore, there were no indirect harmful effects associated with *A. coriaria* adults consuming fungus gnat larvae (Cloyd et al., 2009a). Therefore, other azadirachtin-based products such as Azatin (OHP; Mainland, PA), AzaGuard (BioSafe Systems; East Hartford, CT), Azatrol (PBI/GORDON; Kansas City, MO) and Molt-X (BioWorks; Victor, NY) should not be directly harmful to *A. coriaria* adults or have any detrimental effect on prey consumption. Microbial pesticides including bacterium such as *Bacillus thuringiensis* and entomopathogenic fungi (*Beauveria bassiana* and *Metarrhizium anisopliae*) may be compatible with natural enemies (Croft, 1990). However, compatibility of *B. bassiana* with natural enemies is variable and may depend on whether the natural enemy is a parasitoid or predator. For example, *B. bassiana* was compatible with the parasitoids *Lysiphlebus testaceipes* (Cresson) and *Aphidius colemani* (Viereck) (Murphy et al., 1999) whereas high mortality (>90%) was associated with the predatory ladybird beetle, *Hippodamia convergens* (Guérin-Méneville)

(James and Lighthart, 1994). Indirect toxic effects on natural enemies may be associated with delayed development, reduced fecundity, and decreased prey consumption (Croft, 1990).

Plant growth regulators (PGRs) are natural and synthetic compounds designed to modify plant growth and are used in horticultural cropping systems to regulate plant metabolism and enhance crop productivity (Luckwill, 1981; Nickell, 1994). Plant growth regulators are typically used in very small concentrations, which are degraded rapidly in the plant and soil (Luckwill, 1981). There is minimal information available on the direct and indirect effects of PGRs on natural enemies. Oetting and Latimer (1995) found there were no direct deleterious effects of acymidol (A-Rest; SePRO, Carmel, IN) and paclobutrazol (Bonzi; Syngenta Crop Protection, Greensboro, NC) at the standard and at high rates on *Neoseiulus cucumeris* (Oudemans) and *Orius insidiosus* (Say), which suggests that these PGRs may be compatible with *A. coriaria* adults. However, no such data is available on *A. coriaria*. Therefore, the objective of this study was to determine the compatibility of certain pesticides and PGRs with *A. coriaria* adults, based on both direct and indirect effects associated with development, prey consumption, and reproduction.

Materials and Methods

The direct and indirect effects of selected pesticides and PGRs on the adult rove beetle (RB) *A. coriaria* were evaluated under laboratory conditions [22 to 24°C; 40 to 60% relative humidity (RH), and 0:24 (L:D) hour photoperiod]. Direct effects were assessed based on adult survival and indirect effects were determined based on impact on RB development time from egg to adult, prey consumption, and reproduction (ability to produce offspring). Adult recovery rates were used as an indicator of adult survival. Common name, trade name, rates and company

information associated with the pesticides and PGRs used in this study are presented in Table 1. In total, 15 pesticides and three PGRs were evaluated using standard application rates, although for some treatments; two, three, or four times the labeled rate was used. The selected pesticides are commercially available and classified as “reduced-risk” except for the pyrethroids, lambda-cyhalothrin (CyhaloCap; Whitmire Micro-Gen Research Labs, St Louis, MO) and cyfluthrin (Cy-kick; Whitmire Micro-Gen Research Labs, St Louis, MO). For example, kinoprene (Enstar II; Wellmark International; Schaumburg, IL), pyriproxyfen (Distance; Valent Corp.; Walnut Creek, CA), cyromazine (Citation; Syngenta Crop Protection; Greensboro, NC), organic oil (Zero Tolerance; Natural Garden Solutions; Piedmont, CA) and soybean and rosemary oils (Indoor Pharm; Pharm Solutions; Port Townsend, WA) are labeled for control of arthropod pests of greenhouse-grown crops including fungus gnat larvae. In addition, the three PGRs used [acymidol, paclobutrazol and uniconazole (Sumagic; Valent; Walnut Creek, CA)] are labeled for use in greenhouse production systems as drench applications to the growing medium.

Assessments involved quantification of RB adult survival after exposure to the pesticide treatments for 10 days whereas RB exposure time to the PGR treatments was 96 hours. Rove beetle adult survival was initially assessed after two days of exposure (see Appendix C).

However, the time of exposure was extended based on the pre-sumption that it may take longer than two days for some of the pesticides (microbials and IGRs) to kill insects (Croft, 1990).

A. Direct effects of pesticides and plant growth regulators on Atheta coriaria adults

The direct effects of the pesticides and PGRs on *A. coriaria* adults were assessed in four separate experiments with five pesticides used for experiment 1, seven pesticides for experiment 2, and four pesticides for experiment 3, whereas the three PGRs at three different application rates were used in experiment 4 (Table 1). In addition, direct effects were determined after RB

were applied to the growing medium (GM) 24 hours before, and 24 hours after applying the pesticides, whereas the PGRs were applied 24 hours before releasing the RB adults into 473 mL deli squat containers (Fabri-Kal Corp., Kalamazoo, MI). The experiments were set-up as a completely randomized block design with two blocks (days as blocks) and 10 replications per treatment. The number of treatments for each experiment are presented in Table 1.

The direct effects of pesticides on RB adults were determined following the procedures described by Cloyd et al. (2009a). Three-hundred mL of GM (Sunshine LC1 Professional Growing Mix; Sun Gro Horticulture, Inc.; Bellevue, WA) was measured using a 600 mL glass beaker and placed into a 473 mL deli squat container. Modified lids with insect screening were used to allow for ventilation. Before preparing the pesticide and PGR solutions, individual pesticides and PGRs were measured using a 1 mL sterile syringe (BD; Franklin Lakes, NJ), and 10, 100 and 500 mL graduated cylinders, except for acetamiprid and cyromazine (solid formulations), which were measured using a balance scale (Denver Instrument; Bohemia, NY). Pesticides and PGRs were mixed with water separately using a 600 mL glass beaker in order to obtain the adequate concentration for each treatment. Subsequently, 70 mL of each pesticide and PGR solution was applied uniformly as a drench to the GM in each deli squat container, and 1 tsp of raw oatmeal (≈ 1.5 g) was placed on the GM surface as a food source for the RB adults. In addition, a water control was included in all experiments.

Twenty RB adults (males and females of various ages) were randomly collected from the main colony (maintained at Kansas State University, Manhattan, KS) by sieving the GM using #5 and #10 mesh size sieves, recovered into a 9 dram plastic vial using an aspirator, and then placed into a container. Initially, the containers were warmed by heat lamps (Commercial Electric; Atlanta, GA) situated about 90 cm above the containers for 24 hours, thus allowing the

GM to dry, which facilitated the sieving procedure (Appendix E, Figure 1). After 10 days, RB adults were collected, based on the procedure described previously, and the number of live RB adults was recorded.

B. *Indirect effects of pesticides and plant growth regulators on Atheta coriaria adults*

Indirect effects of pesticides and PGRs were assessed based on *A. coriaria* development time from egg to adult and prey consumption in two separate experiments with 19 and 17 treatments with 7 replications per treatment, using RB adults obtained from the direct toxicity experiments described above.

In order to determine the indirect effects of the pesticides and PGRs on the duration of RB development time from egg to adult, approximately 10 male and female RB adults previously exposed to the treatments (pesticides and PGRs) were placed on a filter paper disk lining the bottom of a 90 x 10 mm Petri dish with 2 tsp (\approx 3 g) of moistened Sunshine LC1 Professional Growing Mix, and 1 to 2 pieces of raw oatmeal as a supplemental food source. In addition, a piece of moistened cotton was placed in the Petri dish in order to maintain constant moisture. Petri dishes were labeled and then placed separately into a 740 mL plastic container (Appendix E, Figure 2). Subsequently, the plastic containers were maintained for one week in an illuminated incubator (Thermo Electron Corporation; Marietta, OH) at $26 \pm 2^\circ\text{C}$ and 50 to 60% RH, under 12:12 (L:D) hour photoperiod for egg deposition. Every 24 hours, the GM was examined under a dissecting microscope (10 x 22) for the presence of eggs. Rove beetle eggs were collected using a moistened, soft fine camel-hair paintbrush and then placed individually on a filter paper disk lining the bottom of a 90 x 10 mm Petri dish with 2 tsp (\approx 3 g) of GM and 1 to 2 pieces of oatmeal as a food source. Petri dishes were placed separately into a 740 mL plastic container and maintained under laboratory conditions [22 to 24°C ; 40 to 60% RH, and 0:24

(L:D) hour photoperiod]. The GM was examined daily under a dissecting microscope to prevent the growth of mold and fungi, and time (days) from oviposition to adult emergence was recorded.

The indirect effects of pesticides and PGRs on adult *A. coriaria* prey consumption was evaluated in a separate experiment using the following procedure. A total of 20 second (six to seven days old) or third (eight to nine days old) instar fungus gnat larvae were collected from a FG-inoculated growing medium (Sunshine LC1 Professional Growing Mix) sample using a 150 mm disposable flint glass non-sterile Pasteur pipet (Fisher Scientific[®]; Pittsburgh, PA), which were then placed into a 50 x 15 mm glass Petri dish filled with 10 to 15 mL of water. Larvae were collected individually using the Pasteur pipet, transferred to the glass Petri dish and counted. Subsequently, 1.6 mL of water was poured into a 100 x 15 mm glass Petri dish using a 10 mL capacity plastic graduated cylinder. The bottom of the Petri dish was lined with 90 mm diameter qualitative filter paper (Whatman Int. Ltd.; Maidstone, UK).

For each of the treatments (pesticides, PGRs and water control), 10 treated RB adults, including males and females, were placed into a 473 mL deli squat container with 300 mL of moistened GM (Sunshine LC1 Professional Growing Mix) and oatmeal as a supplemental food source for approximately three weeks in order to obtain F₁ generation adults, which were used to assess predation. Subsequently, a single F₁ RB adult was placed into the Petri dish and the number of FG larvae consumed from a total of 20 was determined after 24 hours based on the number of head capsules (sclerotized portion of the larval head) in the Petri dish. The number of head capsules was counted under a dissecting microscope (10 x 22) and recorded. Untreated RB adults were used as a control whereas the check consisted of 20 FG larvae and no RB adults.

Similarly, in order to assess the indirect effects of the pesticides and PGRs on RB reproduction, 300 mL of GM (Sunshine LC1 Professional Growing Mix) were measured using a 600 mL glass beaker and placed into a 473 mL deli squat container. The treatments were applied following the procedure described to determine any direct toxic effects using the application rates presented in Table 1. For each of the treatments associated with experiments 1 and 2 (pesticides and the control) 10 RB adults (males and females of various ages) were randomly collected from the main colony by sieving the GM using #5 and #10 mesh size sieves and recovered into a 9 dram plastic vial using an aspirator. Subsequently, RB adults were placed into the container. After three weeks, the ability of RB adults to reproduce was determined based on the number of RB adults and larvae in the containers. The number of live adults and larvae was counted and recorded. There were six replications per each treatment.

Statistical analysis

Data from all experiments were analyzed using a statistical analysis software program SAS Systems for Windows, version 9.2 (SAS Institute 2002). Data associated with the direct toxic effects of pesticides and PGRs on *A. coriaria* were subjected to an analysis of variance (ANOVA) using the PROC ANOVA procedure (SAS Institute 2002) with the number of live RB adults as the response. Additionally, significant differences among the treatments were determined using a Tukey's least significant means test at a significance level of $\alpha = 0.05$. In addition, data associated with the indirect effects of the pesticides and PGRs on *A. coriaria* development time from egg to adult, prey consumption, and reproduction were subjected to an ANOVA using days, number of FG larval head capsules, and number of RB adults and larvae as the response variables. Significant differences among the treatments were determined using a Tukey's least significant means test at a significance level of $\alpha = 0.05$.

Results

A. Direct effects of pesticides and plant growth regulators on *Atheta coriaria* adults

Results associated with experiments 1 to 3, in which the RB adults were applied to the GM 24 hours before and after applying the pesticides, are presented in Tables 2 through 7. In addition, results from experiment 4, in which RB adults were applied to the GM 24 hours after applying the PGRs, are presented in Table 8. Results from experiment 1 are presented in Tables 2 and 3. There were significant differences among the treatments when the rove beetle adults were released both before ($F = 28.57$; $df = 5, 59$; $P \leq 0.0001$) and after ($F = 13.52$; $df = 5, 59$; $P \leq 0.0001$) applying the pesticides. The number of RB adults recovered, after application, from the acetamiprid treatment at the high rate (0.028 g/70 mL) was 10.3 ± 1.3 (mean \pm SEM), which was similar to the recovery rate (13.3 ± 0.6) obtained when acetamiprid was applied at the low rate (0.014 g/70 mL). Rove beetle adult recovery rates were similar (not statistically different) for kinoprene, organic oils and soybean and rosemary oil, whereas a recovery rate of 17.6 ± 0.2 was obtained for the water control (Table 2). When rove beetles were released before applying the pesticides, there were no significant differences among the number of RB adults recovered from the kinoprene and organic oil (13.5 ± 0.6 and 16.0 ± 0.4) treatments and the water control (12.8 ± 0.3) (Table 3). The recovery rate associated with acetamiprid at the low rate was similar to the soybean and rosemary oil treatment, whereas the lowest adult recovery rate was obtained from the acetaprimid treatment at the high rate. In addition, when RB were released after applying the pesticides, RB recovery rates associated with the acetamiprid (at both high and low rates) and kinoprene treatments were significantly lower than the control, whereas when RB adults were released before applying the pesticides, recovery rates for the acetamiprid (at both

high and low rates) and soybean and rosemary oil treatments were significantly lower than the control (Figure 1).

Results from experiment 2 are presented in Tables 4 and 5. There were significant differences among the treatments when rove beetle adults were released both before ($F = 64.51$; $df = 9, 99$; $P \leq 0.0001$) and after ($F = 61.26$; $df = 9, 99$; $P \leq 0.0001$) applying the pesticides. There were no significant differences in the recovery rates among the treatments *Beauveria bassiana* (at low and high rates), metaflumizone, azadirachtin, pyriproxyfen, cyromazine and the water control, when RB were released after applying the pesticides. The lowest recovery rates were associated with cyfluthrin (at both low and high rates), which were significantly lower than lambda-cyhalothrin and the other treatments (Table 4). When RB were released before application, there were no significant differences among the *Beauveria bassiana* (at both low and high rates), azadirachtin, and cyromazine treatments, and the water control. In addition, pyriproxyfen was similar to metaflumizone and both were significantly higher in terms of adult survival than lambda-cyhalothrin and cyfluthrin (at both high and low rates) (Table 5).

Overall, when RB were applied to the GM after applying the pesticides, only the pyrethroids lambda-cyhalothrin and cyfluthrin (at both high and low rates) were significantly lower in regards to adult survival than the control, whereas when RB were applied to the GM before applying the pesticides metaflumizone, pyriproxyfen and lambda-cyhalothrin and cyfluthrin were significantly lower in adult survival than the control (Figure 2).

Results for experiment 3 in which three azadirachtin products (AzaGuard: BioSafe Systems; East Hartford, CT; Azatrol: PBI/GORDON; Kansas City, MO; and Molt-X: BioWorks; Victor, NY) and spirotetramat (Kontos: OHP; Mainland, PA) were used are presented in Tables 6 and 7. The highest RB adult recovery rate was associated with Azatrol and the lowest with

Molt-X when RB were released into the containers after application. Similarly, when RB were released into the containers before applying the pesticides, the highest recovery rate was associated with Azatrol and the lowest with spirotetramat (Table 7). However, there were no significant differences among the treatments when RB were released either before ($F = 0.55$, $df = 4, 24$, $P \leq 0.698$) or after ($F = 1.55$, $df = 4, 39$, $P \leq 0.208$) applying the pesticides (Figure 3).

Results from experiment 4, associated with the nine PGR treatments (three PGRs with three application rates per PGR) are presented in Table 8. Recovery rates for all the treatments were high (>90%) with 18 to 19 RB adults recovered per treatment. As such, there were no significant differences ($F = 0.42$, $df = 9, 99$, $P \leq 0.924$) among the treatments (Figure 4).

B. Indirect effects of pesticides and plant growth regulators on *Atheta coriaria* adults

The indirect effects of the pesticides and PGRs on *A. coriaria* development time from egg to adult are presented in Tables 9 and 10. There were no significant differences ($F = 1.40$, $df = 11, 83$, $P \leq 0.193$) among the pesticide treatments (Table 9). The longest duration (23.7 ± 0.6 days) was associated with kinoprene, the shortest (21.7 ± 0.6 days) with the soybean and rosemary oil treatment. Similarly, there were no significant differences ($F = 2.02$, $df = 7, 55$, $P \leq 0.071$) among the PGR treatments (Table 10). The longest duration (22.7 ± 1.2 days) was associated with uniconazole at the high rate and the shortest duration (21.0 ± 0.0 days) with acymidol at the high rate (Table 10).

The indirect effects of the pesticides and PGRs on RB prey consumption are presented in Table 11. There were significant differences in the number of FG larvae consumed among the treatments ($F = 11.07$, $df = 15, 111$, $P \leq 0.0001$). The number of head capsules present in the Petri dish was significantly lower for kinoprene (15.0 ± 1.0) than the organic oils and water control (18.3 ± 0.5 and 18.9 ± 0.4) but significantly higher than *Beauveria bassiana* (11.4 ± 0.6)

when applied at the high rate. The lowest number of FG larval head capsules occurred in the *Beauveria bassiana* treatments with no significant differences between low and high rates. The number of head capsules was high (17 to 18 out of 20) for the organic oil, soybean and rosemary oil, pyriproxyfen and cyromazine treatments, the four azadirachtin-based products (Azatin, AzaGuard, Azatrol and Molt-X) and spirotetramat treatment. However, there were no significant differences among the pesticide treatments compared to the control (Figure 5). Similarly, there were no significant differences among the PGR treatments (Figure 5).

The indirect effects of the pesticides used in experiments 1 and 2 on *A. coriaria* reproduction are presented in Table 12. Based on the number of RB adults recovered, there were significant differences ($F = 19.47$; $df = 14, 89$; $P \leq 0.0001$) among the treatments. The number of RB adults recovered for the kinoprene, organic oils and soybean and rosemary oil treatments was significantly higher (6 to 7 adults out of 10) than acetamiprid when applied at the low and high rates, whereas the number of adults for the *Beauveria bassiana* (at both low and high rates), azadirachtin, pyriproxyfen and cyromazine treatments was significantly higher than metaflumizone, lambda-cyhalothrin and cyfluthrin (at both low and high rates). There were significant differences ($F = 13.22$; $df = 14, 89$; $P \leq 0.0001$) in the number of RB larvae recovered among the treatments. However, no RB adults were recovered from the cyfluthrin treatment when applied at a high rate. In addition, the number of the RB larvae for the organic oils treatment was significantly higher than the control whereas no larvae were recovered from the metaflumizone, cyfluthrin (at both low and high rates), pyriproxyfen and acetamiprid (at the high rate) treatments. There were no significant differences in the number of larvae recovered among the *Beauveria bassiana* (at both low and high rates), kinoprene, organic oils and soybean and rosemary oil treatments. Furthermore, the number of RB larvae recovered from the azadirachtin,

cyromazine and acetamiprid (at the low rate) treatments was low (1 to 2 larvae out of 10), which was similar to the kinoprene and soybean and rosemary oil treatments, and the control. Rove beetle larvae (approximately 2 to 3 per container) were observed after applying AzaGuard, Azatrol, Molt-X and Kontos (experiment 3). In addition, RB adults and larvae were observed after applying the nine PGR treatments (experiment 4).

Discussion

In this study, the pesticides acetamiprid, kinoprene, lambda-cyhalothrin, and cyfluthrin were harmful to *Atheta coriaria* adults, whereas *Beauveria bassiana*, azadirachtin, organic oils and the plant growth regulators were compatible with *A. coriaria*. Pesticides labeled for control of FG larvae, such as organic oils and soybean and rosemary oil, were not directly harmful when applied at the standard application rates to the GM 24 hours before releasing RB adults. However, kinoprene and acetamiprid, when applied at low and high rates (0.014 ml/70 ml and 0.028 ml/70 ml), were directly toxic to RB adults. As such, it appears that organic oils and soybean and rosemary oil are compatible with *A. coriaria*, whereas kinoprene and acetamiprid are not. However, when the pesticides were applied after releasing RB adults into the containers, soybean and rosemary oil was directly harmful to RB adults whereas organic oil was not. In fact, lower adult survival rates were obtained for the control, acetamiprid (at both low and high rates) and soybean and rosemary oil, which suggests the time that RB adults are released into the containers (whether before or after pesticides are applied) may influence *A. coriaria* compatibility with pesticides. These results are similar to Cloyd et al. (2009b), in which soybean and rosemary oil negatively affected rove beetle survival, when applied at the same rate, and the neonicotinoid-based insecticides clothianidin, dinotefuran and thiamethoxam were also not

compatible with *A. coriaria* adults. It has been shown that soybean and rosemary oil are directly toxic to the predatory mite, *Amblyseius barkeri* (Hughes) (Momen and Amer, 1999). Overall, the organic oil treatment was compatible with *A. coriaria* when applied at the standard application rate, whereas soybean and rosemary oil and acetamiprid (at both low and high rates) were not compatible with *A. coriaria*.

Rove beetle adult survival was lower when adults were released before rather than after applying the pesticides except for kinoprene and organic oils where adult survival was similar. It appears that RB adults may have escaped exposure when released after application of soybean and rosemary oil, and when released before applying kinoprene since survival was not significantly different than the control. Acetamiprid was less toxic when applied at the standard application rate than at the high rate when RB adults were released before application, whereas there was no difference in direct toxicity when RB were released after application.

The pyrethroids, lambda-cyhalothrin and cyfluthrin (applied at low and high rates) were directly harmful to RB adults regardless of the time that adults were released into the containers. *Beauveria bassiana* (applied at low and high rates), azadirachtin, and cyromazine were compatible with the RB. In addition, metaflumizone and pyriproxyfen were directly toxic to RB adults when applied after release in the containers. However, when RB were released after applying the pesticides, both metaflumizone and pyriproxyfen did not negatively affect RB survival, which suggests that RB may have avoided exposure to the pesticides. Metaflumizone has been demonstrated to be compatible with predators such as *Orius insidiosus* (Say) and *Amblyseius swirskii* (Athias-Henriot) (Gradish et al., 2011), whereas pyriproxyfen is compatible with *Chrysoperla carnea* (Stephens) (Medina et al., 2003b), *Stratiolaelaps simitus* (Womersley) (Cabrera et al., 2004) and *Cryptolaemus montrouzieri* (Mulsant) (Cloyd and Dickinson, 2006b).

Although RB survival was similar for metaflumizone and pyriproxyfen, these pesticides were less deleterious to RB adults than both lambda-cyhalothrin and cyfluthrin when applied at low and high rates. In addition, *Beauveria bassiana* when applied at low and high rates, azadirachtin and pyriproxyfen were not directly harmful to *A. coriaria*. However, cyromazine was similar to metaflumizone and pyriproxyfen in terms of direct toxicity when applied after RB were released into the containers.

Although lambda-cyhalothrin was less harmful to RB than cyfluthrin (at low and high rates) when released after applying the pesticides, both were equally harmful when RB were released before application. Furthermore, cyfluthrin was directly harmful to RB adults regardless of the application rate, with no differences in RB adult survival. Overall, RB adult survival in experiment 2 was lower when releases were made before rather than after applying the pesticides. *Beauveria bassiana* (at the standard application rate and at high rate), azadirachtin and cyromazine are compatible with *A. coriaria*. As such, these pesticides can be applied without compromising biological control programs against fungus gnats when using *A. coriaria*. However, reduced numbers of FG larvae due to pesticide applications could affect availability of FG larvae as a food source, which may indirectly influence RB adult survival. Moreover, further evaluations, when RB are released before application, are required before using metaflumizone and pyriproxyfen in combination with *A. coriaria*. The findings of the current study corroborate those of Jandricic et al (2006) in which cyromazine was compatible with *A. coriaria* adults. Similarly, cyromazine was compatible with the parasitoid *Chrysocharis parksi* (Crawford) (Parrella et al., 1983) and diflubenzuron was compatible with the rove beetle, *Aleochara bilineata* (Gordon and Cornect, 1986), whereas kinoprene negatively affected survival of the

parasitoid *Opius dimidiatis* (Ashmead) (Poe, 1974). As such, compatibility with IGRs may vary depending on the active ingredient and possibly natural enemy type.

The azadirachtin-based products evaluated (AzaGuard, Azatrol and Molt-X) and spirotetramat were not harmful to RB adults regardless of the time in which RB were released into the containers (whether before or after applying the pesticides), which suggests that these pesticides are compatible with *A. coriaria*. These results are similar and elaborate on the findings of Cloyd et al (2009b) when using azadirachtin, in which adult *A. coriaria* survival was not directly affected by the pesticide. However, the direct toxic effects of azadirachtin on natural enemies may vary depending on the species. For example, azadirachtin was harmful to *C. carnea* larvae (Medina et al., 2003a) and moderately toxic to *Trichogramma cacoeciae* (Marchall) adults (Saber et al., 2004), whereas azadirachtin was compatible with *L. dactylopii* (Rothwangl et al., 2004). Based on the short residual toxicity exhibited by azadirachtin, releases made shortly after pesticide application were recommended in order to avoid direct toxicity to *Macrolophus caliginosus* (Wagner) (Tedeschi et al., 2001). In addition, there were no direct toxic effects on *A. coriaria* associated with the three PGRs acymidol, paclobutrazol and uniconazole when applied at the standard application rates or high rates, which suggests that these PGRs are very compatible with *A. coriaria* adults. This is similar to the findings of Oetting and Latimer (1995) in which four PGRs including acymidol and paclobutrazol were compatible with the predators *Neoseiulus cucumeris* (Oudemans) and *Orius insidiosus* (Say).

Atheta coriaria development time from egg to adult was not affected by any of the pesticides evaluated. Although two of the pesticides used exhibited direct toxicity to *A. coriaria* adults (kinoprene and soybean and rosemary oil) it appears that these did not arrest larval development or exhibit significant direct effects on duration of development time from egg to

adult in the F₁ generation. Therefore, these pesticides may be applied without affecting predator:prey synchronization (meaning that predacious stages coincide with susceptible development stage of the prey in time), which is an index of natural enemy effectiveness (Bigler, 1989; Fournet et al., 2000). Similarly, Peleg (1983) found that methoprene did not inhibit larval development of *Chilocorus bipustulatus* L. although pupation was negatively affected. Additionally, RB adults exposed to acetamiprid, metaflumizone, pyriproxyfen, lambda-cyhalothrin and cyfluthrin did not produce offspring or the F₁ RB died before completing development. Whether these pesticides indirectly inhibited egg hatch, or larval or pupal development was difficult to determine when using GM as a substrate, which suggests that a different technique may be appropriate. Furthermore, the PGRs, acymidol, paclobutrazol and uniconazole did not affect *A. coriaria* development when applied at the standard application rates or at high rates. These results are important since there is minimal information available associated with the indirect effects of “reduced-risk” pesticides or PGRs on the development of soil-dwelling predators.

Atheta coriaria consumption of FG larvae was indirectly affected by kinoprene, and *Beauveria bassiana* at low and high rates, whereas organic oils, soybean and rosemary oil, azadirachtin, pyriproxyfen, cyromazine and spirotetramat did not negatively affect prey consumption. These results differ from Momen and Amer (1999) in which rosemary oil decreased the food consumption rate of *Amblyseius barkeri* (Hughes). Prey consumption (of FG larvae) was not inhibited by any of the azadirachtin-based products evaluated (Azatin, AzaGuard, Azatrol and Molt-X), which coincides with Cloyd et al. (2009b). In addition, the PGRs acymidol, paclobutrazol and uniconazole were not indirectly toxic to *A. coriaria* in terms of inhibiting FG larval consumption when applied at the recommended labeled rates.

Based on the number of RB larvae after 21 days of exposure to the pesticides, *Beauveria bassiana* (at both low and high rates), azadirachtin, cyromazine, kinoprene, organic oils and soybean and rosemary oil did not negatively affect *A. coriaria* reproduction whereas metaflumizone, lambda-cyhalothrin, cyfluthrin (at both low and high rates), pyriproxyfen and acetamiprid (at the high rate) inhibited RB female reproduction. Low reproduction may be associated with reduced egg hatch or decreased fecundity. For example, pyriproxyfen has been shown to inhibit egg hatch of *C. carnea* when applied at a high rate (150 g/L) (Medina et al., 2003). Furthermore, pyriproxyfen was discovered to decrease citrus mealybug, *Planococcus citri* (Risso) egg production (Cloyd, 2003), whereas *Macrolophus caliginosus* (Wagner) adults exposed to azadirachtin-based products exhibited reduced fecundity (Tedeschi et al., 2001). The findings from this study differ from Momen and Amer (1999) in which soybean and rosemary oil inhibited *Amblyseius barkeri* (Hughes) reproduction. Interestingly, females exposed to lambda-cyhalothrin and metaflumizone that did not produce offspring in treated GM, laid eggs and produced offspring, after they were transferred to untreated GM. In addition, apparently, organic oils numerically increased female RB egg production, which is not an uncommon phenomenon. Since the number of larvae across treatments was low (<10 per container), differences between treatments may be partly explained by the variation associated with individual female fecundity at the beginning of the oviposition period. Therefore, further studies should include the number of eggs laid per individual female in order to assess female fecundity. However, the results obtained in this study demonstrate that some pesticides may inhibit RB female reproduction. In contrast, it appears that the azadirachtin-based products (AzaGuard, Azatrol and Molt-X) and spirotetramat, and the nine PGR treatments (at the standard and higher rates) were not detrimental to RB reproduction.

In summary, acetamiprid (at both low and high rates), lambda-cyhalothrin and cyfluthrin (at both low and high rates) were directly harmful to *A. coriaria* whereas *Beauveria bassiana* (at both low and high rates), azadirachtin, spirotetramat, cyromazine, and the PGRs acymidol, paclobutrazol and uniconazole did not directly affect RB survival. Furthermore, there were no indirect toxic effects of the pesticides on *A. coriaria* development from egg to adult except for acetamiprid, metaflumizone, pyriproxyfen, lambda-cyhalothrin and cyfluthrin, which inhibited the production of F₁ RB adults. In addition, none of the PGR treatments negatively affected RB development. However, kinoprene and *Beauveria bassiana* (at low and high rates) exhibited a negative effect on RB prey consumption (FG larvae). Acetamiprid (at the high rate), metaflumizone, lambda-cyhalothrin, cyfluthrin (at both low and high rates) and pyriproxyfen also inhibited *A. coriaria* reproduction.

Results from this study demonstrate that certain reduced-risk pesticides can be applied against fungus gnat populations without affecting *A. coriaria* performance as a biological control agent whereas pyrethroids are highly toxic to RB adults regardless of the release time. Although the pesticides and plant growth regulators evaluated did not affect *A. coriaria* development time, prey consumption was influenced by *Beauveria bassiana* and kinoprene, whereas pesticides such as metaflumizone and the pyrethroids negatively affected *A. coriaria* reproduction. Further studies are warranted to investigate the direct effect of pesticides under greenhouse conditions, since pesticides that are harmful in laboratory experiments may not be harmful in caged experiments, as noted by Rothwangl et al., (2004) for kinoprene on the parasitoid *L. dactylopii*.

Figures and Tables

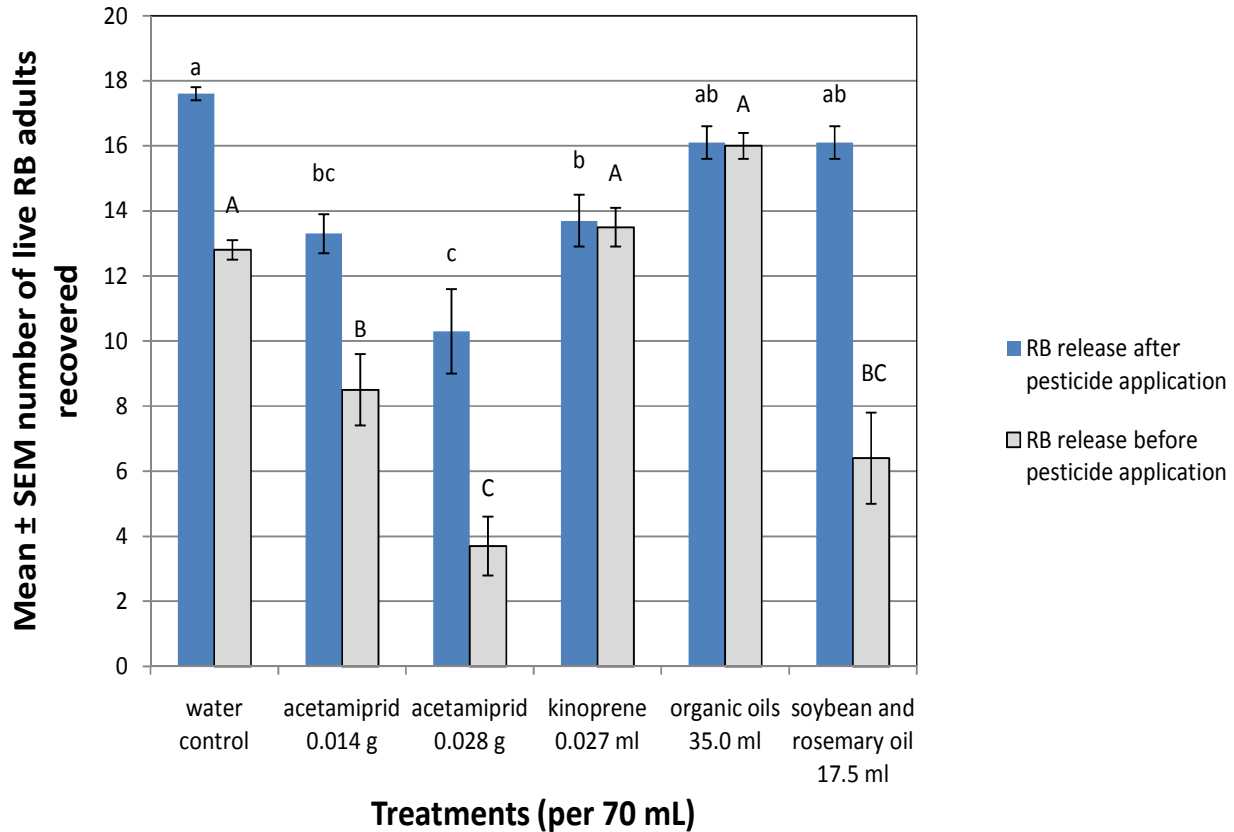


Figure 4-1 Rove beetle (RB), *Atheta coriaria* adult recovery rates 10 days after exposure to pesticides used in experiment 1 in which 20 adults were applied in containers with 300 mL of growing medium (SB300 Universal Professional Growing Mix; Sun Gro Horticulture, Inc.; Bellevue, WA) 24 hours before and 24 hours after applying the pesticides. There were 10 replications per treatment. Means followed by the same letter are not significantly different ($P > 0.05$) as determined by Tukey's mean separation test. Vertical bars represent standard errors of the mean (SEM). The pesticides and rates used were acetamiprid (TriStar; Cleary's, Dayton, NJ) at 0.014 g/70 mL, acetamiprid (TriStar; Cleary's, Dayton, NJ) at 0.028 g/70 mL, kinoprene (Enstar II; Wellmark International, Schaumburg, IL) at 0.027 mL/70 mL, organic oils (Zero Tolerance; Natural Garden Solutions, Piedmont, CA) at 35.0 mL/70 mL and soybean and rosemary oil (Indoor Pharm; Pharm Solutions, Port Townsend, WA) at 17.5 mL/70 mL.

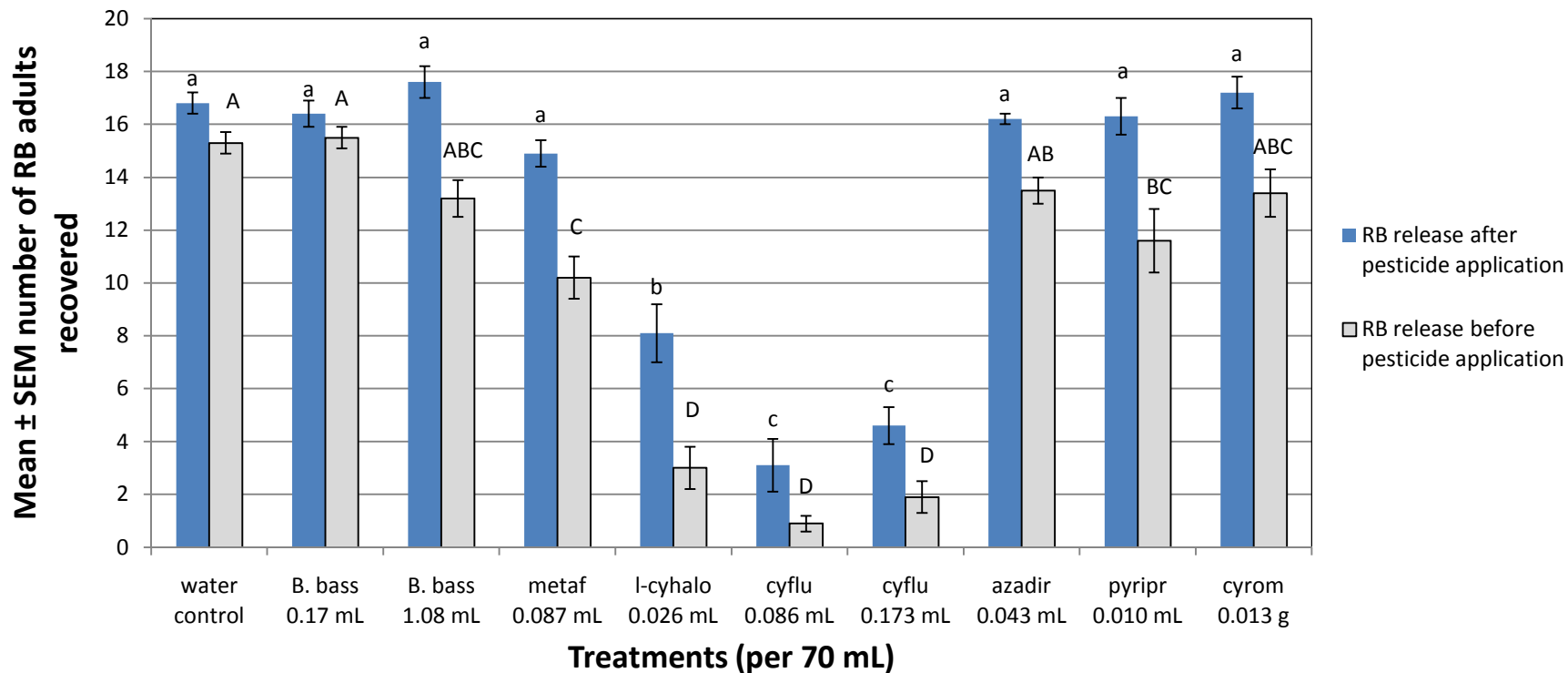


Figure 4-2 Rove beetle (RB), *Atheta coriaria* adult recovery rates 10 days after exposure to pesticides used in experiment 2 in which 20 adults were applied in containers with 300 mL of growing medium (SB300 Universal Professional Growing Mix; Sun Gro Horticulture Inc.; Bellevue, WA) 24 hours before and 24 hours after applying the pesticides. There were 10 replications per treatment. Means followed by the same letter are not significantly different ($P > 0.05$) as determined by Tukey's mean separation test. Vertical bars represent standard errors of the mean (SEM). The pesticides and rates used were *Beauveria bassiana* (*B. bass*) (BotaniGard; BioWorks, Victor, NY) at 0.17 mL/70 mL, *Beauveria bassiana* (*B. bass*) (BotaniGard, BioWorks; Victor, NY) at 1.08 mL/70 mL, metaflumizone (metaf) (Alverde; BASF Corp., Research Triangle Park, NC) at 0.087 mL/70 mL, lambda-cyhalothrin (l-cyhalo) (CyhaloCap; Whitmire Micro-Gen Research Labs, St. Louis, MO) at 0.026 mL/70 mL, cyfluthrin (cyflu) (Cy-kick; Whitmire Micro-Gen Research Labs, St. Louis, MO) at 0.086 mL/70 mL, cyfluthrin (cyflu) (Cy-kick; Whitmire Micro-Gen Research Labs, St. Louis, MO) at 0.173 mL/70 mL, azadirachtin (azadir) (Azatin; OHP, Mainland, PA) at 0.043 mL/70 mL, pyriproxyfen (pyripr) (Distance; Valent Corp., Walnut Creek, CA) at 0.010 mL/70 mL, cyromazine (cyrom) (Citation; Syngenta Crop protection, Greensboro, NC) at 0.013 g/70 mL.

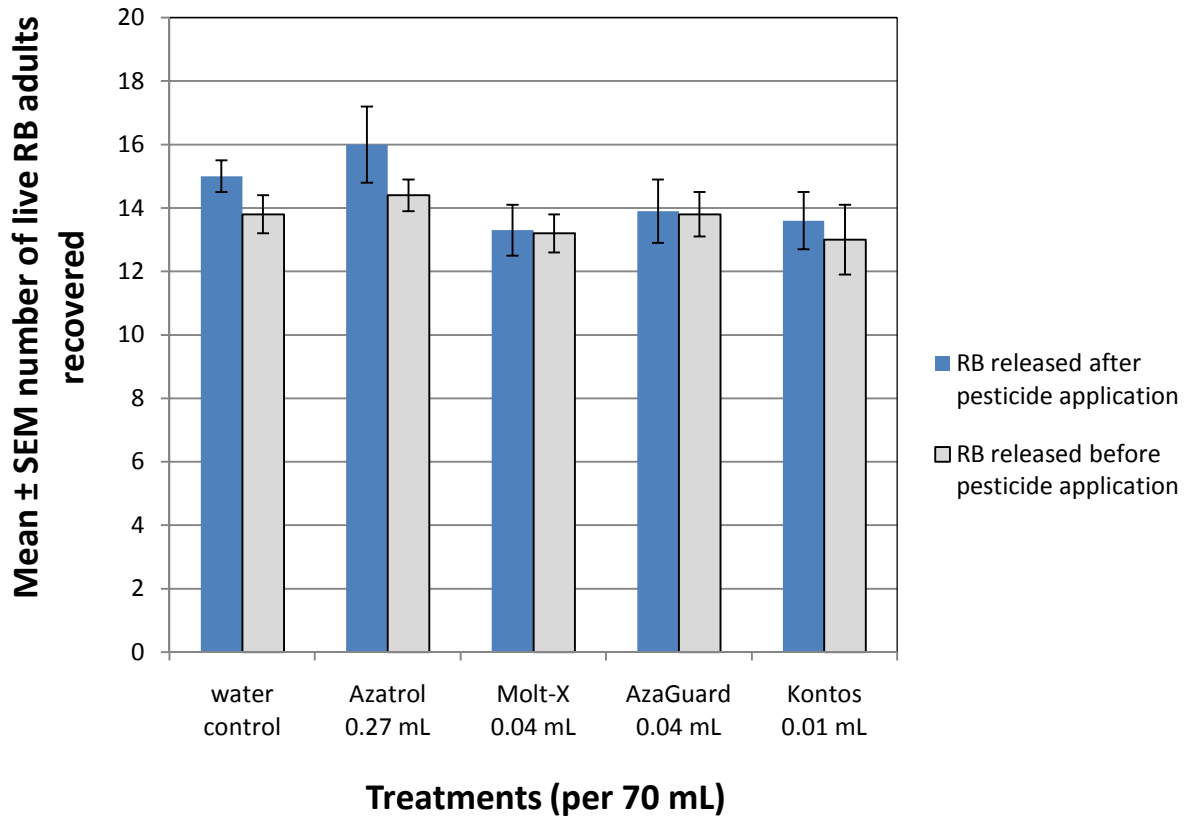


Figure 4-3 Rove beetle (RB), *Atheta coriaria* adult recovery rates 10 days after exposure to pesticides used in experiment 3 in which 20 adults were applied in containers with 300 mL of growing medium (Sunshine LC1 Professional Growing Mix; Sun Gro Horticulture, Inc.; Bellevue, WA) 24 hours before and 24 hours after applying the pesticides. The number of replications when released before and after application was 5 and 8 replications per treatment. None of the treatments associated with rove beetles released either before ($F = 0.55$, $df = 4,24$, $P \leq 0.698$) or after ($F = 1.55$, $df = 4,39$, $P \leq 0.208$) applying the pesticides were significantly different from each other. Vertical bars represent standard errors of the mean (SEM). The pesticides and rates used were azadirachtin (Azatrol; PBI/GORDON, Kansas City, MO) at 0.27 mL/70 mL, azadirachtin (Molt-X; BioWorks, Victor, NY) at 0.04 mL/ 70 mL, azadirachtin (AzaGuard; BioSafe Systems, East Hartford, CT) at 0.04 mL/70 mL, spirotetramat (Kontos; OHP, Mainland, PA) at 0.01 mL/70 mL.

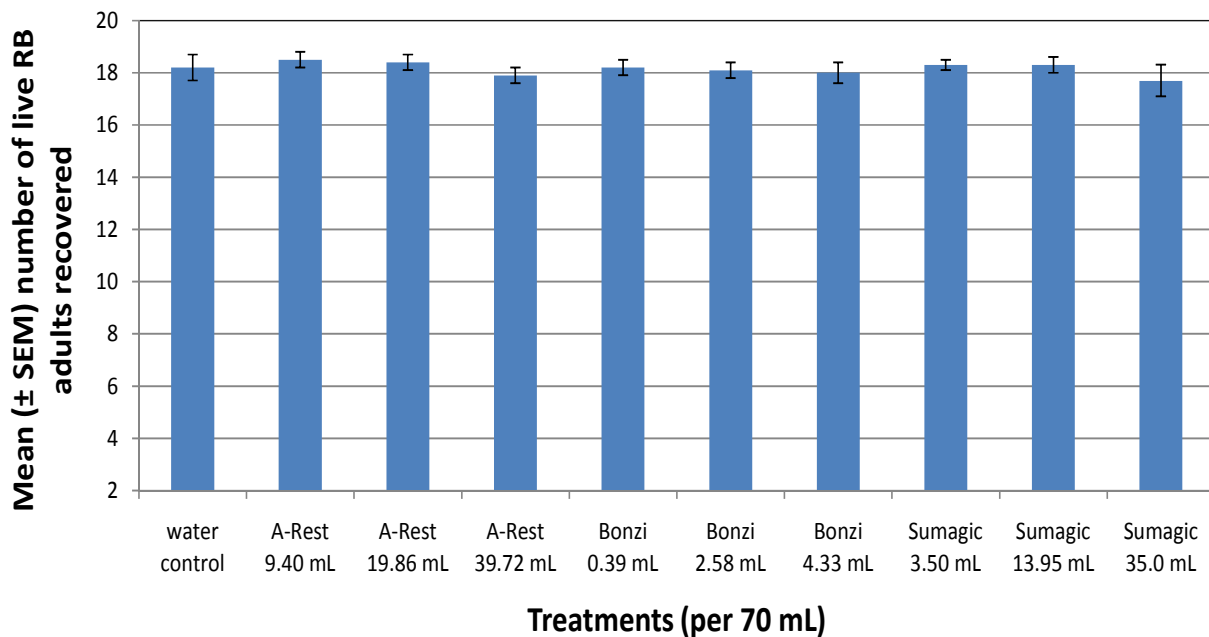


Figure 4-4 Rove beetle (RB), *Atheta coriaria* adult recovery rates 96 hours after exposure to plant growth regulators used in experiment 4 in which 20 adults were applied in containers with 300 mL of growing medium (Sunshine LC1 Professional Growing Mix; Sun Gro Horticulture, Inc.; Bellevue, WA) 24 hours after applying the PGRs. There were 10 replications per treatment. None of the treatments were significantly different ($F = 0.42$, $df = 9, 99$, $P \leq 0.924$) from each other. Vertical bars represent standard errors of the mean (SEM). The PGRs and rates used were acymidol (A-Rest; SePRO, Carmel, IN) at 9.40 mL/70 mL, acymidol (A-Rest; SePRO, Carmel, IN) at 19.86 mL/70 mL, acymidol (A-Rest; SePRO, Carmel, IN) at 39.72 mL/70 mL, paclobutrazol (Bonzi; Syngenta Crop Protection, Greensboro, NC) at 0.39 mL/70 mL, paclobutrazol (Bonzi; Syngenta Crop Protection, Greensboro, NC) at 2.58 mL/70 mL, paclobutrazol (Bonzi; Syngenta Crop Protection, Greensboro, NC) at 4.33 mL/70 mL, uniconazole (Sumagic; Valent Corp., Walnut Creek, CA) at 3.50 mL/70 mL, uniconazole at 13.95 mL/70 mL, and at 35.0 mL/70 mL.

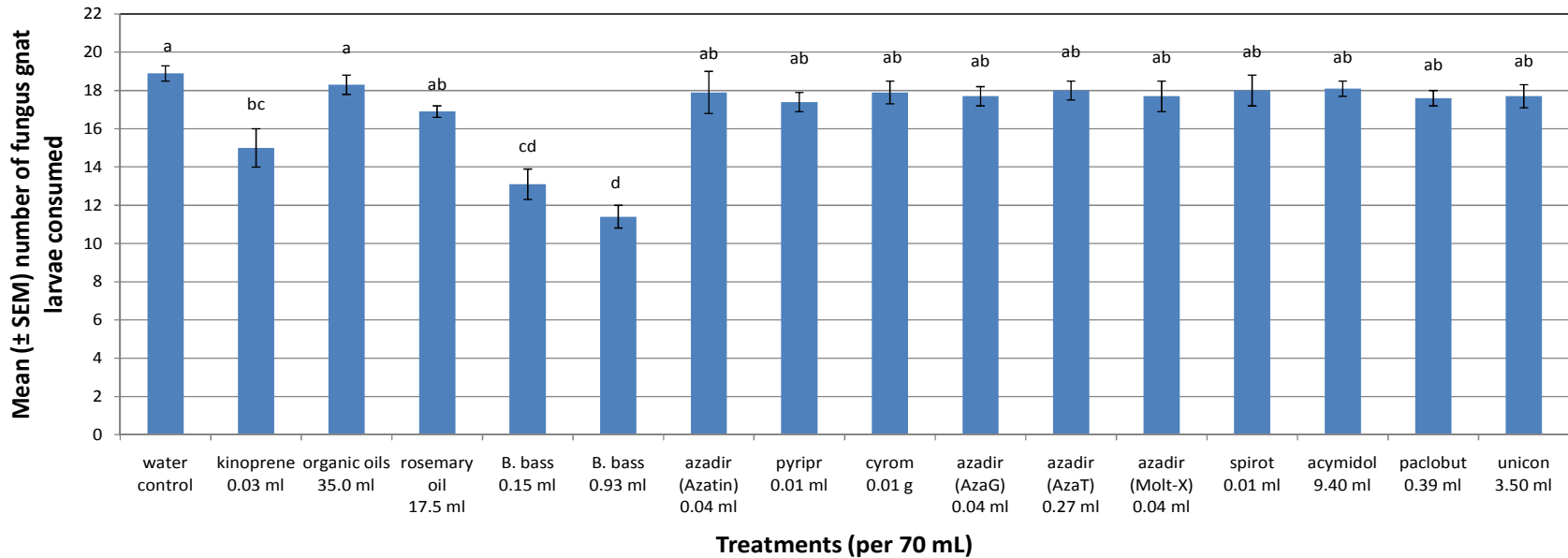


Figure 4-5 Rove beetle, *Atheta coriaria* adult prey (FG larvae) consumption in Petri dishes 24 hours after exposure of pesticide treated adults to 20 fungus gnat (FG) *Bradysia* sp. nr. *coprophila* larvae. There were seven replications per treatment. Means followed by the same letter among columns are not significantly different ($P > 0.05$) as determined by Tukey's mean separation test. Vertical bars represent standard errors of the mean (SEM). The pesticides and rates used were kinoprene (Enstar II; Wellmark International, Schaumburg, IL) at 0.027 mL/70 mL, organic oils (Zero Tolerance; Natural Garden Solutions, Piedmon, CA) at 35.0 mL/70 mL, soybean and rosemary oil (Indoor Pharm; Pharm Solutions, Port Townsend, WA) at 17.5 mL/70 mL, *Beauveria bassiana* (*B. bass*) (BotaniGard; BioWorks, Victor, NY) at 0.17 mL/70 mL, *Beauveria bassiana* (*B. bass*) (BotaniGard; BioWorks, Victor, NY) at 1.08 mL/70 mL, azadirachtin (azadir) (Azatin; OHP, Mainland, PA) at 0.043 mL/70 mL, pyriproxyfen (pyripr) (Distance; Valent, Walnut Creek, CA) at 0.010 mL/70 mL, cyromazine (cyrom) (Citation; Syngenta Crop Protection, Greensboro, NC) at 0.01 g/70 mL, azadirachtin (azadir) (AzaGuard; BioSafe Systems, East Hartford, CT) at 0.044 mL/70 mL, azadirachtin (azadir) (Azatrol; PBI/GORDON, Kansas City, MO) at 0.276 mL/70 mL, azadirachtin (azadir) (Molt-X; BioWorks, Victor, NY) at 0.044 mL/70 mL, spirotetramat (spirot) (Kontos; OHP, Mainland, PA) at 0.009 mL/70 mL, and the plant growth regulator rates were acymidol (A-Rest; SePRO, Carmel, IN) at 9.42 mL/70mL, paclobutrazol (paclobut) (Bonzi; Syngenta Crop Protection, Greensboro, NC) at 0.39 mL /70 mL, and uniconazole (unicon) (Sumagic; Valent Corp., Walnut Creek, CA) at 3.50 mL/70 mL.

Table 4-1 Common name, trade name, rates, and company information associated with pesticides used in experiments 1 to 3, and plant growth regulators (PGR) used in experiment 4.

Common name (trade name)	Pesticide/PGR Type	Rate (per 100 gal)	Rate (per 70 mL)	Company
Experiment 1				
acetamiprid (TriStar)	neonicotinoid	2.66 fl oz	0.014 g	Cleary's; Dayton, NJ.
acetamiprid (TriStar)	neonicotinoid	5 fl oz	0.028 g	
kinoprene (Enstar II)	Insect growth regulator	5 fl oz	0.027 mL	Wellmark International; Schaumburg, IL.
organic oils (Zero Tolerance)	botanical	6,400 fl oz	35.0 mL	Natural Garden Solutions; Piedmont, CA.
soybean and rosemary oil (Indoor Pharm)	botanical	3,200 fl oz	17.5 mL	Pharm Solutions; Port Townsend, WA.
Experiment 2				
<i>Beauveria bassiana</i> (BotaniGard ES)	microbial	33 fl oz	0.17 mL	BioWorks; Victor, NY.
<i>Beauveria bassiana</i> (BotaniGard ES)	microbial	200 fl oz	1.08 mL	
metaflumizone (Alverde)	semicarbazone	16 fl oz	0.087 mL	BASF Corp.; Research Triangle Park, NC.
lambda-cyhalothrin (CyhaloCap)	pyrethroid	5 fl oz	0.026 mL	Whitmire Micro-Gen Research Labs; St Louis, MO.
cyfluthrin (Cy-kick)	pyrethroid	16 fl oz	0.086 mL	
cyfluthrin (Cy-kick)	pyrethroid	32 fl oz	0.173 mL	
azadirachtin (Azatin)	botanical	8 fl oz	0.043 mL	OHP, Mainland, PA.
pyriproxyfen (Distance)	Insect growth regulator	2 fl oz	0.010 mL	Valent Corp.; Walnut Creek, CA
cyromazine (Citation)	Insect growth regulator	2.66 oz	0.013 g	Syngenta Crop Protection; Greensboro, NC.
Experiment 3				
azadirachtin (AzaGuard)	botanical	8 oz fl	0.044 mL	BioSafe Systems; East Hartford, CT.
azadirachtin (Azatrol)	botanical	32 oz fl	0.276 mL	PBI/GORDON; Kansas City, MO.
azadirachtin (Molt-X)	botanical	8 oz fl	0.044 mL	BioWorks, Victor, NY.
spirotetramat (Kontos)	tetramic acid	1.7 oz fl	0.009 mL	OHP, Mainland, PA
Experiment 4				
acymidol (A-Rest)	growth inhibitor	-	9.40 mL	SePRO; Carmel, IN.
acymidol (A-Rest)	growth inhibitor	-	19.86 mL	
acymidol (A-Rest)	growth inhibitor	-	39.72 mL	
paclobutrazol (Bonzi)	growth retardant	-	0.39 mL	Syngenta Crop Protection; Greensboro, NC.
paclobutrazol (Bonzi)	growth retardant	-	2.58 mL	
paclobutrazol (Bonzi)	growth retardant	-	4.33 mL	
uniconazole (Sumagic)	growth retardant	-	3.50 mL	Valent; Walnut Creek, CA.
uniconazole (Sumagic)	growth retardant	-	13.95 mL	
uniconazole (Sumagic)	growth retardant	-	35.0 mL	

Table 4-2 Mean (\pm SEM) number of live rove beetle, *Atheta coriaria* adults recovered associated with experiment 1 in which 20 rove beetle adults were released 24 hours after treatments (pesticides and water control) were applied. There were 10 replications per treatment.

Treatment (common and trade name)	Rate (per 70 ml)	Number of live rove beetle adults recovered
water control	---	17.6 \pm 0.2a ^z
acetamiprid (TriStar)	0.014 g	13.3 \pm 0.6bc
acetamiprid (TriStar)	0.028 g	10.3 \pm 1.3c
kinoprene (Enstar II)	0.027 ml	13.7 \pm 0.8b
organic oils (Zero Tolerance)	35.0 ml	16.1 \pm 0.5ab
soybean and rosemary oil (Indoor Pharm)	17.5 ml	16.1 \pm 0.5ab

^zMeans followed by the same letter are not significantly different ($P > 0.05$) as determined by a Tukey's means separation test.

Table 4-3 Mean (\pm SEM) number of live rove beetle, *Atheta coriaria* adults recovered associated with experiment 1 in which 20 rove beetle adults were released 24 hours before treatments (pesticides and water control) were applied. There were 10 replications per treatment.

Treatment (common and trade name)	Rate (per 70 ml)	Number of live rove beetle adults recovered
water control	---	12.8 \pm 0.3a ^z
acetamiprid (TriStar)	0.014 g	8.5 \pm 1.1b
acetamiprid (TriStar)	0.028 g	3.7 \pm 0.9c
kinoprene (Enstar II)	0.027 ml	13.5 \pm 0.6a
organic oils (Zero Tolerance)	35.0 ml	16.0 \pm 0.4a
soybean and rosemary oil (Indoor Pharm)	17.5 ml	6.4 \pm 1.4bc

^zMeans followed by the same letter are not significantly different ($P > 0.05$) as determined by a Tukey's means separation test.

Table 4-4 Mean (\pm SEM) number of live rove beetle, *Atheta coriaria* adults recovered associated with experiment 2 in which 20 rove beetle adults were released 24 hours after treatments (pesticides and water control) were applied. There were 10 replications per treatment.

Treatment (common and trade name)	Rate (per 70 ml)	Number of live rove beetle adults recovered
water control	---	16.8 \pm 0.4a ^z
<i>Beauveria bassiana</i> (BotaniGard ES)	0.17 ml	16.4 \pm 0.5a
<i>Beauveria bassiana</i> (BotaniGard ES)	1.08 ml	17.6 \pm 0.6a
metaflumizone (Alverde)	0.086 ml	14.9 \pm 0.5a
lambda-cyhalothrin (CyhaloCap)	0.026 ml	8.1 \pm 1.1b
cyfluthrin (Cy-kick)	0.086 ml	3.1 \pm 1.0c
cyfluthrin (Cy-kick)	0.174 ml	4.6 \pm 0.7c
azadirachtin (Azatin)	0.043 ml	16.2 \pm 0.2a
pyriproxyfen (Distance)	0.010 ml	16.3 \pm 0.7a
cyromazine (Citation)	0.013 g	17.2 \pm 0.6a

^zMeans followed by the same letter are not significantly different ($P > 0.05$) as determined by a Tukey's means separation test.

Table 4-5 Mean (\pm SEM) number of live rove beetle, *Atheta coriaria* adults recovered associated with experiment 2 in which 20 rove beetle adults were released 24 hours before treatments (pesticides and water control) were applied. There were 10 replications per treatment.

Treatment (common and trade name)	Rate (per 70 ml)	Number of live rove beetle adults recovered
water control	---	15.3 \pm 0.4 ^z
<i>Beauveria bassiana</i> (BotaniGard ES)	0.17 ml	15.5 \pm 0.4a
<i>Beauveria bassiana</i> (BotaniGard ES)	1.08 ml	13.2 \pm 0.7abc
metaflumizone (Alverde)	0.086 ml	10.2 \pm 0.8c
lambda-cyhalothrin (CyhaloCap)	0.026 ml	3.0 \pm 0.8d
cyfluthrin (Cy-kick)	0.086 ml	0.9 \pm 0.3d
cyfluthrin (Cy-kick)	0.174 ml	1.9 \pm 0.6d
azadirachtin (Azatin)	0.043 ml	13.5 \pm 0.5ab
pyriproxyfen (Distance)	0.010 ml	11.6 \pm 1.2bc
cyromazine (Citation)	0.013 g	13.4 \pm 0.9abc

^zMeans followed by the same letter are not significantly different ($P > 0.05$) as determined by a Tukey's means separation test.

Table 4-6 Mean (\pm SEM) number of live rove beetle, *Atheta coriaria* adults recovered associated with experiment 3 in which 20 rove beetle adults were released 24 hours after treatments (pesticides and water control) were applied. There were eight replications per treatment.

Treatment (common and trade name)	Rate (per 70 ml)	Number of live rove beetle adults recovered
water control	---	15.0 \pm 0.5a
azadirachtin (Azatrol)	0.044 ml	16.0 \pm 1.2a
azadirachtin (Molt-X)	0.276 ml	13.3 \pm 0.8a
azadirachtin (AzaGuard)	0.044 ml	13.9 \pm 1.0a
spirotetramat (Kontos)	0.009 ml	13.6 \pm 0.9a

²Means followed by the same letter are not significantly different ($P > 0.05$) as determined by a Tukey's means separation test.

Table 4-7 Mean (\pm SEM) number of live rove beetle, *Atheta coriaria* adults recovered associated with experiment 3 in which 20 rove beetle adults were released 24 hours before treatments (pesticides and water control) were applied. There were five replications per treatment.

Treatment (common and trade name)	Rate (per 70 ml)	Number of live rove beetle adults recovered
water control	---	13.8 \pm 0.6a
azadirachtin (Azatrol)	0.044 ml	14.4 \pm 0.5a
azadirachtin (Molt-X)	0.276 ml	13.2 \pm 0.6a
azadirachtin (AzaGuard)	0.044 ml	13.8 \pm 0.7a
spirotetramat (Kontos)	0.009 ml	13.0 \pm 1.1a

²Means followed by the same letter are not significantly different ($P > 0.05$) as determined by a Tukey's means separation test.

Table 4-8 Mean (\pm SEM) number of live rove beetle, *Atheta coriaria* adults recovered 96 hours after exposure to treatments (plant growth regulators and water control) associated with experiment 4 in which 20 rove beetle adults were released 24 hours after treatments were applied. There were 10 replications per treatment.

Treatment (common and trade name)	Rate (per 70 ml)	Number of live rove beetle adults recovered
water control	---	18.2 \pm 0.5a ^z
acymidol (A-Rest)	9.40 ml	18.5 \pm 0.3a
acymidol (A-Rest)	19.86 ml	18.4 \pm 0.3a
acymidol (A-Rest)	39.72 ml	17.9 \pm 0.3a
paclobutrazol (Bonzi)	0.39 ml	18.2 \pm 0.3a
paclobutrazol (Bonzi)	2.58 ml	18.1 \pm 0.3a
paclobutrazol (Bonzi)	4.33 ml	18.0 \pm 0.4a
uniconazole (Sumagic)	3.50 ml	18.3 \pm 0.2a
uniconazole (Sumagic)	13.95 ml	18.3 \pm 0.3a
uniconazole (Sumagic)	35.0 ml	17.7 \pm 0.6a

^zMeans followed by the same letter are not significantly different ($P > 0.05$) as determined by a Tukey's means separation test.

Table 4-9 Mean (\pm SEM) *Atheta coriaria* development time (days) from egg to adult in the F₁ generation after exposure of adults to pesticide treatments for 10 days. There were seven replications per treatment.

Treatment (common and trade name)	Rate (per 70 ml)	Development time from egg to adult (days)
water control	---	21.0 \pm 0.3a
kinoprene (Enstar II)	0.027 ml	23.7 \pm 0.6a
organic oils (Zero Tolerance)	35.0 ml	22.1 \pm 1.1a
soybean and rosemary oils (Indoor Pharm)	17.5 ml	21.7 \pm 0.6a
<i>Beauveria bassiana</i> (BotaniGard ES)	0.17 ml	21.7 \pm 0.4a
<i>Beauveria bassiana</i> (BotaniGard ES)	1.08 ml	23.1 \pm 0.9a
azadirachtin (Azatin)	0.043 ml	23.4 \pm 0.8a
cyromazine (Citation)	0.013 g	22.7 \pm 0.6a
azadirachtin (AzaGuard)	0.044 ml	22.4 \pm 0.7a
azadirachtin (Azatrol)	0.276 ml	22.3 \pm 0.8a
azadirachtin (Molt-X)	0.044 ml	23.6 \pm 0.6a
spirotetramat (Kontos)	0.009 ml	23.0 \pm 0.8a

²Means followed by the same letter are not significantly different ($P > 0.05$) as determined by a Tukey's means separation test.

Table 4-10 Mean (\pm SEM) *Atheta coriaria* development time (days) from egg to adult in the F₁ generation after exposure of adults to treatments (plant growth regulators) for 96 hours. There were seven replications per treatment.

Treatment (common and trade name)	Rate (per 70 ml)	Development time from egg to adult (days)
water control	----	21.0 \pm 0.3a ^z
acymidol (A-Rest)	9.40 ml	20.6 \pm 0.2a
acymidol (A-Rest)	39.72 ml	21.0 \pm 0.0a
paclobutrazol (Bonzi)	0.39 ml	21.6 \pm 0.6a
paclobutrazol (Bonzi)	2.58 ml	21.0 \pm 0.0a
paclobutrazol (Bonzi)	4.33 ml	22.1 \pm 0.6a
uniconazole (Sumagic)	3.50 ml	22.7 \pm 1.0a
uniconazole (Sumagic)	13.95 ml	22.7 \pm 1.2a

^zMeans followed by the same letter are not significantly different ($P > 0.05$) as determined by a Tukey's means separation test.

Table 4-11 Mean (\pm SEM) *Atheta coriaria* adult prey consumption (fungus gnat larvae) based on fungus gnat (FG) head capsules, after exposure to treatments (pesticides and plant growth regulators). The initial number of fungus gnat larvae was 20. There were seven replications per treatment.

Treatment (common and trade name)	Rate (per 70 ml)	Prey consumption (number of FG larval head capsules)
water control	---	18.9 \pm 0.4a ^z
kinoprene (Enstar II)	0.027 ml	15.0 \pm 1.0bc
organic oils (Zero Tolerance)	35.0 ml	18.3 \pm 0.5a
soybean and rosemary oil (Indoor Pharm)	17.5 ml	16.9 \pm 0.3ab
<i>Beauveria bassiana</i> (BotaniGard ES)	0.17 ml	13.1 \pm 0.8cd
<i>Beauveria bassiana</i> (BotaniGard ES)	1.08 ml	11.4 \pm 0.6d
azadirachtin (Azatin)	0.043 ml	17.9 \pm 1.1ab
pyriproxyfen (Distance)	0.010 ml	17.4 \pm 0.5ab
cyromazine (Citation)	0.013 g	17.9 \pm 0.6ab
azadirachtin (AzaGuard)	0.044 ml	17.7 \pm 0.5ab
azadirachtin (Azatrol)	0.276 ml	18.0 \pm 0.5ab
azadirachtin (Molt-X)	0.044 ml	17.7 \pm 0.8ab
spirotetramat (Kontos)	0.009 ml	18.0 \pm 0.8ab
acymidol (A-Rest)	9.40 ml	18.1 \pm 0.4ab
paclobutrazol (Bonzi)	0.39 ml	17.6 \pm 0.4ab
uniconazole (Sumagic)	3.50 ml	17.7 \pm 0.6ab

^zMeans followed by the same letter are not significantly different ($P > 0.05$) as determined by a Tukey's means separation test.

Table 4-12 Mean (\pm SEM) number of live rove beetle *A. coriaria* adults and larvae recovered associated with experiments 1 and 2 in which 10 treated adults were released 24 hours after treatments were applied. There were six replications per treatment.

Treatment (common and trade name)	Rate (per 70 ml)	Number of live rove beetle adults recovered	Number of live rove beetle larvae recovered
water control	---	7.3 \pm 0.7a ^z	3.8 \pm 0.6bcd ^z
<i>Beauveria bassiana</i> (BotaniGard ES)	0.17 ml	6.8 \pm 0.6a	5.3 \pm 1.4abc
<i>Beauveria bassiana</i> (BotaniGard ES)	1.08 ml	6.2 \pm 0.5a	5.7 \pm 1.4ab
metaflumizone (Alverde)	0.086 ml	0.8 \pm 0.3b	0.0 \pm 0.0e
lambda-cyhalothrin (CyhaloCap)	0.026 ml	0.7 \pm 0.7b	0.2 \pm 0.2e
cyfluthrin (Cy-kick)	0.086 ml	0.2 \pm 0.2b	0.0 \pm 0.0e
cyfluthrin (Cy-kick)	0.174 ml	0.0 \pm 0.0b	0.0 \pm 0.0e
azadirachtin (Azatin)	0.043 ml	6.3 \pm 0.4a	1.2 \pm 0.5de
pyriproxyfen (Distance)	0.010 ml	6.3 \pm 0.7a	0.0 \pm 0.0e
cyromazine (Citation)	0.013 g	5.7 \pm 0.7a	2.0 \pm 0.7cde
acetamiprid (TriStar)	0.014 g	1.5 \pm 0.6b	0.7 \pm 0.4de
acetamiprid (TriStar)	0.028 g	0.5 \pm 0.5b	0.0 \pm 0.0e
kinoprene (Enstar II)	0.027 ml	6.2 \pm 1.7a	3.8 \pm 1.4bcd
organic oils (Zero Tolerance)	35.0 ml	7.7 \pm 0.7a	8.0 \pm 0.6a
soybean and rosemary oil (Indoor Pharm)	17.5 ml	7.5 \pm 1.0a	2.8 \pm 0.3bcde

^zMeans followed by the same letter are not significantly different ($P > 0.05$) as determined by a Tukey's means separation test.

Chapter 5 - SUMMARY AND CONCLUSIONS

The three objectives associated with this research were 1) quantitatively assess selected life history parameters of the rove beetle, *Atheta coriaria* (Kraatz), including duration of the life stages, female fecundity and longevity; 2) evaluate predation on the fungus gnat *Bradysia* sp. nr. *coprophila* under laboratory conditions; and 3) determine the direct and indirect effects of reduced-risk pesticides and plant growth regulators (PGR) on *A. coriaria* adults. Findings from the first two studies will contribute to improving biological control of the fungus gnat, *B.* sp. nr. *coprophila* by enhancing rearing methods and refining the appropriate number of RB adults to release. The third study will assist greenhouse producers in integrating pesticides and PGR into their biological control programs.

In study one, *A. coriaria* development time from egg to adult was 17.0 days at 26°C when using growing medium (GM) as a substrate and oatmeal as a supplemental food source. This is in contrast to Miller and Williams (1983) in which development time from egg to adult in Petri dishes with eggs of Nitidulidae as the only food source was 12.5 days at 26.7°C. Therefore, factors associated with the GM and food source may influence the duration of certain life stages such as larva and pupa. The synchronization of predator and prey life cycles is important in terms of effectiveness. As such, the duration of *A. coriaria*'s life cycle at 26°C is similar to the fungus gnat (FG). In addition, this study is the first to determine *A. coriaria* adult longevity and female fecundity. For instance, male and female adult longevity was 50 to 60 days with the maximum longevity of 80 to 90 days, indicating that adults can prey on FG larvae and reproduce throughout the crop production cycle. Fecundity is lower than other rove beetle (RB) species including *Aleochara bilineata*, *A. bipustulata* (Fournet et al., 2000) and *Oligota pygmaea* (Perumalsamy et al. 2009) with females capable of laying up to 8 eggs per day throughout their

50 to 60 days lifespan. Furthermore, there is variability associated with adult longevity and female fecundity, which may be a problem when assessing potential RB colony productivity for mass-rearing. For inundative releases, natural enemy fecundity may not be as important as it is for inoculative releases, which is more common in greenhouses. Moreover, RB quality control may be important, as infertile females as well as females with short longevity (10 to 30 days) and low fecundity (< 10 eggs per female) may affect the number of progeny and reduce the likelihood of colony establishment. Based on the findings of the current study, RB colonies will start increasing after one generation (approximately three weeks after release). Therefore, understanding the life history of *A. coriaria* will be useful in enhancing the efficiency of augmentative biological control, mass-rearing, and quality control.

In study two, *A. coriaria* predation efficacy was 70 to 80% in Petri dishes, with no effect of larval age when using second and third instar FG larvae on RB adult prey consumption. However, prey consumption was lower when using GM as a substrate, indicating that predation was influenced by factors associated with the substrate. As such, this requires further investigation. Overall, assessments on predation suggest that prey consumption increased as FG larval densities increased. This is important because, although FG populations increase faster than RB populations, any rise in FG populations may be compensated by RB prey consumption (functional response). Nevertheless, knowing the potential prey consumption of *A. coriaria* will be important in assessing potential effectiveness against FG when released in greenhouse production systems.

Predator:prey ratios assessed in this study ranged from 1:2 to 1:40 with different numbers of predator and prey used. Total predation was slightly higher when using one to four RB adults and decreased at five adults for any FG larval density (10, 20 30 and 40 FG larvae).

Consequently, releasing RB adults at a density of 1 to 4 adults per container may be a viable and cost-effective strategy whereas higher densities may not result in effective regulation of FG larval populations. However, when using 15 RB adults and 20 FG larvae (predator-prey ratio of 3:4), the number of FG adults recovered was lower than the control (0 RB adults), indicating that some predation occurred. A decline in predation at higher RB densities may be due to mutual interference occurring as RB density increased, which has been demonstrated with other predators such as *Paederus fuscipes* (Curtis) (Shen and Pang, 1989) and *Coccinella septempunctata* L. (Pandey et al., 1984). Therefore, further investigations are warranted in order to assess and quantify this phenomenon.

As the number of predator and prey increased, when maintaining the same predator:prey ratio, using 1 to 4 RB adults and 10, 20, 30 and 40 FG larvae (ratio of 1:10), prey consumption was similar, indicating that the number of RB adults should be increased only if there is a change in the predator:prey ratio in order to obtain a higher prey consumption. In addition, predation efficacy may be high at a predator-prey ratio of 1:20; however, further research, including greenhouse studies are needed in order to actually determine predation efficacy at higher FG larval densities. The results affiliated with predation and predator-prey ratios will be useful in promoting more efficient use of *A. coriaria* as a biological control agent.

In study three, the direct toxic effects of certain reduced-risk pesticides and PGR was investigated with assessments based on adult survival ten days after exposure of RB adults to the treatments when applied at different application rates. In addition, the indirect effects of the pesticides and PGR on RB development time from egg to adult, prey consumption and reproduction were determined. Overall, the number of RB adults recovered was higher when released after, rather than before applying the pesticides. The pesticides associated with organic

oils, and soybean and rosemary oil were compatible with RB whereas acetamiprid and kinoprene were harmful. However, when RB were released before applying the pesticides, soybean and rosemary oil were harmful, which is similar to the findings of Cloyd et al. (2009). The pyrethroid insecticides lambda-cyhalothrin and cyfluthrin were directly harmful to RB adults whereas *Beauveria bassiana*, metaflumizone, azadirachtin, pyriproxyfen and cyromazine were compatible with RB adults. All the azadirachtin-based products evaluated and spirotetramat were compatible with RB adults regardless of the release time (pre- and post-application). In addition, the PGR acymidol, paclobutrazol and uniconazole were compatible with RB when applied at both standard and high application rates.

The pesticides *Beauveria bassiana*, kinoprene, organic oils, soybean and rosemary oil, cyromazine, the azadirachtin-based products, and spirotetramat did not indirectly affect RB development time from egg to adult. However, kinoprene and *B. bassiana* appeared to be indirectly harmful based on prey consumption. The pesticides metaflumizone, lambda-cyhalothrin, cyfluthrin, pyriproxyfen and acetamiprid apparently inhibited female reproduction. Results from this study will help greenhouse producers to select those pesticides (*Beauveria bassiana*, azadirachtin, and spirotetramat) that can be integrated with *A. coriaria* whereas those that were harmful to adults such as lambda-cyhalothrin, cyfluthrin, and acetamiprid should be avoided. All the PGR assessed were compatible with *A. coriaria*. In addition, it is recommended that greenhouse producers apply pesticides before rather than after releasing RB adults.

In conclusion, the use of *A. coriaria* in augmentative biological control programs will help regulate FG populations. However, effective FG management requires efficient release rates and implementation of additional pest management techniques including pesticides. The findings from these studies will enhance current management strategies against FG in greenhouse

production systems by 1) improving the efficiency of rearing methods; 2) refining release rates of RB adults, which will avoid extraneous costs; and 3) allowing greenhouse producers to time pesticide applications accordingly without disrupting biological control programs.

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APPENDICES

**APPENDIX A. TEMPERATURE (°C) AND RELATIVE HUMIDITY (RH) FROM APRIL
TO DECEMBER 2009 – 2011.**

Table A-1 Mean (\pm SEM) temperature ($^{\circ}$ C) in laboratory (Kansas State University, Department of Entomology, Manhattan, KS) from April to November 2009 to 2011. The temperature ($^{\circ}$ C) was recorded using HOBO data loggers (Onset, MicroDaq; Contoocook, NH) at 30-minute intervals.

YEAR	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	TOTAL MEAN
2009	26.9 \pm 0.2	27.4 \pm 0.2	26.7 \pm 0.1	25.9 \pm 0.1	26.3 \pm 0.2	27.0 \pm 0.2	27.2 \pm 0.2	26.5 \pm 0.1	26.0 \pm 0.2	26.7 \pm 0.2
2010	26.0 \pm 0.0	26.1 \pm 0.0	26.8 \pm 0.1	23.2 \pm 0.0	23.5 \pm 0.0	22.6 \pm 0.0	21.3 \pm 0.0	23.3 \pm 0.1	23.3 \pm 0.0	24.0 \pm 3.4
2011	22.4 \pm 0.1	23.3 \pm 0.2	23.5 \pm 0.1	23.6 \pm 0.1	23.7 \pm 0.6	22.7 \pm 0.2	22.7 \pm 0.2	23.4 \pm 0.1	--	23.2 \pm 0.2

Table A-2 Mean (\pm SEM) percent relative humidity (% RH) in laboratory (Kansas State University, Department of Entomology, Manhattan, KS) from April to November 2009 to 2011. The relative humidity (RH) was recorded using HOBO data loggers (Onset, MicroDaq; Contoocook, NH) at 30-minute intervals.

YEAR	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	TOTAL MEAN
2009	28.4 \pm 1.4	34.5 \pm 1.1	39.7 \pm 1.0	44.6 \pm 0.6	41.9 \pm 0.5	38.8 \pm 1.0	36.5 \pm 0.8	38.1 \pm 1.2	41.5 \pm 1.0	38.2 \pm 0.5
2010	50.2 \pm 0.2	51.6 \pm 0.1	56.5 \pm 0.1	61.6 \pm 0.1	59.0 \pm 0.1	61.6 \pm 0.1	54.2 \pm 0.1	54.1 \pm 0.2	51.9 \pm 0.2	56.1 \pm 0.1
2011	51.3 \pm 0.7	46.4 \pm 1.3	46.5 \pm 0.5	48.2 \pm 1.2	55.8 \pm 1.6	58.0 \pm 1.0	46.9 \pm 0.5	43.0 \pm 0.6	--	49.5 \pm 0.5

APPENDIX B. EFFECT OF GROWING MEDIUM MOISTURE CONTENT ON ROVE BEETLE *ATHETA CORIARIA* ADULT SURVIVAL.

Materials and Methods

Rove beetle (RB) adult survival was assessed in an experiment with five treatments, consisting of five SB300 Universal Professional Growing Mix growing medium (Sun Gro Horticulture Inc.; Bellevue, WA) moisture contents (48%, 64%, 70%, 72% and 76%), and 10 replications per treatment. Data were collected seven and 14 days after inoculating the GM with 10 RB adults. Three-hundred mL of GM were placed in a 473 mL deli squat container using a 600 mL glass beaker. In addition, 0, 40, 60, 80, and 100 mL of water were added separately to the GM. The weight of both, empty deli squat container and deli squat container with 300 mL of moist GM, was recorded before inoculating the GM with 10 RB adults. Additionally, 1 tsp (≈ 1.5 g) of raw oatmeal was placed on the surface of the GM. After seven and 14 days, the adults were recovered by sieving the GM using #5 and #10 sieves, and then aspirated into a 9 dram plastic vial. The number of adults was counted and recorded before placing the adults back into the container. Percent GM moisture content was calculated using the following equation: $(A-B)-(C-B)/(A-B) \times 100$, where A = initial weight (g) (empty deli squat container with moist GM), B = weight (g) of empty deli squat container, and C = final weight (g) (empty deli squat container with dry GM). In order to obtain the final weight (C), the weight of the container with the GM was recorded daily until an equivalent weight was observed for three consecutive days. Data were analyzed using a statistical analysis software program SAS Systems for Windows, version 9.2 (SAS Institute, 2002). Data associated with the number of adults recovered for each GM

moisture content were subjected to an analysis of variance (ANOVA) using the PROC ANOVA procedure (SAS Institute, 2002) with the number of live RB adults as the response variable.

Additionally, differences among the treatments were determined using a Tukey's least significant means test at a significance level of $\alpha = 0.05$.

Results and discussion

There were 5.9 ± 1.1 and 0.5 ± 0.2 adults recovered seven and 14 days after inoculating the GM, with a GM moisture content of 48%, while 9.4 ± 0.3 and 6.5 ± 1.0 adults were recovered seven and 14 days from the GM with a moisture content of 72%. In addition, initial GM percent moisture contents decreased from 48, 64, 70, 72 and 76% to 30, 53, 60, 66 and 72% seven days after inoculating the GM, and 6, 36, 47, 56 and 62%, 14 days after inoculating the GM, respectively (Table 1). High RB adult recovery rates were associated with higher initial GM moisture contents ($>60\%$), while low RB recovery rates were associated with low GM moisture contents ($<60\%$) suggesting that GM moisture content may influence RB adult survival. The number of RB adults recovered when the initial moisture content was 48% was not significantly different than the number of RB adults recovered when the initial moisture content was 70% although significantly lower compared to initial moisture contents of 64, 72 and 76% seven days after inoculating the GM (Table 1). Additionally, initial moisture contents of 64, 72 and 76% were not significantly different in terms of number of RB adults recovered although these were significantly higher compared to initial moisture contents of 48% and 70%, 14 days after inoculating the GM (Table 1). The mean number of RB adults recovered seven and 14 days after inoculation decreased when the initial moisture content was $<64\%$.

Table B-1 Rove beetle (RB), *Atheta coriaria* adults recovered seven and 14 days after inoculating the growing medium (GM) (SB300 Universal Professional Growing Mix; Sun Gro Horticulture, Inc.; Bellevue, WA) with 10 rove beetle adults associated with five initial growing medium moisture contents. There were 10 replications per growing medium moisture content.

Percent initial GM moisture content	Data collected seven days after inoculating GM with 10 RB adults		Data collected 14 days after inoculating GM with 10 RB adults	
	% GM moisture content	Number of RB adults recovered (mean \pm SEM)	% GM moisture content	Number of RB adults recovered (mean \pm SEM)
48	30	5.9 \pm 1.1 b ^z	6	0.5 \pm 0.2 c ^z
64	53	8.5 \pm 0.4 a	36	7.9 \pm 0.7 a
70	60	8.2 \pm 0.4 ab	47	4.1 \pm 1.2 b
72	66	9.4 \pm 0.3 a	56	6.5 \pm 1.0 ab
76	70	8.8 \pm 0.7 a	62	5.9 \pm 1.2 ab

^zMeans followed by the same letter within a column are not significantly different ($P \leq 0.05$) as determined by a Tukey's means separation test.

APPENDIX C. DIRECT EFFECTS OF PESTICIDES ON THE ROVE BEETLE *ATHETA CORIARIA* ADULTS TWO DAYS AFTER EXPOSURE.

Materials and Methods

The direct effects of pesticides on *Atheta coriaria* adults were assessed in two separate experiments with five pesticides used for experiment 1 and seven pesticides for experiment 2. In addition, direct effects were determined after rove beetles (RB) were applied to the growing medium (GM) 24 hours before, and 24 hours after applying the pesticides. The experiments were set-up as a completely randomized block design with two blocks (days) and 10 replications per treatment. The number of treatments for each experiment is presented in Table 1.

Three-hundred mL of GM (SB300 Universal Professional Growing Mix; Sun Gro Horticulture, Inc.; Bellevue, WA) was measured using a 600 mL glass beaker and placed into a 473 mL deli squat container. Modified lids with insect screening were used to allow for ventilation. Before preparing the pesticide solutions, individual pesticides were measured using a 1 mL sterile syringe (BD; Franklin Lakes, NJ), and 10, 100 and 500 mL graduated cylinders, except for acetamiprid and cyromazine (solid formulations), which were measured using a balance scale (Denver Instrument; Bohemia, NY). Pesticides were mixed with water separately using a 600 mL glass beaker in order to obtain the adequate concentration for each treatment. Subsequently, 70 mL of each pesticide solution was applied uniformly as a drench to the GM in each deli squat container, and 1 tsp of raw oatmeal (≈ 1.5 g) was placed on the GM surface as a food source for the RB adults. In addition, a water control was used in both experiments.

Twenty RB adults (males and females of various ages) were randomly collected from the main colony (Kansas State University, Manhattan, KS) by sieving the GM using #5 and #10

mesh size sieves, then recovered into a 9 dram plastic vial using an aspirator, and placed into the container. Initially, the containers were warmed by heat lamps (Commercial Electric; Atlanta, GA) installed with a 125-W clear medium-based (BR40) heat light bulb (Phillips Lighting Comp.; Somerset, NJ) placed approximately 90 cm above the containers for 24 hours, thus allowing the GM to dry, which facilitated the sieving procedure. After two days, RB adults were collected, based on the procedure described previously, and the number of live RB adults was recorded. Data from all experiments were analyzed using a statistical analysis software program SAS Systems for Windows, version 9.2 (SAS Institute, 2002). Data associated with the direct toxic effects of pesticides on *A. coriaria* were subjected to an analysis of variance (ANOVA) using the PROC ANOVA procedure (SAS Institute, 2002) with the number of live RB adults as the response variable. Additionally, differences among the treatments were determined using a Tukey's least significant means test at a significance level of $\alpha = 0.05$.

Results and discussion

Twenty RB adults were exposed to the pesticides 24 hours after application, with high adult survival rates (>90%) obtained for acetamiprid when applied at a low rate (0.014 g/70 mL), kinoprene, organic oils, soybean and rosemary oil, and the control, while an adult survival of 16.5 ± 1.0 (mean \pm SEM) was obtained for acetamiprid when applied at a high rate (0.028 g/70 mL) (Figure 1). Rove beetle adult recovery rates associated with kinoprene, organic oils, and soybean and rosemary oil were higher but not significantly different compared to acetamiprid when applied at a low rate (Figure 1).

When RB were released into the containers before applying the pesticides, the highest adult survival rates were obtained with kinoprene (16.3 ± 0.9) and organic oils (17.3 ± 0.5). Rove beetle adult recovery rates affiliated with acetamiprid applied at both low and high rates, and

soybean and rosemary oil were significantly lower than the control, while kinoprene and organic oils were not significantly different from the control (Figure 2). It appears that *A. coriaria* was compatible with kinoprene and organic oils, while acetamiprid at both low and high rates, and soybean and rosemary oil were harmful to adult *A. coriaria* based on the low recovery rates.

In the second experiment, the effect of seven pesticides (Table 1) on RB survival was investigated. Results, in which 20 RB adults were exposed to pesticides 24 hours after application are presented in Figure 3. Rove beetle adult recovery rates were similar for azadirachtin, pyriproxyfen, and cyromazine, compared to the control, while there was a direct toxic effect associated with lambda-cyhalothrin (7.5 ± 0.9), and cyfluthrin when applied at a low (0.086 mL/70 mL) (12.3 ± 0.8) and high (0.173 mL/70 mL) (10.7 ± 0.8) rate. *Beauveria bassiana*, at both low and high rates, and metaflumizone were similar to the control (Figure 3). Low RB survival (seven to 12 adults out of 20) (35 to 60%) was associated with lambda-cyhalothrin and cyfluthrin. Using the same treatments, RB adult survival was evaluated in another experiment in which the pesticides were applied 24 hours after releasing 20 RB adults into the containers. The highest RB adult survival was associated with *Beauveria bassiana* (17.1 ± 1.0) when applied at a high rate (1.08 mL/70 mL), while the lowest RB adult survival was associated with cyfluthrin (4.4 ± 1.2) when applied at a high rate (0.173 mL/70 mL). Results were similar when the RB were released into the deli squat containers before or after applying the pesticides. However, when the RB were released before application, there was no significant difference in RB survival between lambda-cyhalothrin (9.2 ± 1.2) and cyfluthrin (10.2 ± 0.8) when applied at a low rate (0.086 mL/70 mL) (Figure 4).

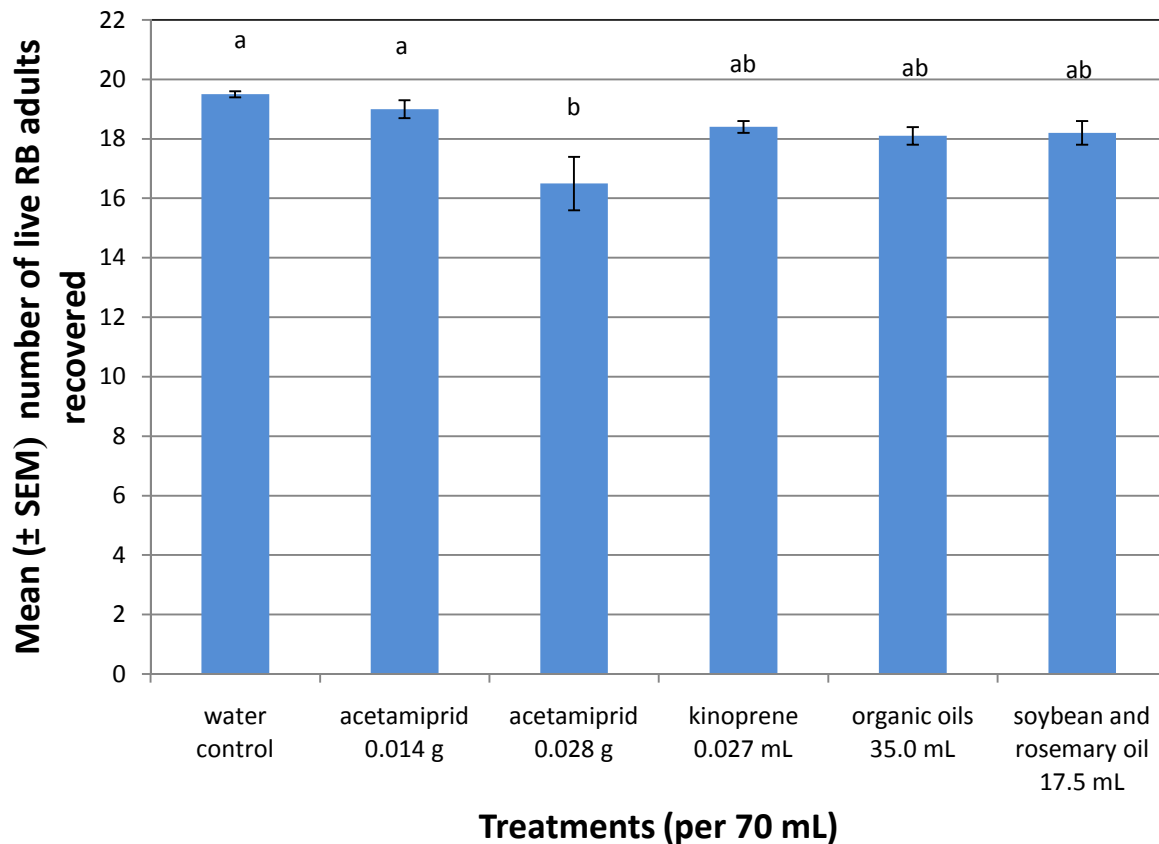


Figure C-1 Rove beetle (RB), *Atheta coriaria* adult recovery rates two days after exposure to pesticides used in experiment 1 in which 20 adults were applied to containers with 300 mL of growing medium (SB300 Universal Professional Growing Mix; Sun Gro Horticulture, Inc.; Bellevue, WA) 24 hours after applying the pesticides. There were 10 replications per treatment. Means followed by the same letter are not significantly different ($P \leq 0.05$) as determined by Tukey's mean separation test. Vertical bars represent standard errors of the mean (SEM). The pesticides and rates used were acetamiprid (TriStar; Cleary's, Dayton, NJ) at 0.014 g/70 mL, acetamiprid (TriStar; Cleary's, Dayton, NJ) at 0.028 g/70 mL, kinoprene (Enstar II; Wellmark International, Schaumburg, IL) at 0.027 mL/70 mL, organic oils (Zero Tolerance; Natural Garden Solutions, Piedmont, CA) at 35.0 mL/70 mL and soybean and rosemary oil (Indoor Pharm; Pharm Solutions, Port Townsend, WA) at 17.5 mL/70 ml rate.

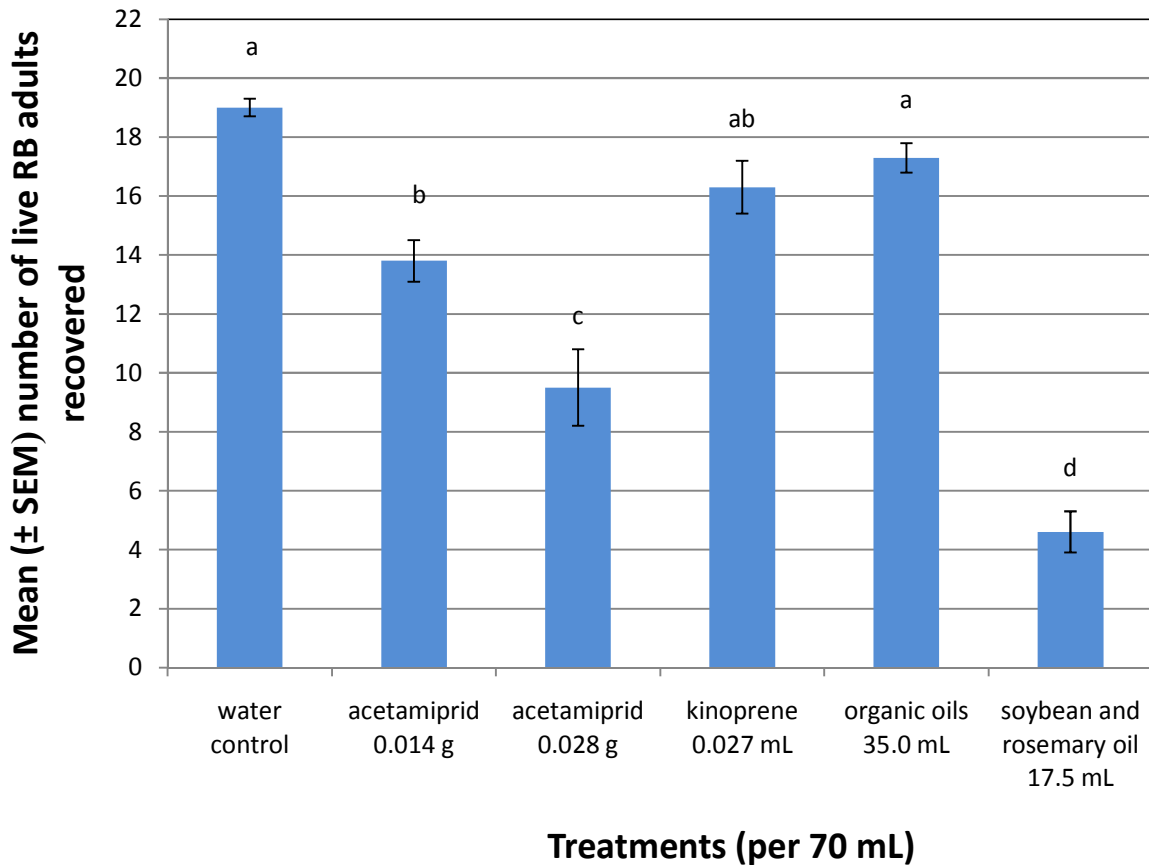


Figure C-2 Rove beetle (RB), *Atheta coriaria* adult recovery rates two days after exposure to pesticides used in experiment 1 in which 20 adults were applied to containers with 300 mL of growing medium (SB300 Universal Professional Growing Mix; Sun Gro Horticulture Inc.; Bellevue, WA) 24 hours before applying the pesticides. There were 10 replications per treatment. Means followed by the same letter are not significantly different ($P \leq 0.05$) as determined by Tukey's mean separation test. Vertical bars represent standard errors of the mean (SEM). The pesticides and rates used were acetamiprid (TriStar; Cleary's, Dayton, NJ) at 0.014 g/70 mL, acetamiprid (TriStar; Cleary's, Dayton, NJ) at 0.028 g/70 mL, kinoprene (Enstar II; Wellmark International, Schaumburg, IL) at 0.027 mL/70 mL, organic oils (Zero Tolerance; Natural Garden Solutions, Piedmont, CA) at 35.0 mL/70 mL and soybean and rosemary oil (Indoor Pharm, Pharm Solutions; Port Townsend, WA) at 17.5 mL/70 mL rate.

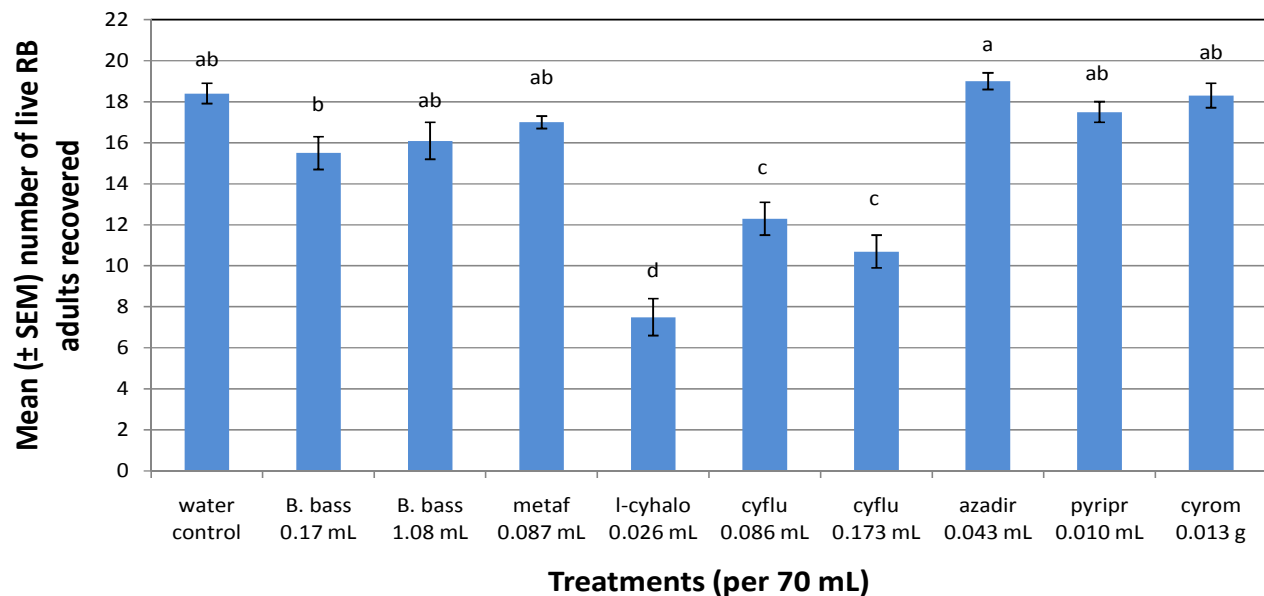


Figure C-3 Rove beetle (RB), *Atheta coriaria* adult recovery rates two days after exposure to pesticides used in experiment 2 in which 20 adults were applied to containers with 300 mL growing medium (SB300 Universal Professional Growing Mix; Sun Gro Horticulture Inc.; Bellevue, WA) 24 hours after applying the pesticides. There were 10 replications per treatment. Means followed by the same letter are not significantly different ($P \leq 0.05$) as determined by Tukey's mean separation test. Vertical bars represent standard errors of the mean (SEM). The pesticides and rates used were *Beauveria bassiana* (*B. bass*) (BotaniGard; BioWorks, Victor, NY) at 0.17 mL/70 mL, *Beauveria bassiana* (*B. bass*) (BotaniGard; BioWorks, Victor, NY) at 1.08 mL/70 mL, metaflumizone (metaf) (Alverde; BASF Corp., Research Triangle Park, NC) at 0.087 mL/70 mL, lambda-cyhalothrin (l-cyhalo) (CyhaloCap; Whitmire Micro-Gen Research Labs, St. Louis, MO) at 0.026 mL/70 mL, cyfluthrin (cyflu) (Cy-kick; Whitmire Micro-Gen Research Labs, St. Louis, MO) at 0.086 mL/70 mL, cyfluthrin (Cy-kick; Whitmire Micro-Gen Research Labs, St. Louis, MO) at 0.173 mL/70 mL, azadirachtin (azadir) (Azatin; OHP, Mainland, PA) at 0.043 mL/70 mL, pyriproxyfen (pyripr) (Distance; Valent Corp., Walnut Creek, CA) at 0.010 mL/70 mL, cyromazine (cyrom) (Citation; Syngenta Crop protection, Greensboro, NC) at 0.013 g/70 mL.

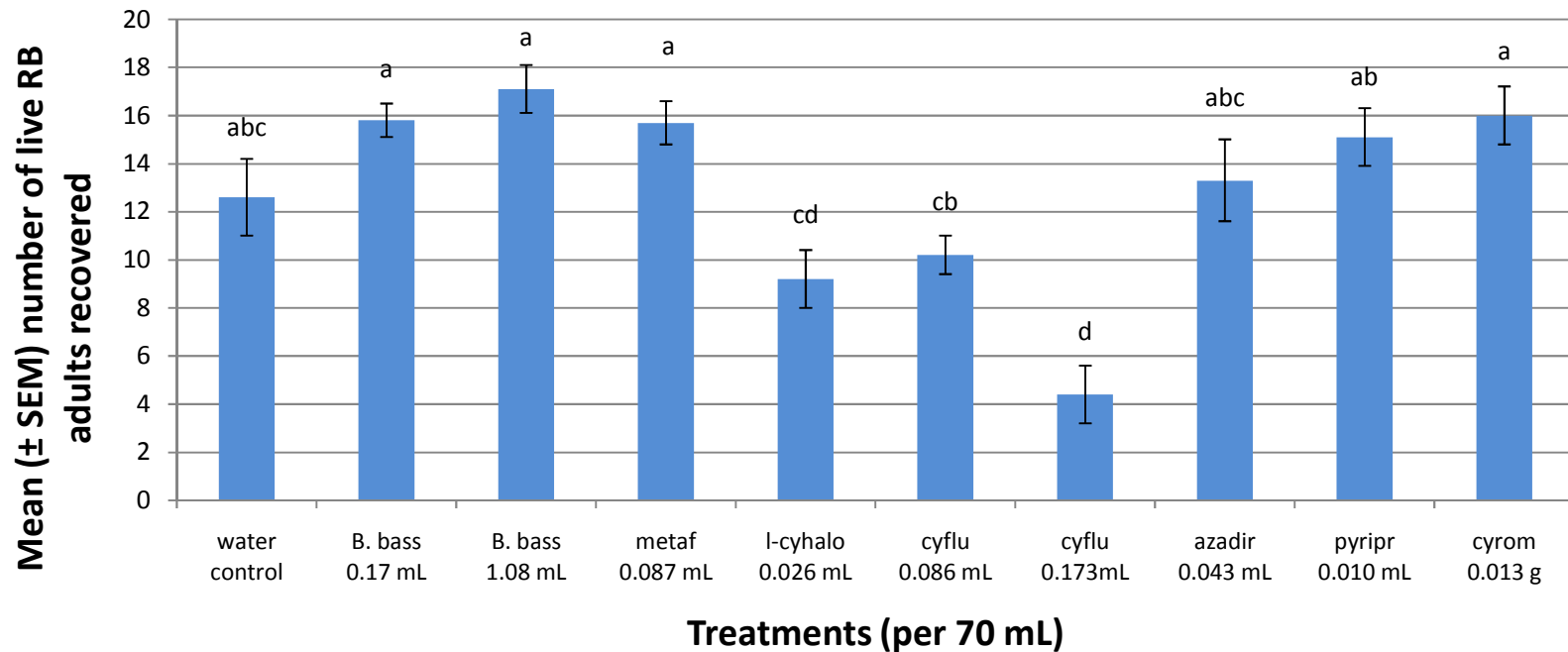


Figure C-4 Rove beetle (RB), *Atheta coriaria* adult recovery rates two days after exposure to pesticides used in experiment 2 in which 20 adults were applied to containers with 300 mL growing medium (SB300 Universal Professional Growing Mix; Sun Gro Horticulture Inc.; Bellevue, WA) 24 hours before applying the pesticides. There were 10 replications per treatment. Means followed by the same letter are not significantly different ($P \leq 0.05$) as determined by Tukey's mean separation test. Vertical bars represent standard errors of the mean (SEM). The pesticides and rates used were *Beauveria bassiana* (*B. bass*) (BotaniGard; BioWorks, Victor, NY) at 0.17 mL/70 mL, *Beauveria bassiana* (*B. bass*) (BotaniGard; BioWorks, Victor, NY) at 1.08 mL/70 mL, metaflumizone (metaf) (Alverde; BASF Corp., Research Triangle Park, NC) at 0.087 mL/70 mL, lambda-cyhalothrin (l-cyhalo) (CyhaloCap; Whitmire Micro-Gen Research Labs, St. Louis, MO) at 0.026 mL/70 mL, cyfluthrin (cyflu) (Cy-kick; Whitmire Micro-Gen Research Labs, St. Louis, MO) at 0.086 mL/70 mL, cyfluthrin (Cy-kick; Whitmire Micro-Gen Research Labs, St. Louis, MO) at 0.173 mL/70 mL, azadirachtin (azadir) (Azatin; OHP, Mainland, PA) at 0.043 mL/70 mL, pyriproxyfen (pyripr) (Distance; Valent Corp., Walnut Creek, CA) at 0.010 mL/70 mL, cyromazine (cyrom) (Citation; Syngenta Crop protection, Greensboro, NC) at 0.013 g/70 mL.

Table C-1 Common name, trade name, rates, and company information associated with the pesticides used in experiments 1 and 2.

Common name (trade name)	Rate (per 100 gal)	Rate (per 70 mL)	Company
Experiment 1			
acetamiprid (TriStar)	2.66 fl oz	0.014 g	Cleary's; Dayton, NJ.
acetamiprid (TriStar)	5 fl oz	0.028 g	
kinoprene (Enstar II)	5 fl oz	0.027 mL	Wellmark International; Schaumburg, IL.
organic oils (Zero Tolerance)	6,400 fl oz	35.0 mL	Natural Garden Solutions; Piedmont, CA.
soybean and rosemary oil (Indoor Pharm)	3,200 fl oz	17.5 mL	Pharm Solutions; Port Townsend, WA.
Experiment 2			
<i>Beauveria bassiana</i> (BotaniGard ES)	33 fl oz	0.17 mL	BioWorks; Victor, NY
<i>Beauveria bassiana</i> (BotaniGard ES)	200 fl oz	1.08 mL	
metaflumizone (Alverde)	16 fl oz	0.087 mL	BASF Corp.; Research Triangle Park, NC.
lambda-cyhalothrin (CyhaloCap)	5 fl oz	0.026 mL	Whitmire Micro-Gen Research Labs; St Louis, MO.
cyfluthrin (Cy-kick)	16 fl oz	0.086 mL	
cyfluthrin (Cy-kick)	32 fl oz	0.173 mL	
Azadirachtin (Azatin)	8 fl oz	0.043 mL	OHP, Mainland, PA.
pyriproxyfen (Distance)	2 fl oz	0.010 mL	Valent Corp.; Walnut Creek, CA.
cyromazine (Citation)	2.66 oz	0.013 g	Syngenta Crop Protection; Greensboro, NC.

APPENDIX D. NUMBER OF ROVE BEETLE *ATHETA* *CORIARIA* ADULTS AFTER ONE GENERATION USING SAME- AGE ADULTS AS INITIAL POPULATION.

Materials and Methods

In order to obtain same-aged rove beetle cohorts, 10 rove beetle adults were collected randomly from the main colony into a 9 dram vial using an aspirator, and then transferred to a 90 x 10 mm Petri dish lined with filter paper, plus 2 tsp (\approx 3 g) of moistened SB300 Universal Professional Growing Mix growing medium (GM), and 1 to 2 pieces of oatmeal for egg deposition. One week after inoculating the GM, twenty eggs were collected using a soft fine camel-hair paintbrush, which were placed into a 473 mL deli squat container with 300 mL of moistened GM and oatmeal. After approximately one week, the GM was examined for the presence of larvae. Larvae were collected by sieving the GM using #5 and #10 mesh size sieves and then recovered using a 9 dram vial using an aspirator. The total number of larvae recovered per deli squat container was recorded. Subsequently, larvae were transferred to a 90 x 10 mm Petri dish containing 2 tsp (\approx 3 g) of moistened GM and 1 to 2 pieces of oatmeal. After 10 days, the GM was examined daily for 5 to 10 days to detect the presence of adults. Adult rove beetles were collected into a 9 dram vial using an aspirator and gender (male or female) was determined. Immediately after emergence, male and female adults were grouped together to form mated pairs. The total number of adults emerged per Petri dish and the development time (days) from egg to adult was recorded. In addition, twenty late instar (approximately 4.0 mm in size) larvae (Miller and Williams 1983) were collected from the main colony using the procedure described previously. After 10 days, the GM was examined daily for 5 to 10 days to detect the presence of

adults. Adults were collected into a 9 dram vial using an aspirator and the number of adults that emerged per Petri dish was recorded.

In order to ascertain the number of RB adults in the F_1 generation associated with two different RB densities, the GM in a 473 mL deli squat container was inoculated with 1 and 5 RB pairs. Sex of the adults was determined based on the eighth abdominal tergite as described by Klimaszewski and Peck (1998). There were eight replications per initial RB density. Based on availability of RB adults, recently emerged males and females (< 24 hours old) were used to form mated pairs, which were then separately placed into 473 mL deli squat containers filled with moistened GM. To prepare the substrate, 300 mL of GM was measured using a 600 mL beaker and placed into the container. Subsequently, 60 mL of water and approximately 1.5 g of raw oatmeal was added onto the top of the GM as a supplemental food source.

After 18 to 21 days, assuming a generation period of approximately three weeks under the experimental conditions, the P_1 adults were collected by sieving the GM, then recovered into 9 dram vials using an aspirator, and placed on a filter paper disk lining the bottom of a 90 x 10 mm Petri dish with 2 tsp (\approx 3 g) of moistened GM and 1 to 2 pieces of oatmeal. The GM in the deli squat containers was examined daily for 7 to 10 days to detect the presence of RB adults (F_1 generation) and the total number of RB adults in the F_1 generation for each rove beetle density (1 and 5 RB pairs) was recorded.

In a separate experiment, three individual RB colonies were established at 12-day intervals to ensure availability of sufficient RB adults for each colony ($P_1 = 50$). Rove beetle adults were randomly obtained from the main colony assuming that the ratio of males to females was equal (sex ratio 1:1). The three colonies were maintained in plastic containers (6.0 L capacity) using Universal SB300 Professional Growing Mix growing medium (GM) as a

substrate. Preparation of the substrate was as follows; the GM was mixed with water, and approximately 2 tsp (≈ 3 g) of raw oatmeal was placed onto the surface of the GM. In order to maintain constant moisture, water was sprayed every 2 to 3 days onto the surface of the substrate using a 946 mL spray bottle.

To allow for mating and egg deposition, RB adults were maintained in the containers for 10 days. Adults were collected by sieving the GM using mesh sieve sizes of #5 and #10 and then recovered into a 9 dram vial using an aspirator. Consequently, only larvae (F_1 generation) remained in the containers. After larvae developed into adults (approximately one week), the F_1 RB adults were used to establish new individual colonies. Thus, a total of nine replications were obtained from three F_1 colonies (three replications from colony 1, one replication from colony 2, and five replications from colony 3, using 25 same-aged adults per colony as the initial population). The nine replications were established based on the availability of adults within 3 days.

All replications (F_2 colonies) were evaluated after 21 days, assuming a generation period would be completed after approximately three weeks based on the findings of Carney et al. (2002). Adults and larvae were collected separately as described above, using an aspirator, and counted using a manual hand-held counter. In order to facilitate the counting process, adults were placed into a 90 x 10 mm Petri dish and positioned on crushed ice to inhibit movement. The total number of adults and larvae was recorded.

Results and discussion

Rove beetle cohorts (individuals approximately the same age) were obtained after inoculating the GM in deli squat containers with 20 eggs laid within 2 to 3 days. The number of late instar larvae recovered per deli squat container was 10.9 ± 0.1 (Mean \pm SEM) and the mean

number of RB adults obtained per cohort was 5.3 ± 0.4 . Total development time from egg to adult per cohort using SB300 Universal Professional Growing Mix as a substrate was 20.3 ± 0.8 days. Percent recovery of third instar RB larvae per deli squat container was approximately 50% (10 out of 20) and the percent that developed from the egg to the adult stage per cohort was 25 to 30% (≈ 5 out of 20), suggesting that there may have been some cannibalism during the larval stage. For the nine cohorts obtained after inoculating the GM with late instar RB larvae in a Petri dish, the highest percent adult emergence based on the initial number of larvae was 85% (17 out of 20).

The number of RB adults recovered four weeks after inoculating the GM in the deli squat containers with 1 and 5 RB pairs was 38.4 ± 6.8 and 22.4 ± 9.3 . In addition, the number of adults recovered three weeks after inoculating the GM with 1 RB pair was 27.3 ± 5.6 . In three individual colonies, the total number of RB adults recovered 8 weeks after inoculating the GM with 1 RB pair was 120, 129 and 114; while in one colony, the total number of RB adults 8 weeks after inoculating the GM with 5 RB pairs was 272. Furthermore, the number of rove beetle adults (F_1 generation) four weeks after inoculating the GM with 25 rove beetle adults was 222.9 ± 25.4 .

The number of RB adults in the F_1 generation, three to four weeks after initial inoculation of the GM with 1 and 5 RB pairs, was relatively low (38 and 22 adults), however, assessments of F_1 populations did not include eggs, larvae and pupae. In fact, in only three out of eight replications, the number of adults was higher than 20 (28, 33 and 62 adults) when using 1 RB pair as an initial density, with high variability observed in the number of RB adults recovered. However, the number of adults recovered after one generation was lower when using 5 RB pairs

than 1 RB pair, which may be associated with mutual interference, lower female fecundity at high RB density, higher female mortality, or females unable to produce offspring.

APPENDIX E.



Figure E-1 Heat lamps (Commercial Electric; Atlanta, GA) and 473 mL deli squat containers (Fabri-Kal Corp., Kalamazoo, MI) used to assess the direct effects of pesticides and plant growth regulators on adult *Atheta coriaria*.



Figure E-2 Petri dishes and 740 mL plastic containers used to assess *Atheta coriaria* fecundity and development time from egg to adult.