Effects of Farm Management Practices on Pest Slugs and Slug Predators in Field Crops

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Academic Abstract

Mid-Atlantic crop producers are increasingly transitioning to soil conservation methods that include reducing or eliminating tillage and planting high residue cover crops. These practices are associated with an increase in moderate to severe damage to field crops by slugs. Conserving, and even enhancing, natural enemy populations is a desirable way to manage slug infestations because remedial control measures are limited. To better understand how cover crop usage and tillage practices affect slug and natural enemy populations, 43 Virginia fields with different combinations of tillage practices and cover crop use were intensively sampled in 2018 and 2019. Fields were sampled over a six-week period during the early planting season when slugs are most problematic. Shingle traps and pitfall traps were used to sample slugs and natural enemies, respectively. To determine how multiple farming practices, soil composition, landscape features, and field history affect slug feeding injury to seedling plants, over 1,000 hectares of commercial production fields in the Shenandoah Region of Virginia were scouted for slug feeding injury to seedling plants. Corresponding crop producers were then surveyed on management methods. Our goal was to determine if slug feeding risk could be predicted by a single factor and or a combination of factors. Behavioral assays were performed with a common slug pest, *Deroceras laeve*, to determine if this species prefers feeding on maize, soybean, daikon radish, crimson clover, rye, or hairy vetch leaf tissue. Our sampling study found that cover crop use and conservation tillage type

did not affect slug presence and damage, but that these factors affected various slug predators in different ways. We also observed that fields with more Phalangiidae and total predators overall had fewer slugs. Average slug feeding injury in both years was low and no factor or interaction of factors in our broader survey affected slug feeding injury ratings in fields. Behavioral assays indicated that slugs fed more on soybean tissue compared with maize, slugs consumed less maize when it was offered with hairy vetch or crimson clover, and slugs consumed less soybean when it was offered with hairy vetch or daikon radish.

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General Audience Abstract

Invasive slug species the gray field slug, *Deroceras reticulatum* (Muller), and the marsh slug, *Deroceras laeve,* are major pests of maize and soybean grown in Virginia no-till systems. Slug feeding causes injury to young plants that, when severe, can reduce crop yield and necessitate replanting a field. Chemical control options are limited, and farmers may not be willing to use tillage as a control measure. Conserving arthropod slug predators may suppress pest populations below economic levels. Research is needed to understand how farming methods such as tillage type (e.g., no-till, strip tillage, vertical tillage) and cover crop use affect slugs and their predators. Our study investigated 1) how tillage type and cover crop use affected slug and slug predators, 2) if certain farming practices and field characteristics can be used to predict slug injury, and 3) if slugs preferred feeding on commonly-used cover crop species when offered with maize or soybean seedlings. Commercial production fields with different combinations of no tillage or reduced tillage, and planted with or without cover crops, were sampled to evaluate how these farming practices affect slugs and slug predators. Shingle traps and frames were used before planting and during early plant growth stages to collect and identify slug species; pitfall traps were used throughout the growing season to collect and identify slug predator species. To determine if slug feeding risk could be predicted, a survey of commercial production fields in the Shenandoah area was conducted and used to identify factors, if any, that influence slug feeding. Laboratory assays were used to determine feeding preference by offering slugs opportunity to feed on leaf tissue from a maize or soybean seedling or a mature cover crop species. We found that tillage type and use of cover crops did not affect the abundance of slugs, but that they did influence predator populations. The highest number of total predators were found in fields with reduced tillage, cover crops, and no insecticide use prior to crop emergence. Harvestmen were potentially the most impactful slug predator in the region. Field surveys suggested that no tested factor or interaction of factors affected slug injury to plants in commercial fields. Finally, we observed that slugs fed differently on soybean and maize tissue depending on cover crop species present.

DEDICATION

My husband, Adam Alford.

My advisor, Sally Taylor.

My cat, Jasmine.

My slugs that didn't die mid-experiment (number 14, you were incredible. RIP.)

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TABLE OF CONTENTS

List of Tables and Figures

Chapter 1

Chapter 2

Chapter 4

CHAPTER 1: LITERATURE REVIEW

General Slug Life History and Behavior

Slugs are a polyphyletic group of gastropods that includes a range of families (Godan, 1983). They are distinguished from snails by absence of a shell into which they can fully retract their body, although most slugs have a vestigial shell remaining inside of their mantle. Some slug species are crop pests including *Deroceras reticulatum* (Müller) (Mollusca: Pulmonata), a slug distributed worldwide that feeds on several cultivated plants; *Deroceras laeve* (Müller) (Mollusca: Pulmonata); and *Arion subfuscus* (Gastropoda: Pulmonata).

The gray garden slug or gray field slug, *D. reticulatum,* is a medium-sized slug that can grow up to 5 cm in length. It can be colored various shades of gray, cream, and brown with dark blackish spotting and mottling observed in most individuals (Figure 1). This slug will produce a thick milky white mucus and hunch its back when disturbed (Douglas & Tooker, 2012). It is a member of the Agriolimacidae family, a group of slim and active slugs with a keel that is limited to the tail (i.e., does not reach the mantle) and the pneumostome located behind the midpoint of the mantle (Godan, 1983). *Deroceras reticulatum* prefer areas that are more arid and disturbed than other slug species, a factor contributing to their potential to be important crop pests.

Deroceras reticulatum has an annual life cycle and potentially overlapping generations in the eastern US. Adult *D. reticulatum* generally mate in the late summer and or fall and lay eggs in the soil. Slugs are hermaphrodites and *D. reticulatum* is capable of successful self-fertilization when suitable mates are not available. Eggs, along with some juveniles and adults, will overwinter in sheltered areas including crop residues in fields. Surviving adults will become active in the spring and resume laying eggs; eggs laid the prior year will hatch in late April to early May in Virginia (Douglas & Tooker, 2012).

The marsh slug, *D. laeve*, is a smaller slug in the Agriolimacidae family that grows up to 2.5 cm in length. It is dark gray to nearly black with no obvious markings (Figure 2), and will produce watery clear mucus and quickly move away when disturbed. *Deroceras laeve* is found in a wide range of habitats including greenhouses, crop fields, gardens, moist woodlands, and marshes (Douglas & Tooker, 2012).

Deroceras laeve also has an annual life cycle. *Deroceras laeve* overwinter as adults and it typically lays eggs in the spring, although some eggs are laid in the fall and hatch in early spring (Douglas & Tooker, 2012). Large adults become active in mid to late April and most eggs begin to hatch in mid to late May in Virginia.

Both *D. reticulatum* and *D. laeve* are generalists and feed on a wide variety of plant species. They feed on cultivated crops such as maize, rye, wheat, soy, and oilseed rape, as well as on cover crops and weeds. Slugs prefer dying and decaying plants to seedling maize in some instances, such as when dying rye is presented with spring planted maize or soybeans (Gall & Tooker, 2017). In addition to feeding on living plants, they consume organic matter and crop residues in the soil.

Slugs have skin that consists of a single epithelial cell layer. Accordingly, avoiding desiccation via water loss greatly influences slug activity and density. *Deroceras reticulatum* and *D. laeve* are most active during the evening and overnight and after rainfall. These are times when humidity and soil moisture level are high, reducing the risk of desiccation. Slugs shelter in the soil, under crop residues, and in similar shelters that provide cool, moist refuges during warm and dry periods. Both species gain the most weight and have the highest reproductive capacity around 16° Celsius. As temperatures approach 5°C and 26°C, slug growth, life span, and developmental speed decrease and mortality increases (South, 1981). Slugs are most abundant and active in crop fields in years with mild winters and cool, wet springs. These conditions lower overwintering mortality and are optimal for maintaining body water levels while feeding and reproducing in the spring (South, 1992).

Slug Feeding in Cultivated Crops

Slugs are generalist herbivores and several species are pests of crops including maize, *Zea mays* L., and soybean, *Glycine max* (L.) Herr. The four most common pest species of slugs in the eastern United States are *Deroceras reticulatum, Deroceras laeve, Arion subfuscus,* and *Arion fasciatus* (Nilsson) (Gastropoda: Pulmonata). Of these, *D. reticulatum* is considered the most important pest slug species in maize and soybean grown in the mid-Atlantic US, with *A. subfuscus* and *D. laeve* occasional pests (Douglas & Tooker, 2012).

Deroceras reticulatum causes economic damage to maize and soybean in the early season following planting and crop emergence when adult and juvenile slugs feed on seeds and seedling plants. This is particularly problematic when planting date is closely aligned with slug hatch and small plants and seed are exposed to a growing population of young slugs. Slugs cause injury to plants by scraping leaf surfaces with their radula, a tongue-like organ covered in rows of chitinous teeth (Godan, 1983). This feeding behavior causes a characteristic “windowpane” injury to plant leaves (Figure 3). Sustained feeding can result in shredded leaves in maize and destroyed cotyledons and associated seedling death in soybean. Yields decrease when high levels of injury occur, and farmers may be forced to replant sections, or entire fields, to ensure optimal stands and uniform plant growth. Once plants are larger in size (i.e., V4-V5) and or conditions favor rapid plant growth, plants are capable of outgrowing injury that the slugs may cause, regardless of whether adults or juveniles are the dominant life stage (Douglas & Tooker, 2012).

Slug Management Strategies

Chemical control options for slug control are limited. Insecticides are largely ineffective against slugs – carbamates have limited effectivity and are only registered for ornamental use. Molluscicide baits including metaldehyde and iron phosphate are effective, but they are also relatively costly, difficult to broadcast because of granular formulations, and water soluble. Baits cannot be applied in wet conditions and are likewise rendered ineffective by rainfall; thus, they frequently cannot mitigate a problematic pest population under the conditions that favor slugs. Tilling (i.e., cultivation of soil) is the most effective cultural method of slug control, as it reduces the amount of moist shelter to which slugs rely on for survival (Gall & Tooker, 2017). Tillage is not always an attractive option, because it contributes to loss of soil integrity and subsequent erosion. Planting in warm soil favorable for plant growth, and practices that promote plant growth and health stands early season (e.g., seeding rate, row cleaners, fertilization) can mitigate slug damage. Row cleaners are used to clear residue and allow the most sunlight to seed furrows and warm the soil, and fertilizer can be applied to encourage plant growth. If plants grow past the seedling stage (i.e., V4-V5) before slugs are abundant and active in the spring, yield losses can be reduced or avoided.

Planting into living cover crops, or "planting green", is currently being explored as a management practice to reduce slug feeding on crops (Gall & Tooker, 2017). Following planting and prior to seedling emergence, cover crops are killed with herbicides or destroyed with mechanical rollers. Ideally, slugs will consume the cover crop equally or more than the emerging seedlings, improving the odds that most seedlings will escape feeding injury. Planting green is growing in popularity with no-till farmers despite little or no empirical research supporting its benefits (Gall & Tooker, 2017).

Slug Arthropod Natural Enemies

Multiple groups of arthropods prey upon slugs and some species are gastropod specialists. Ground beetles (Carabidae), harvestmen (Opiliones), and some marsh flies (Sciomyzidae) are some known slug predators in the United States. Other potential predators observed worldwide include rove beetles (Staphylinidae), firefly larvae (Lampyridae), centipedes (Chilopoda), and some spiders (Arachnida) (Barker, 2004; Nyffeler & Symondson, 2001).

Carabids are perhaps the most common arthropod predator of gastropods with several species specializing in slugs and snails. European species such as *Carabus violaceus* (Linnaeus) and *Cychrus caraboides* (L.) will selectively feed on slugs (Barker, 2004). These beetles have been observed using their mandibles like "forks" and stabbing slugs either in the head or behind the mantle. This paralyzes or kills the prey, thus negating defensive mucus production that would make feeding and continued attacks difficult (Pakarinen, 1993).

Other carabid generalists, such as *Pterostichus niger* (Schaller), *Harpalus rufipes* Degeer, and *Pterostichus melanarius* (Illiger) will consume slug juveniles and eggs when available and prefer slugs that have been stressed and are unable to produce defensive mucus (Pakarinen, 1993). Some generalist carabids feed on slug eggs and small juveniles and avoid larger adult slugs, likely because larger slugs produce more mucus (El-Danasoury, Cerecedo, Córdoba, & Iglesias-Piñeiro, 2017). Without the specialized hunting strategies of the slug-specialists, generalist carabids risk having mouthparts and tarsi immobilized with mucus if they cannot kill slugs quickly. This makes an unstressed adult slug difficult prey. Generalist predators prefer easier prey, like dipteran larvae and aphids, to slugs when available, and the presence of such prey in macrocosms can reduce slug control by carabids significantly (El-Danasoury et al., 2017; Symondson et al., 2006). It not known how this affects predation in a field setting where it is difficult to measure the availability and consumption of alternative prey.

Arachnids have been observed predating on slugs. Several species of harvestmen specialize in snails or slugs, such as *Phalangium opilio* (Linnaeus) and some other members of the Trogulidae family. Spiders will also consume slugs, though no specialists have been documented. In general, members of Mygalomorphae tend to include gastropods in their diet, possibly due to their larger size and time spent on the ground. A handful of species in Araneomorphae have been recorded consuming slugs, but gastropods make up an insignificant amount of their diet (Nyffeler & Symondson, 2001).

Two species of marsh flies (Sciomyzidae), *Tetanocera plebeja* (Loew) and *Tetanocera valida* (Loew), are larval parasitoids of slugs. *Tetanocera plebeja* larvae feed and grow in a single slug host until completion of the first instar, and *T. valida* will feed and grow until its third instar. When the initial host dies, larvae will finish consuming its remains and actively predate on other slugs and snails until pupation. In order to complete development, larvae may kill four to nine additional gastropods (Barker, 2004).

Conservation Tillage and Cover Crop Use

Conventional tillage practices (i.e., cultivation) are used to prepare the seed bed for planting, reduce competition from weeds, lessen soil compaction, and control some soil dwelling pests and disease (Hobbs et al. 2008). Detrimental effects of tillage include compaction, unfavorable pore size and distribution, and reduced biological activity (Titi, 2003). An unavoidable consequence of tillage is loosened topsoil that, in turn, leads to increased soil erosion, reduced capacity of soil to retain moisture, and increased water and nitrate runoff. Soil conservation strategies that do not use tillage (commonly "no-till" strategies) or use tillage minimally (i.e., reduced or conservation tillage), focus on increasing the organic matter in soil, which improves soil structure, reduces soil erosion, increases soil moisture capacity, and moderates soil temperature. Thus, no-till and conservation tillage strategies stratify the soil against drought, but they may also delay planting in some years because of cool, wet soils. Although adoption of soil conservation strategies can initially be more expensive due to the purchase of specialized machinery, additional herbicide applications, and initial yield deficits, no-till fields typically achieve yields comparable to conventionally managed fields within three to ten years (Powelson et al. 2014, Pittelkow et al. 2015). A multi-year study determined that reduced tillage and no-till had beneficial effects on soil health and structure compared to conventional tillage (Tebrugge, 1999). Benefits of conservation practices can be offset by increased slug feeding injury in maize and soybeans (Wilson, 1992). This is because increased residue on the soil surface provides food, humidity, and shelter for slugs (Titi, 2003).

The USDA defines conservation tillage by a minimum of 30% coverage of soil surface with crop residue (USDA, 2018). This is typically achieved by reducing the amount of soil turnover during tillage, which conserves residue on the soil surface. Conservation tillage methods may include vertical tillage, strip tillage, and no till. Vertical tillage uses disks set at an angle that reduce the amount of horizontal disturbance to the soil. Thus, most soil disturbance is vertical. Disks can be set at a range of angles, which leads to a range of soil disturbance. Nearly vertical disks will have minimal disturbance, while more angled disks will more aggressively cultivate soil. Strip tillage is a method of tillage where a thin strip of soil, ideally where the seeds will be planted, is cultivated and the area between is undisturbed (Al-Kaisi, 2012). The tilled area is generally 15-20 cm wide and 15-20 cm deep. By tilling only where the seeds will be planted, the soil there warms faster and better drains in wet environments. Ideally, this encourages faster germination. No-till fields do not use any type of tillage and the only soil disturbance consists of a furrow being cut in the soil, seeds dropped in, and the furrow pressed closed. Row cleaners may be used to remove surface residue away from the seed slot, allowing more sunlight to reach and warm the soil above germinating seeds.

Mid-Atlantic US crop producers are increasingly transitioning to no-till or conservation tillage management systems. Virginia has one of the highest no-till adoption rates in the US (Wade et al. 2015). According to the Soil Health Institute, the number of no-till farm acres in the US have increased by 8.3% from 2012 to 2017, with more than 100 million acres of no-till reported (LaRose, 2017). Many growers additionally plant cover crops to expand the soil-health benefits that no-till adoption provides. In Virginia, state and local governments, the Natural Resource Conservation Service (NRCS), and non-governmental organizations (e.g., Chesapeake Bay Foundation) emphasize soil management as a critical mechanism to reduce agricultural runoff into the Chesapeake Bay. The NRCS Environmental Quality Incentives Program (EQIP) promotes the adoption of no-till and cover crop usage. In Virginia in 2018, for example, EQIP producers are compensated, per acre, $70 for no-till and between $40-48 (wheat and rye, respectively) for planting cover crops.

Cover crops are used in agriculture for a variety of beneficial effects and are not harvested and sold as the main cash crop in an operation. Benefits from cover crops can include reduced erosion (Roth, 2017; Schipanski, 2013), improved soil health (Roth, 2017; Schipanski, 2013), pest and disease control (Chen, 2019; Palhano, 2017), increased biodiversity (Schipanski, 2013), and potentially increased cash crop yields (Bergtold, 2017). They can be used in most crop production systems, including no-till, reduced till, and conventional tillage operations. A variety of cover crop species may be planted including legumes (e.g., crimson clover and hairy vetch), cereals (e.g., wheat, rye, and other grasses), and broadleaf species (e.g., radishes and mustards) (Clark, 2015). Cover crop adoption has increased an average of 8.4% a year between 2012 and 2017, and by 2017, a total of 153,402 US farm operations planted cover crops (Soil Health Institute, 2017).

Pre-Plant Insecticide use in Virginia

Crop producers may broadcast insecticides to the field before or at planting, or prior to crop emergence with an herbicide application. These applications are designed to target multiple pests including cutworms, billbugs, seedcorn maggot, and armyworms. Broad-spectrum insecticides in the pyrethroid class are often used (Herbert, 2016). Pyrethroids and other insecticides applied to soil have no activity against slugs and can be highly-toxic to slug predators including ground beetles and spiders (Brust et al., 1985; Everts et al., 1989). In the Shenandoah region, insecticides are commonly applied with herbicides during cover crop termination or pre-emergence weed control. Insecticide use prior to and or at the time of planting may increase slug feeding, likely by recusing natural enemy populations (Douglas, 2016).

Figures

Figure 1. Adult *Deroceras reticulatum* with light brown coloration and dark brown mottling, Shenandoah county, Virginia, 2018. Photo K. Brichler.

Figure 2. Adult *Deroceras laeve* with nearly black coloration, Montgomery county, Virginia, 2018. Photo K. Brichler.

Figure 3. Window pane injury to maize leaf caused by *Deroceras laeve*, Shenandoah county, Virginia, 2019. Photo K. Brichler.

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CHAPTER 2: EFFECTS OF FARM MANAGEMENT PRACTICES ON SLUGS AND SLUG PREDATORS

Introduction

The gray garden slug or gray field slug, *Deroceras reticulatum* (Müller) (Mollusca: Pulmonata), and the marsh slug, *Deroceras laeve* (Müller) (Mollusca: Pulmonata), feed on corn *Zea mays* (L.), and soybean, *Glycine max* (L.), grown in the Shenandoah region of Virginia. *Deroceras reticulatum* is a medium-sized slug (up to 5 cm in length), colored various shades of gray, cream, and brown with dark blackish spotting and mottling in most individuals (Figure 1). It produces a thick milky white mucus and hunches its back when disturbed. *Deroceras laeve* is a smaller slug (up to 2.5 cm in length), and colored dark gray to nearly black with no obvious markings. It produces watery clear mucus and quickly moves away when disturbed. (Douglas & Tooker, 2012). Both species have overlapping generations through the year, and both overwintering adults and newly hatched juveniles are active in fields in the spring.

Both *D. reticulatum* and *D. laeve* are generalists and feed on a wide variety of plant species. They feed on both cultivated crops (e.g., maize, rye, wheat, soy, and oilseed rape) and cover crops and weeds. They consume organic matter and crop residues in the soil in addition to feeding on living plants. Slugs prefer dying and decaying plants to seedling maize in some instances (Gall & Tooker, 2017). Slugs can cause economic damage to maize and soybean in the early growing season when juvenile slugs feed on seed and newly emerged seedling plants. Damage may be extensive when planting date is closely aligned with slug hatch and small plants and seed are exposed to a growing population of young slugs. Sustained slug feeding can result in shredded leaves in maize and destroyed cotyledons and associated seedling death in soybean. Yields decrease when high levels of injury occur, and farmers may be forced to replant sections, or entire fields, to ensure optimal stands and uniform plant growth. Once plants are larger in size (i.e., V4-V5) and or conditions favor rapid plant growth, plants are capable of outgrowing injury that the slugs may cause, regardless of whether adults or juveniles are the dominant life stage (Douglas & Tooker, 2012).

Adequate chemical control of pest slugs may be difficult to achieve. Molluscicide slug baits, including metaldehyde and iron phosphate, labeled for slug control in maize and soybean fields are relatively expensive compared to other pesticides and are difficult to broadcast because of granular formulations. Baits are water soluble and are rendered ineffective by rainfall. Extensive damage to plant stands frequently occurs before a grower identifies a slug presence although scouting efforts have increased.

Biological management methods of slugs, such as the conservation or supplementation of natural enemies, could suppress slug pests below economic levels. Arthropod natural enemies of slugs include spiders, harvestmen and ground beetles (Barker, 2004). Generalist predators known to consume slugs could provide concurrent management of other invertebrate pests. Conversely, intraguild predation and the availability of alternative prey many decrease the effectiveness of generalist slug predators (Symondson et al., 2002). The role of slug predators in biological control and the predator species present in the Shenandoah valley crop production fields are largely unexplored.

Field management methods, including how the soil is tilled or cultivated, have the potential to affect slugs and slug predators. Tillage (i.e., the cultivation or turning over of soil) is used to prepare the seed bed for planting, reduce competition from weeds, lessen soil compaction, and control some soil dwelling pests and disease (Hobbs et al. 2008). Reduced or no tillage (commonly “no-till”) strategies are designed to increase the amount of organic material within and covering the soil. Reducing or eliminating tillage is associated with increased slug feeding. Larger *D. reticulatum* populations have been observed when tillage is reduced or removed (Mabbett, 1991), perhaps because tillage buries or damages slug eggs (Chabert and Gandrey, 2005). Increasing predator abundance and diversity may benefit slug control although there are few studies that document this phenomenon (Symondson et al., 2002).

There are documented increases in predator abundance associated with increased residue coverage in no-till systems (Prasifka et al., 2006 Pullaro et al., 2006); however, this effect is not always observed (Szendrei and Weber, 2009). Crop residues may provide a more suitable environment for alternative prey species that sustain predator populations year-round (Xu, Fujiyama and Xu, 2011). Further, some predators, like spiders, may leave crop fields when soil or plants are mechanically disturbed (Thorbek and Bilde, 2004).

Cover crops are used in agriculture for a variety of beneficial effects and are not harvested and sold as the main cash crop in an operation. They can be used in most crop production systems, including no-till, reduced till, and conventional tillage operations. Benefits from cover crops include reduced erosion (Roth, 2017; Schipanski, 2013), improved soil health (Roth, 2017; Schipanski, 2013), pest and disease control (Chen, 2019; Palhano, 2017), increased biodiversity (Schipanski, 2013), and potentially increased cash crop yields (Bergtold, 2017). Cover crop use has been documented to aid in control of armyworm in maize production systems by supporting natural enemy populations (Laub and Luna, 1992). Conversely, use of certain cover crop species (i.e., red clover or vetch compared to rye alone) increased slug feeding on the subsequent cash crop (Vernavà et al, 2005).

Broad spectrum insecticide use has been well-documented as a contributing factor to pest outbreaks, often because of detrimental effects on invertebrate predators (Dutcher, 2007; Knight et al, 2007; Zanardi et al, 2018). It is possible that farm management practices that include the broadcast of insecticides prior to plant emergence benefit slug pests by killing their natural enemies. In Virginia, these applications are designed to target multiple pests including cutworms, billbugs, seedcorn maggot, and armyworms. Broad-spectrum insecticides in the pyrethroid class are often used (Herbert, 2016). Pyrethroids and most other insecticides applied in maize and soybean have no activity against slugs and can be highly-toxic to slug predators including ground beetles and spiders (Brust et al., 1985; Everts et al., 1989). Insecticides applied to the seed coating may increase slug feeding and decrease yield, likely by recusing natural enemy populations (Douglas, 2016).

In 2018 and 2019, we sampled commercial maize and soybean fields in the Shenandoah region of Virginia that used different combinations of farm management methods (tillage, cover crops, and insecticide use) for slugs and arthropod predators in order to determine 1) the species of slugs and predators present, and 2) the effects, if any, of these management methods on slug and predator populations. We focused on no-till and reduced tillage systems, planting any species of cover crop, and broadcast pre-plant pyrethroid use, as these are common practices in the Shenandoah valley region where perennial slug pest populations are problematic. We used shingle and pitfall traps to sample slugs and predators, respectively. We determined that management practices affect slugs and their predators differently, and that only certain species of predators were affected. Our findings have the potential to impact how maize and soybean are managed for slug pests in this region of the mid-Atlantic US.

Materials & Methods

Field selection

Commercial maize and soybean fields in the Central Shenandoah Valley region of Virginia (i.e., Rockingham, Augusta, and Shenandoah counties) were sampled for slugs and arthropod predators. Fields were chosen based on tillage (i.e., no tillage or reduced tillage) and cover crops use (i.e., with and without cover crops planted). Reduced tillage included vertical tillage and strip tillage. Twenty fields were sampled in 2018: 10 planted without cover crops and 10 planted with cover crops (e.g., wheat, barley, daikon radishes, crimson clover). Within each group, five used reduced tillage (e.g., vertical tillage, strip tillage) and five were not tilled. Twenty-four fields were sampled in 2019; 12 planted without cover crops and 12 planted with cover crops (e.g., wheat, barley, daikon radishes, crimson clover). Within each group, six used reduced tillage and six were not tilled. Insecticide use was taken into consideration in 2019 and three fields in each sub-group used a broadcast insecticide prior to crop emergence (i.e., a pyrethroid application timed with a burn down or pre-emergence herbicide application) and three did not.

Slug sampling, collection, and identification

To quantify slug abundance and species composition, artificial shelters and timed visual observations were used. Artificial shelters consisted of a single 30.5 cm^2 roofing shingle placed on a cleared patch of soil and held in place with a metal flag (Douglas & Tooker, 2012). Four shelters were placed in each location along a diagonal transect. Shelters remained in the field for 48 h. They were then flipped over and the bottom of the shelter and ground beneath it searched for slugs for two minutes. Timed visual observations were taken using a 30.5 cm^2 wooden frame. Four areas per field were sampled along a diagonal transect perpendicular to the transect used for shelters. Frames were placed on the ground and the area within them, including any plant residues and or live plants, were searched for slugs for two minutes by visually scanning the area within the frame and moving residue as necessary to check for slugs. Adult slugs were identified to species in 2018. Adults and juveniles were identified to species in 2019. Locations were sampled every two weeks to cover the time period before planting through when plants were small (V1 – V3) and most vulnerable to slug injury. In 2018, slugs were sampled and collected 18 April - 15 June. In 2019, locations were sampled 15 April - 28 June.

Natural enemy sampling and collection

To quantify natural enemy abundance and species composition, four pitfall traps were placed along a diagonal transect. Pitfall traps consisted of two nested 473 ml clear polypropylene containers (Fabri-kal Corporation, Kalamazoo, MI) placed in the soil, with the cup lip level with the ground. The top container was filled with 3 to 5 centimeters of soapy water or propylene glycol. Soapy water was used April – May 2018. Propylene glycol was used June – August 2018 and 2019 to better preserve specimens in hot, humid conditions. A paper plate secured by wooden stakes was placed four cm above the trap to protect from rainfall and debris (Figure 1). After two days, all arthropods and slugs in the traps were collected, returned to the lab, and stored in 70% ethanol until identification. Carabidae and Lycosidae were identified to genus, Phalangiidae to species, and other insects and spiders to family. Voucher specimens were preserved and submitted to the Virginia Tech Insect Collection in Blacksburg, Virginia. Locations were sampled every two weeks in conjunction with slug sampling. Natural enemies were sampled for a longer period than slugs and were collected until 16 August 2018 and 28 June 2019.

Data Analysis

All statistical analyses were performed in R Studio (version 3.5.0) (R Core Team, 2018). Negative binomial generalized linear mixed-effects models with random effects (GLMER) were used to analyze the abundance (i.e., the total number of individuals summed from all traps in a field) of slugs (all species combined), slug predators (all species combined), Carabidae, Philangiidae, and Lycosidae. Years 2018 and 2019 were analyzed separately because of large differences between the number of slugs caught (i.e., 2019 had over three times as many slugs as 2018). Slug density data from 2018 were analyzed with a zero-inflated generalized linear mixed-effects model with random effects due to a high number of zero counts. Fixed effects for 2018 models included tillage type (reduced tillage or no tillage), cover crop use (yes or no), and their interaction. Slug density from 2019 was analyzed with a negative binomial generalized linear mixed-effects model with random effects. Fixed effects for 2019 models included tillage type (reduced tillage or no tillage), cover crop use (yes or no), insecticide use (yes or no), and all possible interactions. Location was treated as a random effect in both years. Means were separated using emmeans (Tukey) at $\alpha = 0.05$. Correlations between slugs caught and predators caught were determined using Spearman's rank correlation rho.

Results

Slug Abundance and Species Composition

2018:

We collected 71 adult slugs from traps: 25 *Deroceras laeve*, 42 *Deroceras reticulatum*, and 4 *Arion subfuscus.* We collected 273 unidentified juvenile slugs. Total slug count for the year was

344 slugs. Highest total slug count was recorded on May 16 (Fig. 2). No variable alone had an effect on the number of slugs caught per field [tillage type (Pr (>|z|) = 0.7017, z = -0.383), cover crop use (Pr (>|z|) = 0.0533, z = -1.932)]. There was an interaction between tillage type and cover crop use (Pr (>|z|) = 0.000319, z = 3.599). No tillage fields with cover crops had fewer slugs than no tillage fields without cover crops (df = inf, p = 0.0034) and reduced tillage fields with cover crops (df = inf, p = 0.0372).

2019

We collected 548 *Deroceras laeve* and 169 *Deroceras reticulatum.* We collected no *Arion* subfuscus. Slugs caught in April had not been identified – total slug count for the year was 1026 slugs. Highest total slug count was recorded on May 16 (Fig. 2). No variable alone had an effect on the number of slugs caught per field [tillage type (Pr(>Chisq) = 0.2077, df = 1), cover crop use (Pr(>Chisq)= 0.2718, df = 4), insecticide use (Pr(>Chisq)= 0.8587, df = 4)]. There were no significant interactions [tillage type and cover crop use (Pr(>Chisq) = 0.9757); tillage type and insecticide use (Pr(>Chisq) = 0.0837); cover crop use and insecticide use (Pr(>Chisq) = 0.3021); tillage type, cover crop use, and insecticide use (Pr(>Chisq)= 0.2614, df = 12)].

Natural Enemy Abundance and Species Composition

2018:

No variable alone affected the total number of slug predators collected per field [tillage type (Pr(>Chisq) = 0.269, df = 1), cover crop use (Pr(>Chisq) = 0.6484, df = 4)]. There was an interaction between tillage type and cover crop use (Pr(>Chisq) = 0.0006, df = 8). The regression between total slugs collected per field and total predators caught per field was significant (p = 0.04212, correlation rho = -0.1368) (Fig. 3).

We collected 500 Carabidae from 15 genera (Table 1). No variable affected the total number of Carabidae collected per field [tillage type (Pr(>Chisq) = 0.6599, df = 1), cover crop use (Pr(>Chisq) = 0.3268, df = 1)]. Data were insufficient to determine any effects from interactions. The regression between total slugs caught per field and total Carabidae collected per field was not significant (p = 0.3155, correlation rho = -0.0678) (Fig. 4).

We collected 872 Phalangiidae and all were identified to a single species, *Phalangium opilio.* Tillage type affected (Pr(>Chisq) = 0.03264) total number of Phalangiidae caught per field, with more harvestmen collected in reduced tillage fields. There was not an effect from cover crop use (Pr(>Chisq) = 0.74, df = 4). Data were insufficient to determine any effects from interactions. The regression between total slugs collected per field and total Phalangiidae collected per field was significant (p = 0.0404, correlation rho = -0.1380) (Fig. 5).

We collected 373 Lycosidae from 12 genera (Table 2). Tillage type affected (Pr(>Chisq) = 0.0047, df = 1) total number of Lycosidae collected per field, with more spiders collected in no till fields. There was no effect from cover crop use (Pr(>Chisq) = 0.5786, df = 4) or PPI use (Pr(>Chisq) = 0.1395, df = 4). Data were insufficient to determine any effects from interactions. The regression between total slugs collected and total Lycosidae collected per field was not significant (p = 0.6402, correlation rho = -0.0316) (Fig. 6).

2019:

No variable alone affected the total number of slug predators collected per field [tillage type (Pr(>Chisq) = 0.5987, df = 1), cover crop use (Pr(>Chisq) = 0.3952, df = 6), insecticide use

(Pr(>Chisq) = 0.7706, df = 6)]. There was an interaction between tillage type, cover crop use, and insecticide use on total predator numbers caught per field (Pr(>Chisq)= 0.0079, df = 12). No till fields without cover crops and insecticide use had more predators than reduced tillage fields without cover crops and insecticide use (Pr(>Chisq) = 0.0377, z ratio = -3.125). There were no other significant interactions [tillage type and cover crop use (Pr(>Chisq) = 0.4064, df = 6); tillage type and insecticide use (Pr(>Chisq) = 0.08138, df = 6); cover crop use and insecticide use (Pr(>Chisq) = 0.9785, df = 6)]. The regression between total slugs collected per field and total predators collected per field was significant (p = 0.04212, correlation rho = -0.1368) (Fig. 3).

We collected 140 Carabidae from 12 genera (Table 1). No variable affected the total number of Carabidae [tillage type (Pr(>Chisq) = 0.3287, df = 1), cover crop use (Pr(>Chisq) = 0.3287, df = 4), insecticide use (Pr(>Chisq) = 0.5328, df = 6)]. Data were insufficient to determine any effects from interactions. The regression between total slugs collected per field and total Carabidae collected per field was not significant (p = 0.3155, correlation rho = -0.0678) (Fig. 4).

We collected 105 Phalangiidae and all were identified to a single species, *P. opilio.* No variable affected the total number of Phalangiidae [tillage type (Pr(>Chisq) = 0.6542, df = 1), cover crop use (Pr(>Chisq) = 0.6542, df = 4), insecticide use (Pr(>Chisq) = 0.7337, df = 4)]. Data collected were insufficient to determine any effects from interactions. The regression between total slugs collected per field and total Phalangiidae collected per field was significant (p = 0.0404, correlation rho = -0.1380) (Fig. 5).

We collected 480 Lycosidae from 13 genera (Table 2). No variable affected the total number of Lycosidae [tillage type (Pr(>Chisq) = 0.8278, df = 1), cover crop use (Pr(>Chisq) = 0.4011, df = 4), insecticide use (Pr(>Chisq) = 0.4722, df = 4)]. Data were insufficient to determine any effects from interactions. The regression between total slugs collected per field and total Lycosidae collected per field was not significant (p = 0.6402, correlation rho = -0.0316).

Discussion

Results of our study indicate that tillage type, cover crop use, and insecticide use alone do not affect the abundance of slugs in soybean and maize fields in the Shenandoah region of Virginia although interactions of multiple practices occurred. We observed fewer slugs in no-till fields that also had cover crops in 2018, but this effect was not consistent between years. This contrasts with previous studies that found no-till to increase slug abundance and activity (Voss, Ulber, and Hoppe, 1998; Wilson and Eisley, 1992). Slug numbers were over three times higher in 2019 compared to 2018, likely due to a mild winter in 2018 – 2019. Prolonged colder temperatures in the winter of 2017 likely killed many slugs because slugs do not survive freezing temperatures for long periods of time (Cook, 2004). No significant interactions occurred in 2019 when slug populations were larger, perhaps indicating that affects from management practices are measurable when slug pest populations are low or moderate.

Notably, slug feeding injury to crops in both years of this project was low for this region. Low injury in 2018 reflects the low number of slugs collected. Importantly, higher numbers of slugs in 2019 did not result in higher plant injury. Early season climatic conditions in 2019 were favorable to rapid plant growth (i.e., adequate moisture and sunlight, warmer temperatures) and plants matured rapidly through susceptible stages before injury could accumulate. Accurately characterizing risk from slug pests will depend on both the size of the pest population and spring climate. Results of our study could vary in cooler, wetter years with higher slug populations.

Three species of slugs were collected. *Deroceras reticulatum* and *D. laeve* were the most abundant species in 2018 and 2019, respectively. Lower *D. laeve* numbers in 2018 may have resulted, in part, from not identifying juvenile slugs. We collected few *Arion subfuscus* in 2018 and none in 2019. It is possible that plant injury from slugs is more severe when *D. reticulatum* populations are higher; *D. reticulatum* is frequently associated with crop injury in other regions in the mid-Atlantic (Douglas, 2012). There are no prior data on slug species composition in this area.

Similar to slug abundance, tillage type, cover crop use, and insecticide use alone did not affect the total number of potential slug predators collected. This is consistent with a recent meta-analysis that found arthropod predator abundance to not be affected by tillage type (Rowen et al., 2020). Cárcamo (2012) observed predators to be more abundant in conventionally tilled fields, but other studies observed more abundant predators in no tillage fields (Rodríguez et al., 2006, Roberston, Kettle, and Simpson, 1994). Multiple studies document higher numbers of generalist predators in fields with cover crops (Carmona and Landis, 1999, Lundgren and Fergen, 2010) and in no-till fields with intercropping (i.e. planting multiple crop types together) (Tonhasca Jr., 1993). Likely, effects on predator populations vary depending on multiple factors including cropping system, species composition, and climate. There were some combinations of practices that favored higher predator populations. Reduced tillage fields with cover crops and no insecticide use had the highest number of predators. No-till fields without cover crops that had insecticides applied had fewest slug predators identified in our study. We did not test insecticide use in 2018 and additional research is needed on how the impact of broad-spectrum insecticides affects natural enemies in different tillage systems. The detrimental effect of pesticides on arthropod natural enemy communities is well-documented (Hill, 2017; Whalen, 2016).

Several known slug predators were collected in our study. All harvestmen were the species *P. opilio*. *Phalangium opilio* has been recorded as a slug predator, as well as a beneficial predator of multiple other crop pests (Allard, 2005; Barker, 2004; Newton, 2001). Of the Carabidae collected, genera *Chlaenius* (Eskelson 2011), *Harpalus* (Eskelson 2011), *Poecilus* (El-Danasoury, 2018), *Pterostichus* (Wendland, 2017), and *Scarites* (Tulli, 2009) include generalist predators that have been documented to occasionally feed on slugs. The Lycosidae genera we collected did not include known slug predators and the total number of Lycosidae collected was not correlated with slug abundance. The small size of the most abundant Lycosidae genera, such as *Pardosa*, may keep these spiders from easily predating on slugs.

The number of total predators and number of Phalangiidae alone, specifically *P. opilio*, had a significantly negative correlation with the number of slugs collected. Our data support other studies that document the value of arthropod predators to slug control (Asteraki, 1993; Barker, 2004; Oberholzer, 2003; Wendland, 2017). Slug feeding has been shown to increase, and soybean yields decrease, when their predators are killed or intoxicated by insecticides (Douglas, 2016). The number of Carabidae and Lycosidae alone was not correlated with slug numbers in our study. Thus, different predators may be more integral to slug control in different crop growing regions and *P. opilio* populations could be contributing more significantly to slug control than Lycosid or Carabid species in the Shenandoah region.

Results of our project demonstrate that no single management method affects slug and slug predator abundance, but some combinations of methods did impact these populations. Effects on slug populations are likely dependent on year. Further, climatic conditions may be a better predictor of slug pest abundance. We were not able to measure the impact of climate because of the proximity of fields and number of reporting weather stations. Research is needed to support this hypothesis. We identified generalist predators are who may provide control of other crop pests in addition to slugs. Importantly, relationships between slug and slug predator populations, including the total number of slug predators and number of *P. opilio*, indicate that there is a pest control benefit to supporting larger populations of natural enemies.

Tables and Figures

Figure 1. Illustration of pitfall trap design used to sample for arthropod predators of slugs in Shenandoah region of Virginia, 2018-2019. Illustration by K. Brichler.

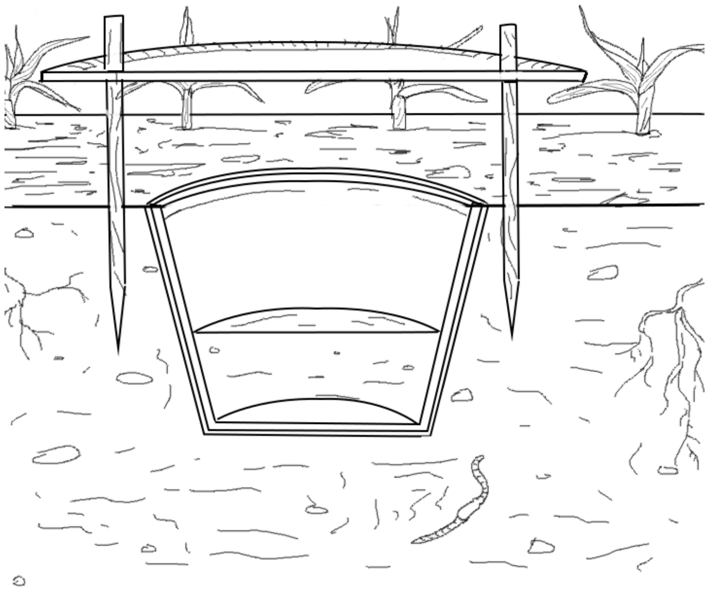


Figure 2. Total slugs (adult and juvenile *Deroceras laeve*, *Deroceras reticulatum*, *Arion subfuscus*) collected by sampling date in 2018 and 2019. Slugs were collected from shingle and frame traps in maize and soybean fields in the Shenandoah region of Virginia, 2018-2019.

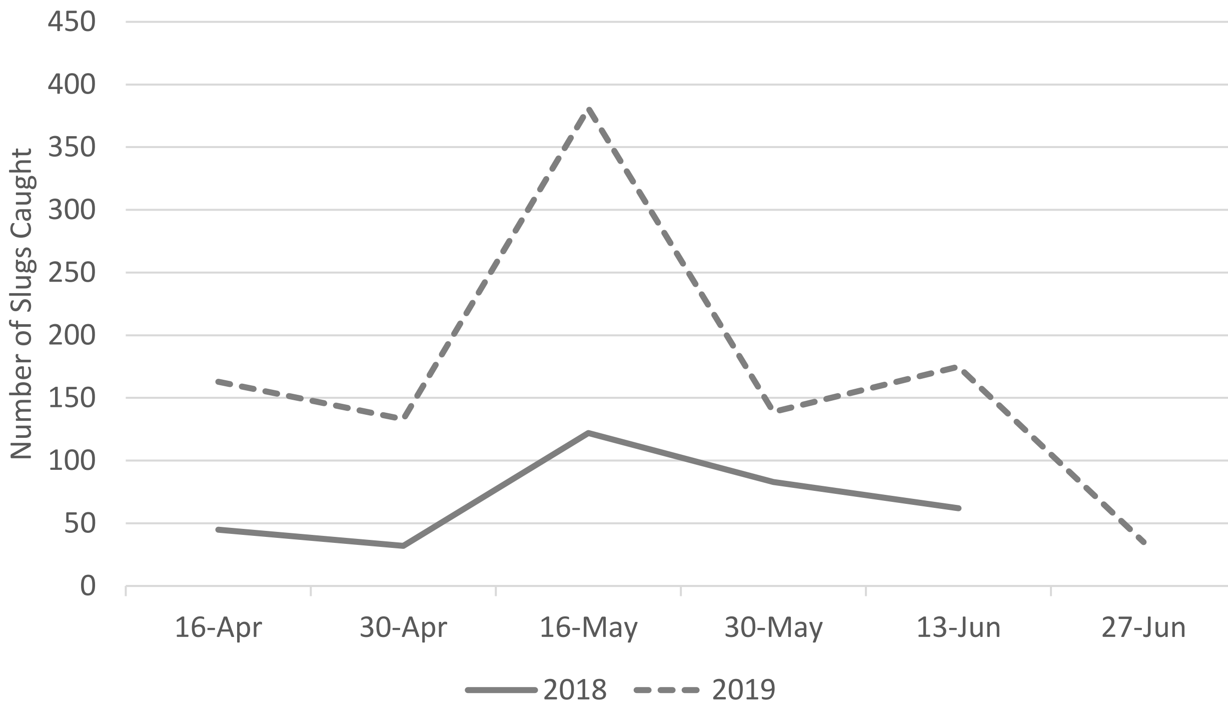


Table 1. Carabidae genera collected and identified from pitfall traps in maize and soybean fields in the Shenandoah region of Virginia in 2018 (April – August) and 2019 (April – June).

Carabidae Genera		
Genus	2018	2019
Acupalpus	6	0
Agonum	2	11
Amara	252	49
Anisodactylus	4	2
Atranus	0	1
Chlaenius	19	12
Cicindelidia	1	0
Dicaelus	2	0
Galerita	1	0
Harpalus	3	14
Notiophilus	1	0
Platynus	10	1
Poecilus	193	19
Pterostichus	1	13
Scarites	4	6
Selenophorus	0	4
Stenopholus	0	1
Trichotichnus	1	0

Table 2. Lycosidae genera collected and identified from pitfall traps in maize and soybean fields in the Shenandoah region of Virginia in 2018 (April – August) and 2019 (April – June).

Lycosidae Genera		
Genus	2018	2019 (20% identified)
Allocosa	1	2
Arctosa	0	1
Gladiocosa	0	1
Hesperocosa	3	3
Hogna	4	1
Pardosa	246	76
Pirata	6	1
Piratula	4	1
Rabidosa	1	1
Schizochosa	32	5
Tigrosa	32	5
Trochosa	10	1
Varacosa	1	1

Figure 3. Correlation between total slugs and total predators (Carabidae, Lycosidae, and Phalangiidae) collected and identified from shingle and pitfall traps in maize and soybean fields in the Shenandoah region of Virginia in 2018 (A) (April – August) and 2019 (B) (April – June).

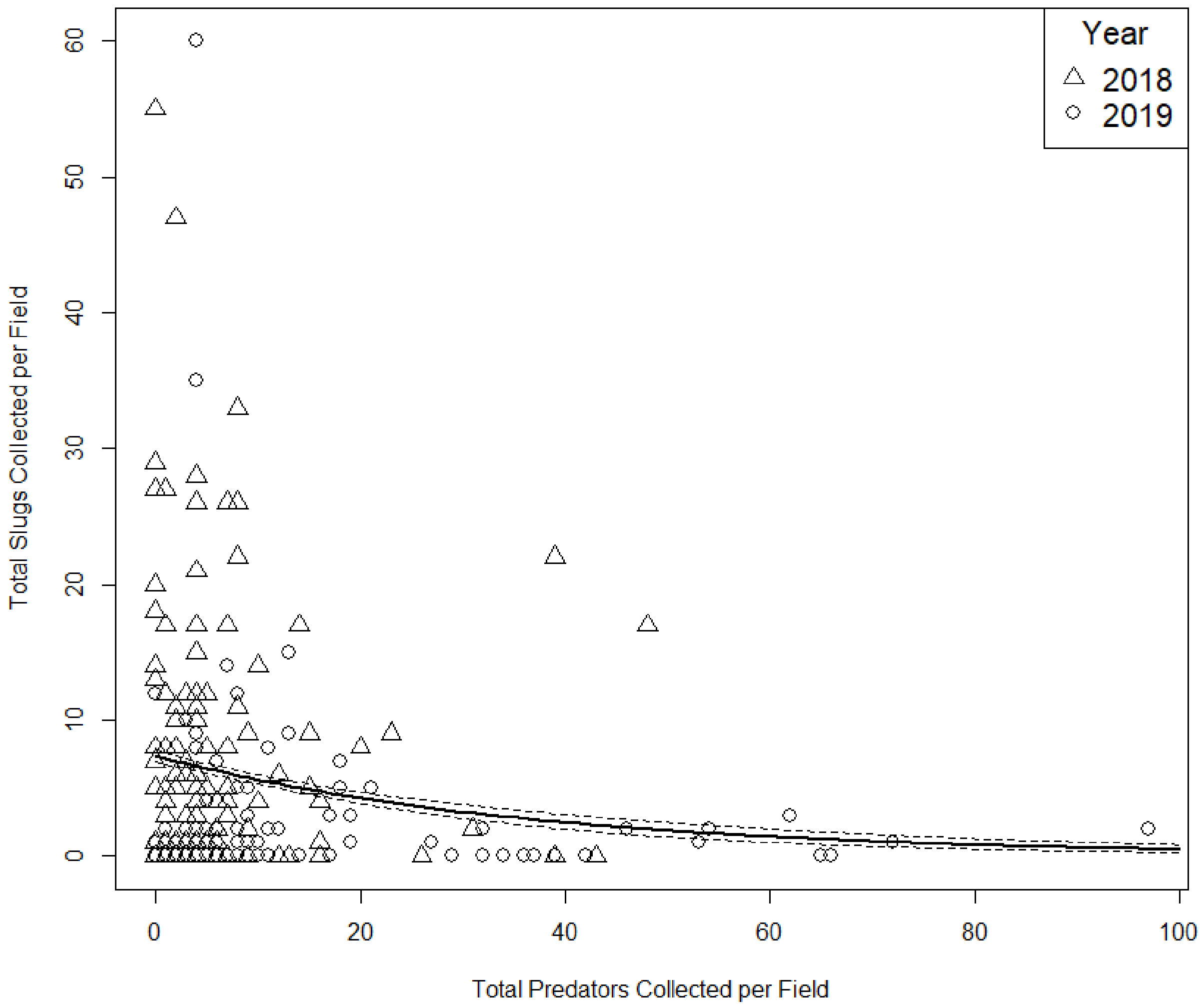


Figure 4. Correlation between total slugs and total Carabidae collected and identified from shingle and pitfall traps in maize and soybean fields in the Shenandoah region of Virginia in 2018 (A) (April – August) and 2019 (B) (April – June).

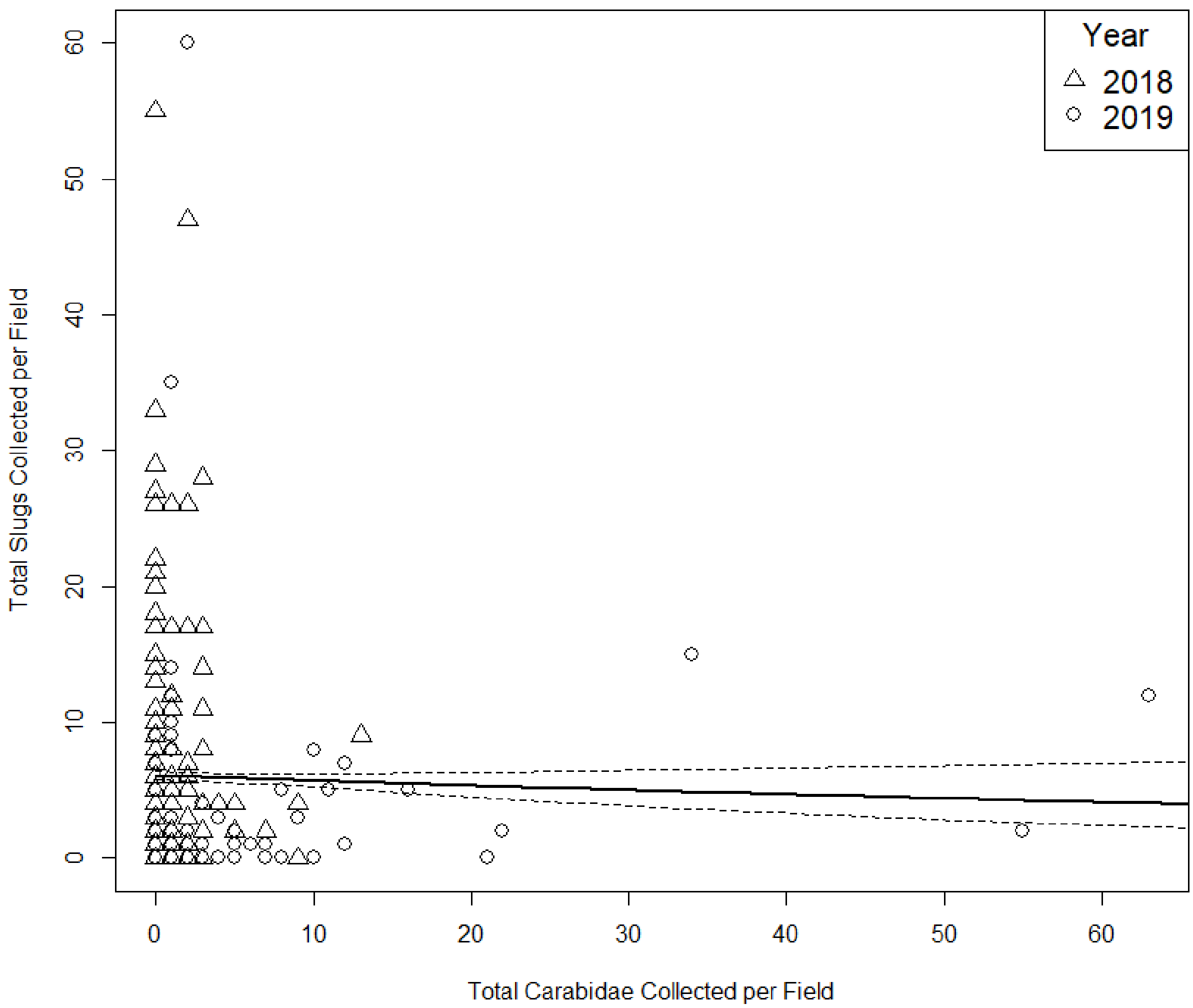


Figure 5. Correlation between total slugs and total Phalangiidae collected and identified from shingle and pitfall traps in maize and soybean fields in the Shenandoah region of Virginia in 2018 (A) (April – August) and 2019 (B) (April – June).

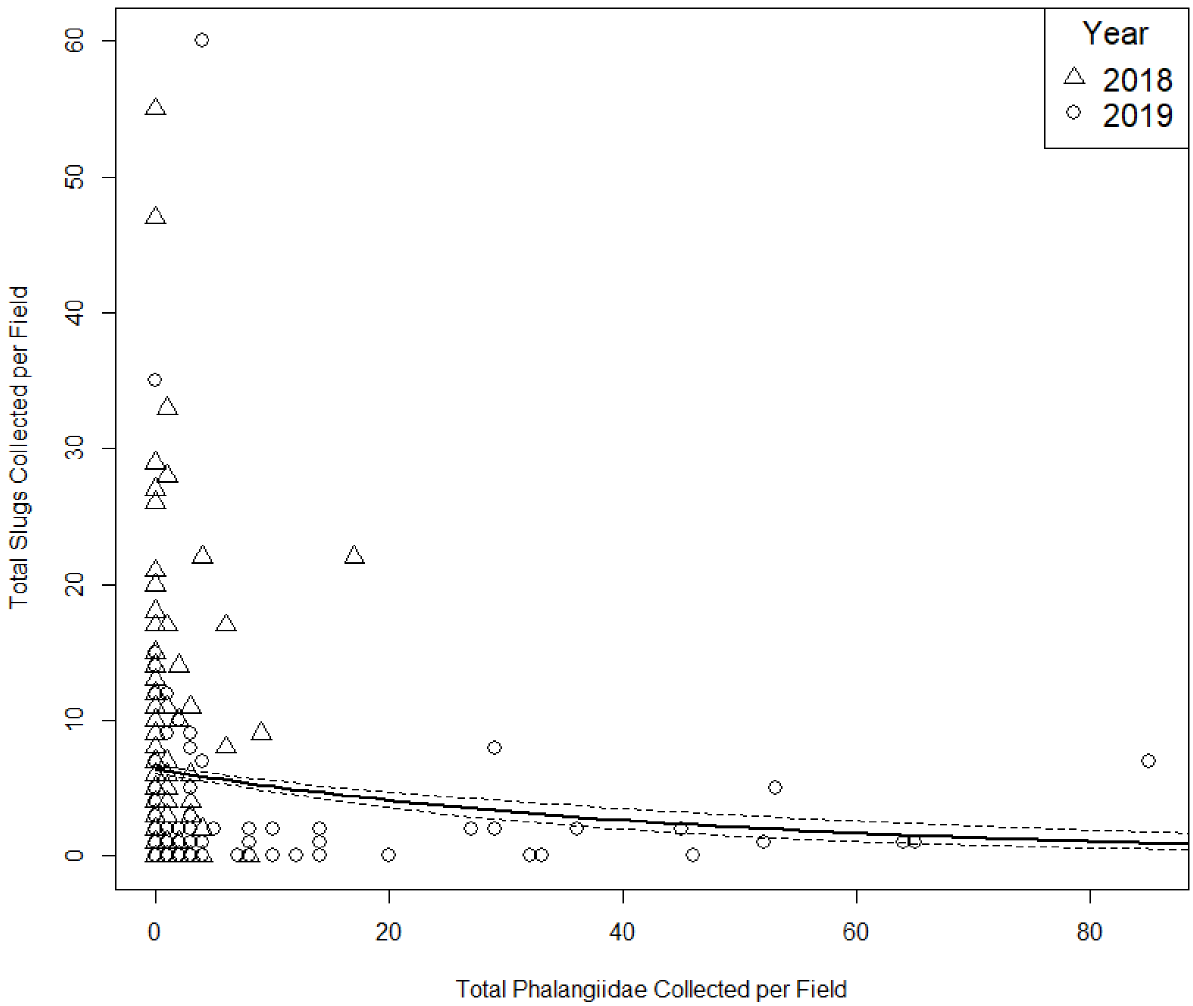
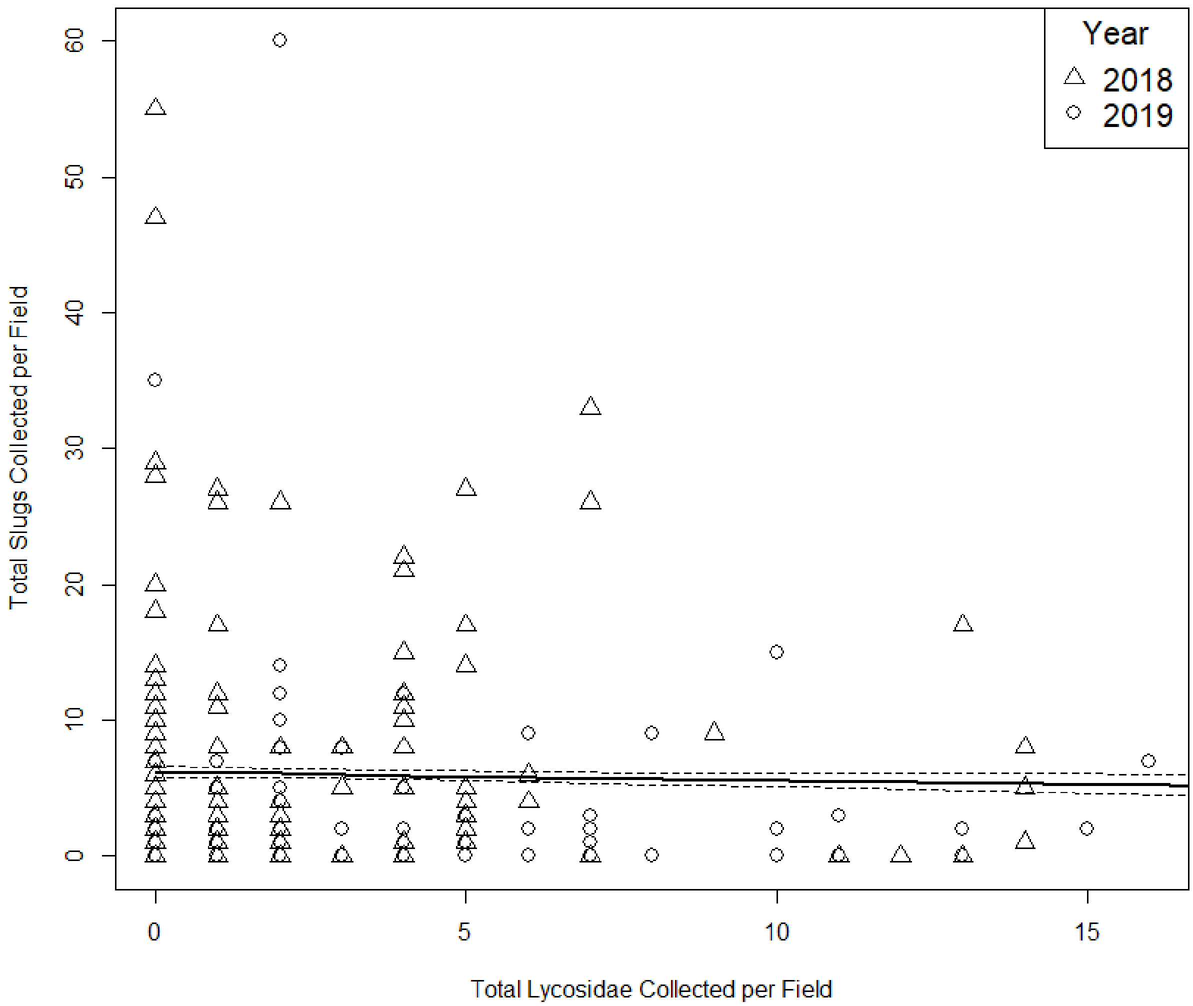


Figure 6. Correlation between total slugs and total Lycosidae collected and identified from shingle and pitfall traps in maize and soybean fields in the Shenandoah region of Virginia in 2018 (A) (April – August) and 2019 (B) (April – June).



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CHAPTER 3: CROP MANAGEMENT PRATICES, FIELD CHARATERISTICS, AND RISK OF SLUG FEEDING INJURY

Introduction

The gray field slug, *Deroceras reticulatum*, and the marsh slug, *Deroceras laeve*, are pests of maize, *Zea mays* (L.), and soybean, *Glycine max* (L.) grown in the mid-Atlantic US. Both species are generalist herbivores and consume a wide variety of plants, including maize, soybean, cover crops, and weeds (Douglas & Tooker, 2012). Slug feeding injury during the seedling stage of these crops (i.e., VE-V3 for maize, VC-V3 for soybean) can reduce grain yield and may necessitate replanting sections or entire fields. In the Shenandoah valley region of Virginia (i.e., Rockingham, Augusta, and Shenandoah counties), slug damage can be widespread and severe in some years.

Chemical control of slug pests may be difficult to achieve based on several factors. Molluscicides labeled for use in row crops include metaldehyde and iron phosphate baits. These products are water soluble and are rendered ineffective by rainfall. Slugs are abundant and active in crop fields in years with cool, wet springs because these conditions are optimal for maintaining body water levels while feeding and reproducing (South, 1992). Thus, wet conditions that favor slug pest outbreaks may also prohibit effective chemical control. Because of the sporadic nature and early season timing of slug outbreaks, problem fields are often not identified until severe damage occurs. Additionally, slug baits are granular and difficult to broadcast mechanically over large sections of a field.

Biological and cultural control methods of slug pests can reduce dependency on chemical control. Multiple management methods (e.g., tillage type, cover crop use, weediness, and row cleaner use) and field characteristics (e.g., soil type, residue coverage, moisture levels) can affect the amount of organic matter and crop residues available to slugs and slug predators to use as shelter and food sources. Pesticides can affect the health and abundance of arthropod predators that may help control slug populations (Douglas, 2016). Knowledge of practices or field characteristics that affect slug populations and the amount of crop damage that they inflict would greatly benefit farms in regions where slugs are a perennial pest and on years of severe damage occur. Ideally and when logistically possible and practical, farm management practices could be modified to reduce risk of slug feeding.

Increased slug populations are associated with the adoption of no tillage, or “no-till”, or reduced tillage management practices and the associated increase in field residues and organic matter (Hammond and Stinner, 1987; Hammond el al., 1999). Virginia has one of the highest no-till adoption rates in the US (Wade et al. 2015). No-till strategies or strategies that use tillage minimally (i.e., reduced or conservation tillage) focus on increasing the organic matter in soil, which improves soil structure, reduces soil erosion, increases soil moisture capacity, and moderates soil temperature.

To expand the soil-health benefits that no-till adoption provides, crop producers may additionally plant cover crops (i.e., crops that are not harvested and sold). Benefits from cover crops can include reduced erosion (Roth, 2017; Schipanski, 2013), improved soil health (Roth, 2017; Schipanski, 2013), pest and disease control (Chen, 2019; Palhano, 2017), increased biodiversity (Schipanski, 2013), and potentially increased cash crop yields (Bergtold, 2017). Cover crops may provide habitat that supports higher pest densities in some systems. There is evidence that injury to maize from caterpillar pests is greater following rye cover crop (Dunbar, O’Neal, and Gassman, 2016). In vineyards, higher leafhopper pressure was associated with using a buckwheat cover crop (Irvin, Bistline-East, and Hoddle, 2016). Multiple insect pests were found to be abundant in a variety of common cover crops throughout the year, including aphids, thrips, big-eyed bugs, plant bugs, and insidious flower bugs (Bugg, Phatak, and Dutcher, 1990). High pest densities in cover crops does not necessarily translate to higher pest densities in the cultivated crop (Tillman et al., 2004). Natural enemies populations may increase with cover crop use because of the availability of prey species year-round (Prasifka et al. 2006, Schmidt et al. 2007); however, larger numbers of natural enemies may or may not provide control of pests in the cultivated crop (Altieri and Schmidt, 1986).

Insecticides are not labeled to control mollusk pests and may kill or intoxicate arthropod natural enemies of slugs. Insecticides have been well-documented causal factors in secondary pest outbreaks and pest resurgences (Metcalf, 1980). Insecticidal seed coatings intoxicate ground beetle predators when slugs consume treated seeds (Douglas, Rohr, and Tooker, 2015) and natural enemy populations are reduced by both seed coatings and broadcast insecticides (Douglas and Tooker, 2016). Crop producers may apply insecticides targeting cutworm at or around the time of cover crop termination (Harris, Svec, and Sans, 1971). This practice is discouraged in Virginia Cooperative Extension recommendations, but it still occurs because of the relative low cost of some insecticides. Insecticides may contribute to slug outbreaks because timing of these applications coincide with spring slug hatch and seedling plant susceptibility to slug injury.

In 2018 and 2019, we conducted a survey of commercial maize and soybean fields in the central Shenandoah Valley of Virginia to determine the effects of various management methods and field qualities on slug injury to seedling plants. Over 3,500 hectares was surveyed over both years of the study. We measured slug feeding injury to plants, field residue coverage, and weediness. Farmer owners and or farm managers were questioned on their management practices and field history including crop rotation, tillage methods, cover crop use, row cleaner use and insecticide use. Our goal was to determine if any factor, or combination of factors, affected average slug feeing injury in a field. Ideally, knowledge of risk could be used by farmers to use to determine where to invest more heavily in scouting and slug management, thus saving time and reducing costs.

Materials & Methods

Field surveys

Commercial maize and soybean production fields in the central Shenandoah Valley of Virginia (e.g. Rockingham, Augusta, and Shenandoah counties) were surveyed for slug feeding injury to seedling plants in 2018-2019. One hundred fifty-eight (totaling approximately 1,500 hectares) and 192 (approximately 2,060 hectares) were surveyed in 2018 and 2019, respectively. Each field was surveyed once in the two weeks following plant emergence when seedlings were at highest risk of severe slug injury (mid-May – mid-June). Methods for estimating slug feeding were based on the "Scouting Protocol: Monitoring Fields for Slug Damage" (available from https://shenandoah.ext.vt.edu/content/dam/shenandoah_ext_vt_edu/files/ag/scouting-protocol-for-slug-management.pdf) (Appendix 1). We examined and rated plant injury on 3.048 m of a single row in four quadrants of each field. Plants were rated on a 0-4 scale (0= no damage; 1= only one leaf showing damage (less than 25% defoliation); 2= all leaves showing moderate damage (25 – 50% defoliation); 3= all leaves consumed except one remaining intact (greater than 75% defoliation); 4= seedling completely cut off at the ground level). Ratings from quadrants were averaged to calculate a single injury score per field. Crop type (maize or soybean), soil characteristics (e.g., moisture, type) and landscape factors (e.g., surrounding landscape, topography, field location) were recorded on the day of sampling. Residue coverage was measured using a marked 100-foot tape measure (NRCS). Weediness was estimated by periodically assessing levels of weeds throughout the field, and ranking the field as no weeds, low weediness, moderate weediness, or high weediness. Farmers and or farm operators for surveyed fields were given a questionnaire on their management practices including tillage type, cover crop use, cover crop type, row cleaner use, previous crop, molluscicide use, and insecticide use (Appendix 2).

Data Analysis

All statistical analyses were performed in R Studio (version 3.5.0) (R Core Team, 2018). Data were analyzed using a linear mixed-effects model with random effects to determine any interactions between variables (tillage type, cover crop use, insecticide use) and significant effects from individual variables. Data were pooled from both years for analysis because results from individual years were consistent with the combined analysis. Fixed effects included tillage type, cover crop use, pre-plant insecticide use, crop type, row cleaner use, soil moisture, topography, weediness, and percent ground cover. Not enough data was collected on previous crop or molluscicide use to run analyses. Year was considered a random effect. An ANOVA was performed to determine any difference between the null and interaction models. Means were separated using emmeans (Tukey HSD) at $\alpha = 0.05$. Correlation between residue coverage and plant injuries were determined using Spearman's rank correlation rho.

Results

Slug injury in fields was not affected by tillage type (df = 6, p = 0.9385), cover crop use (df = 14, p = 0.6118), insecticide use (df = 5, p = 0.3419), cover crop type (df = 2, p = 0.3699), row cleaner use (df = 1, p = 0.2425), soil moisture (df = 2, p = 0.1386), topography (df = 4, p =

0.5763). There were no significant interactions [tillage type, cover crop use, and insecticide use (df = 13, p = 0.4981); tillage type and cover crop use (df = 8, p = 0.7614); tillage type and insecticide use (df = 8, p = 0.918); cover crop use and insecticide use (df = 6, p = 0.4867). Weediness had a significant effect in the overall model (df = 3, p = 0.02559); however, there were no significant differences between weediness levels using Tukey's HSD at α=0.05. Percent ground cover had a low correlation with damage rating (p = 0.7659, correlation rho = -0.0095).

Discussion

We did not identify any land characteristics or farm management methods that affected the degree of slug feeding injury on seedling plants. There was a correlation between increased ground cover and increased slug feeding injury, but the overall effect was small. Our findings suggest that row crop producers in the Shenandoah region may not be able to mitigate risk by changing the management methods that they use. Findings from our study support a meta-analysis that found tillage type did not affect slug and other arthropod pest abundance (Rowan et al., 2020), as well as a study that found conservation tillage to not affect insect pest abundance in cabbage (Hoyt and Walgenbach, 1995). Similarly, cover crops do not affect broccoli pest abundance (Wyland et al., 1996). Multiple studies document that tillage type (Hammond and Stinner, 1987; Tonhasca, Jr. and Stinner, 1991; Troxclair, Jr. and Boethel, 1984), cover crop use (House and Alzugaray, 1989; Koch et al., 2012; Koch et al., 2015), soil moisture (Brust and House, 1990) and weediness (Altieri and Whitcomb, 1980) have significant effects on insect pest abundance in corn and soybean fields. Likely, effects on pests are dependent on factors other than the cropping system. Encouragingly, crop producers in the Shenandoah region of Virginia can use the combination of tillage and cover crop practices that work best for their operation without increasing risk of severe slug damage.

Using management practices that promote rapid plant growth rate and healthy stands may be the most useful strategy to reduce the impact of slug feeding and avoid economic losses.

Deroceras reticulatum and *D. laeve* are invasive pests to North America and thus, must be capable of range dispersion; however, little is known on the dispersal capacity of these species as it relates to movement between crop fields. Studies on *Arion lusitanicus* Mabille (Gastropoda: Arionidae) indicated a range for that species between 12.4 and 45.4 m^2 when population density was high and low, respectively; an area much smaller than a commercial crop production field (Grimm and Paile, 2001). Slugs have the capacity to move towards areas of a field with more favorable conditions (i.e., shelter, moisture, available food sources) (South, 1992). Thus, we focused our efforts on estimating how individual field characteristics, and not landscape and surrounding land use patterns, affected the amount of slug feeding injury. Slug feeding injury was considered a proxy for slug population abundance because of the difficulty in scouting for pest populations. Slugs are more active at night and shelter under residues and, in some instances, under the soil. Trapping requires multiple field visits and sampling only in morning hours before temperatures in shelters cause slugs to disperse from traps. It is possible that feeding injury did not reflect slug abundance if there were alternative food sources available or if seedling emergence and growth occurred rapidly and before slug injury could accumulate.

Slug feeding injury was low in both years of our study. Few slugs were collected in 2018 following a cold winter and, although populations were higher in 2019, favorable conditions for plant growth occurred that allowed crop stands to establish and mature rapidly through susceptible growth stages. It is possible that findings may differ when high slug populations coincide with cool, wet springs. We did not account for temperature or rainfall in our models. Climate has affected abundance of arthropod pests across multiple cropping systems (O’Rourke et al., 2011;

Stack Whitney et al., 2016; Vaidya et al., 2017; Karp et al., 2018, Dorman et al., 2020). More studies are needed to determine if local weather conditions are a useful factor for predicting risk from mollusk pests.

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CHAPTER 4: SLUG FEEDING PREFERENCE

Introduction

The marsh slug, *Deroceras laeve* (Müller) (Gastropoda: Agriolimacidae), is a sporadic pest that can cause economic damage to maize *Zea mays* (L.), and soybean, *Glycine max* (L.), grown in the mid-Atlantic US. Feeding injury to seedling maize and soybeans can reduce yield and necessitate replanting in severe instances (Douglas & Tooker, 2012). *Deroceras laeve*, and closely related pest species *Deroceras reticulatum*, are generalists and feed on a wide variety of plant species including cultivated crops (e.g., maize, rye, wheat, soy, and oilseed rape) and cover crops and weeds. In addition to feeding on living plants, they consume organic matter and crop residues in the soil and prefer dying and decaying plants to seedling maize in some instances (Gall & Tooker, 2017).

Deroceras laeve is found in a wide range of habitats including greenhouses, crop fields, gardens, moist woodlands, and marshes (Douglas & Tooker, 2012). *Deroceras laeve* has an annual life cycle and overwinters as an adult. It typically lays eggs in the spring, although some eggs are laid in the fall and hatch in early spring (Douglas & Tooker, 2012). Large adults become active in mid to late April and most eggs begin to hatch in mid to late May in Virginia.

Avoiding desiccation via water loss greatly influences slug activity and density. *Deroceras laeve* are most active during the evening and overnight and after rainfall when humidity and soil moisture level are high. Slugs shelter in the soil, under crop residues, and in similar shelters that provide cool, moist refuges during warm and dry periods. Slugs are most abundant and active in crop fields in years with mild winters and cool, wet springs. These conditions lower overwintering mortality and are optimal for maintaining body water levels while feeding and reproducing in the spring (South, 1992).

Planting into living cover crops, or "planting green", is being explored as a management practice to reduce slug feeding on crops despite little or no empirical research supporting its benefits (Gall & Tooker, 2017). Cover crops are killed with herbicides or destroyed with mechanical rollers following planting and prior to seedling emergence. Ideally, slugs will feed equally, or more, on cover crop tissue than the emerging seedlings, improving the odds that most seedlings will escape feeding injury.

Feeding preference studies can give key insights into pest behavior in a field setting. For example, one study found the gray garden slug, *Deroceras reticulatum* (Müller) to exhibit a hierarchy of preferences for wheat and common weeds in fields, and the preferences for various weeds to affect how much wheat slugs consumed when offered in conjunction. Weeds preferred to winter wheat would decrease the amount of wheat a slug ate, while weeds less palatable than the wheat would lead to slugs consuming more wheat (Cook, Bailey, and McCrohan, 1996). Another study has documented fall armyworms, *Spodoptera frugiperda* (Smith), prefer bermudagrass that has not been sprayed with various inorganic compounds, and treats sprayed grass as a nonpreferred host. This suggested that sprayed grass would have fewer armyworms, and those remaining would have lower fecundity (Leuck, Wiseman, and McMillian, 1974). Studies have also found various trap crops to be promising tools in managing pests like the diamondback moth, *Plutella xylostella* (L.). The moths would lay fewer eggs in the cash crop (cabbage) when simultaneously offered glossy collards, Indian mustard, or yellow rocket (Badenes-Perez, Shelton, and Nault, 2004).

While cover crops have the potential to reduce cash crop feeding from certain pests, considerations must be made on the effects of a green bridge for other pests. A more recent study found that peal millet as a cover crop in maize and soybean systems in Brazil created a bridge through the winter for multiple lepidopteran pests to infest future maize and soybean plantings (Favetti et al., 2017). Similarly, use of winter oilseed rape in Brussels sprouts production has been found to create a green bridge for Brassica pest species (Belder et al., 2008).

Our study was designed to measure if adult *Deroceras laeve* feed differently on soybean and maize in the presence of four common cover crop species: rye, crimson clover, daikon radish, and hairy vetch. Our goal was to determine if certain species of cover crop, if living in the field when seedlings emerged, could lower feeding severity on maize and soybean.

Materials & Methods

Slug Maintenance:

Deroceras laeve juveniles and adults were collected from fields in Shenandoah County, Virginia, in 2018. Slugs were maintained in a 75 l aquarium at ambient room temperature (approximately 20.5 C). A 2.5 cm layer layer of river rock (collected in Montgomery County, Virginia and rinsed thoroughly with tap water) covered the bottom of the enclosure and was covered by a 5 cm layer of Miracle-Gro Potting Mix (Scotts Miracle-Gro Company, Marysville, OH) and a 1-2 cm layer of leaf litter (leaves and twigs collected in Montgomery County, Virginia rinsed thoroughly in tap water). A mixed diet of carrot, sprouted corn seed, sprouted black beans, sprouted great northern beans, and CRAVE chicken grain free cat food (Mars, Incorporated, McLean, VA) was provided. The enclosure was sealed with plastic wrap to reduce moisture loss. Enclosure was misted with dH_2O approximately once a week and dead slugs were removed as needed. Natural sunlight from nearby windows determined the light/dark cycle. Slugs were maintained for approximately 10 months prior to use in experiments to standardizes size and age of individuals and allow for mortality of field collected slugs. Overlapping generations, likely F1 or F2 generations, were used.

Sixteen slugs of a uniform size were selected and placed into individual labelled 473 ml clear polypropylene containers (Fabri-kal Corporation, Kalamazoo, MI) lined with moist paper towel (Proctor & Gamble, Cincinatti, OH). Cat food and carrot pieces were provided for two days prior to experimental treatments and replaced as needed. Containers were cleaned to remove waste and misted with water to maintain moisture and humidity. If an individual died, it was replaced with a similar sized slug.

Choice Assays:

The experiment was designed as a choice assay. Slugs were presented with an approximately one cm^2 piece of either maize or soybean seedling and one of four cover crops species: daikon radish, crimson clover, hairy vetch, rye. Each slug was presented with each crop and cover crop combination for a total of seven exposure periods per individual slug. Cover crops, maize, and soybean were grown from seed in the greenhouse. Seeds were planted in potting mix (Miracle Gro Potting Mix, Scotts Miracle-Gro Company, Maryville, OH) in 9.8 L pots (1200 series injection molded nursery container, Greenhouse Megastore, Danville, IL) and watered as needed with overhead irrigation. Leaf tissue was removed from maize seedlings in the V2 stage and cotyledons were used from soybean seedlings. Daikon radish and hairy vetch were grown for four months to simulate the maturity of cover crop plants in the field during cash crop emergence. Crimson clover and rye were grown for one month because mold and aphid infestations necessitated replanting. Leaf tissue was removed from rye, crimson clover, and hairy vetch. Slices of root were removed from frozen daikon radish because daikon radish is frost-killed and thus, not-living during crop emergence. Growth stages and source material were chosen to best represent field conditions when maize and soybean seedlings are emerging and cover crops may still be present.

Slugs were starved for 24 hours before being offered experimental treatments. A one $centimeter^2$ piece of either maize or soybean tissue and cover crop tissue were placed on opposite sides of a clean 55 mm diameter, 15 mm deep plastic petri dish (TOOGOO, ShenZhen, Guangdong, China). Slugs were allowed to feed for 48 hours. Amount of feeding on each food source was scored after 24 and 48 hours. The scoring system was as follows: 0 = No feeding, 1 = Small signs of feeding (1-9% sample consumed), 2 = Moderate feeding (10 – 50% sample consumed), 3 = Heavy feeding (50 – 99% sample consumed), 4 = Food source entirely consumed. Following each feeding treatment, slugs were returned to the grain-free cat food and carrot diet for a minimum of four days before the next feeding treatment. Slugs did not receive the same food consecutively to reduce risk of preference from previous exposure. Slugs were randomly assigned to the initial food combination. The remaining order of treatments are shown in Figure 9.

Data Analysis:

Statistical analyses were performed in R Studio (version 3.5.0). An analysis of variance (ANOVA) was performed to determine any difference between the amount of feeding on maize or soybean tissue when different species of cover crop tissue is simultaneously offered. Data on maize and soybean were analyzed separately. Explanatory variables included cover crop type and replicate (i.e., the individual slug used). Means were separated using emmeans (Tukey HSD) at $\alpha = 0.05$.

Results

Feeding Experiment:

Slugs fed more on soybean compared to maize (df=1, F=23.51, P<0.0001)(Fig.2). Cover crop type affected the amount of feeding on maize (df = 3, F=22.01, P<0.0001). Maize was fed on least when hairy vetch was offered (Fig. 3). Cover crop type affected the amount of feeding on soybean (df =3, F=5.674, P=0.0013). Soybean was fed on least when hairy vetch or daikon radish was offered (Fig. 4).

Discussion

Our laboratory experiment demonstrated that adult *D. laeve* fed differently on soybean and maize in the presence of different cover crop species. *Deroceras laeve,* when given two options, fed less on maize when it was offered with hairy vetch and fed less on soybean when it was offered with hairy vetch or daikon radish. When overall consumption of maize and soybean was compared, *D. laeve* fed more on soybean tissue. It is possible that slugs prefer soybean and hairy vetch because of the similarity between these species; both soybean and hairy vetch are legumes in the family Fabaceae. As early as 1942 there were reports in the US of slugs being especially damaging to legumes in fields, particularly hairy vetch and crimson clover, which coincides with our findings (Thompson, 1942).

It is possible that herbivores feeding in hairy vetch cover will transfer to soybean plants following cover crop termination increasing risk of yield loss to mollusk or arthropod pests. Studies have found these cover crop-created green bridges to pose risks in increased lepidopteran pest pressure in maize and soybean fields (Favetti et al., 2017). Similar risks have also been reported in other cropping systems (Belder et al., 2008, Danne et al., 2010). In contrast, some have found hairy vetch to increase soybean yield when compared to no cover or a wheat cover crop, in both conventional and no till fields (Adusumilli, 2020). Another consideration for slugs is that they will feed on other organic matter, and can remain in a field even without green cover through the winter and early spring (Gall and Tooker, 2017).

If the cover crop residues remain in the field long enough, slugs may have reduced feeding on the cash crops during their most critical growth stages. Maize is most vulnerable to slug damage from VE to V3 stages, and soybean from VC to V3. Even if slugs transfer to feeding on the cash crop after cover crop degradation, if the maize or soybean has grown past these stages, effects on yield from slug injury will be minimal.

There are considerations that limit the scope of our findings. We were unable to replicate this experiment because of poor health of slugs in laboratory colonies. Slugs in field settings may have developed feeding preferences based on prior exposure. It is also possible for slugs to feed on other organic matter when living tissue is unavailable (Gall and Tooker, 2017). We did not measure the amount of feeding on maize and soybean tissue alone when no other option was provided. We did not compare feeding on maize and soybean tissue offered together because this does not represent a field relevant scenario (i.e., both crops are grown as monocultures). Slugs are mobile and can migrate to obtain a preferred food source (South, 1982). The range of another similarly sized slug species, *Arion lusitanicus* Mabille (Gastropoda: Arionidae), varies between 12.4 and 45.4 m^2 when population density was high and low, respectively; an area much smaller than a commercial crop production field (Grimm and Paile, 2001).

Feeding preference assays performed in a laboratory setting may provide insight on feeding selection in crop fields. When maize or soybean are planted into a cover crop stand, it may be possible to use a cover crop that reduces slug feeding on the cash crop. This would allow time for seedlings to grow past their most vulnerable growth stages with lower slug pressure while the slugs consume the dead or dying cover. Our experiment provides some support for this strategy. Further studies are needed to understand how slugs feed in crop production environments when various and plentiful food sources are available.

Tables and Figures

Figure 1: Order of crop combination treatments offered to *D. laeve* adults in feeding assays. Initial treatment was randomly assigned.

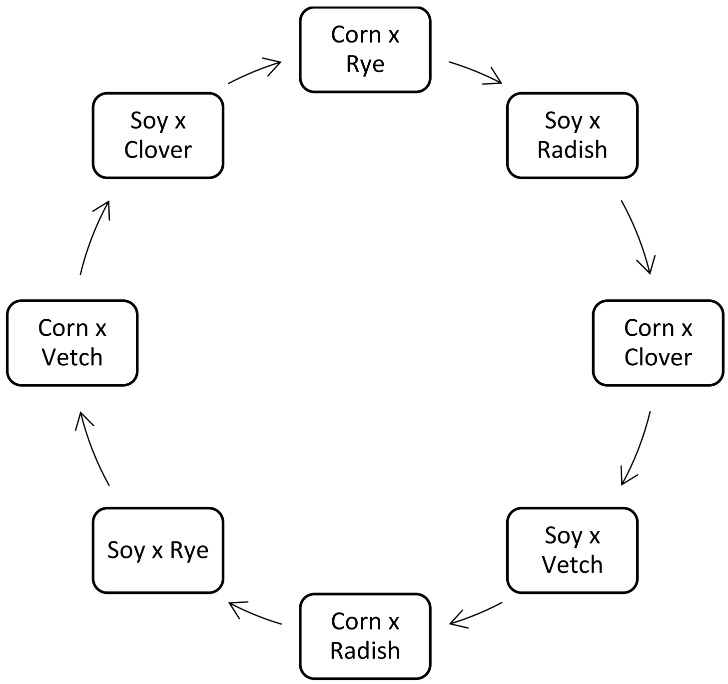


Figure 2. Mean consumption of soybean and maize tissue by adult *Deroceras laeve* when it was offered along with plant tissue from different cover crop species. Feeding score on a 1-4 scale [0=no feeding, 1=small signs of feeding (1-9% sample consumed), 2=moderate feeding (10-50% sample consumed), 3=heavy feeding (50-99% sample consumed), 4=food source entirely consumed].

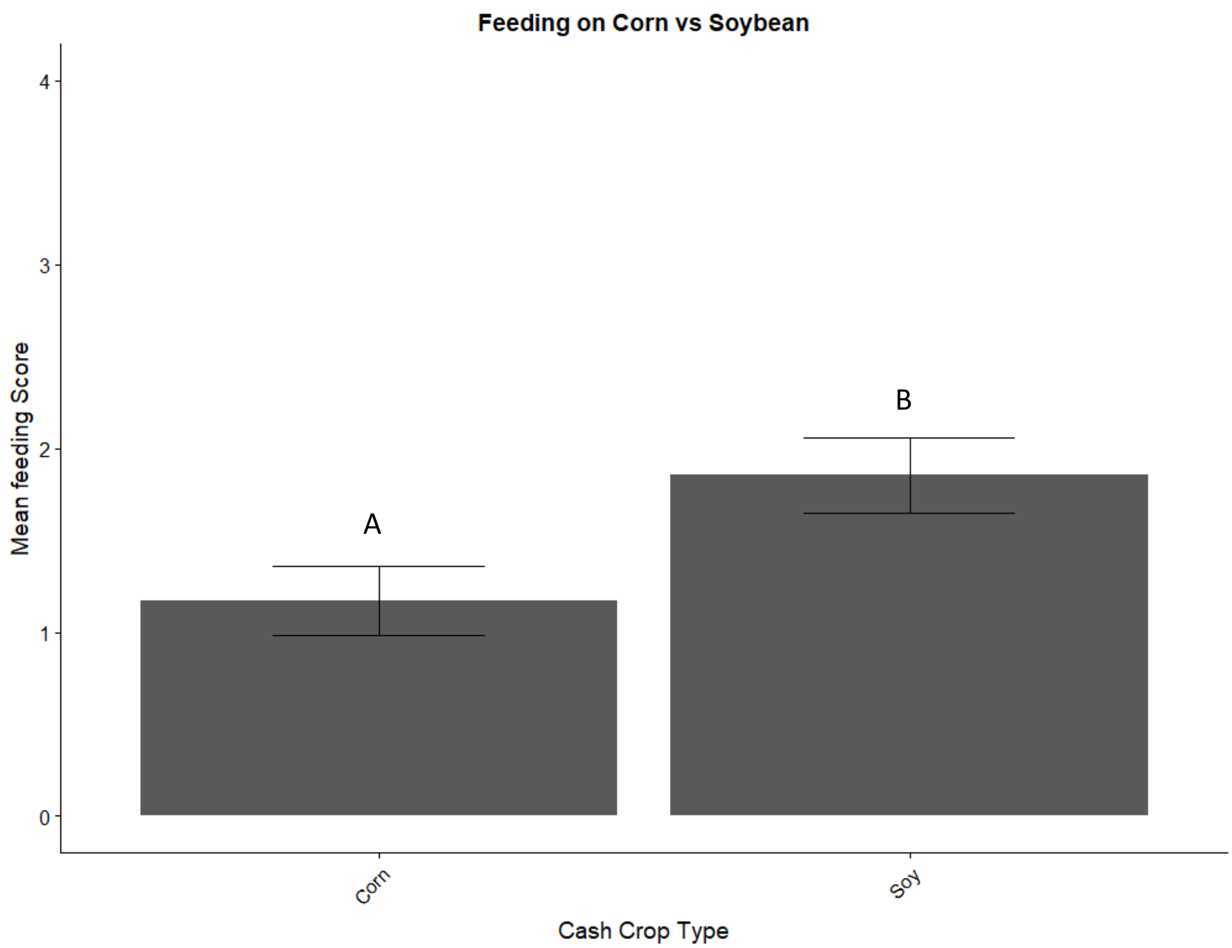


Figure 3. Mean consumption of maize tissue by adult *Deroceras laeve* when it was offered along with plant tissue from different cover crop species (crimson clover, daikon radish, hairy vetch, rye). Feeding score on a 1-4 scale [0=no feeding, 1=small signs of feeding (1-9% sample consumed), 2=moderate feeding (10-50% sample consumed), 3=heavy feeding (50-99% sample consumed), 4=food source entirely consumed].

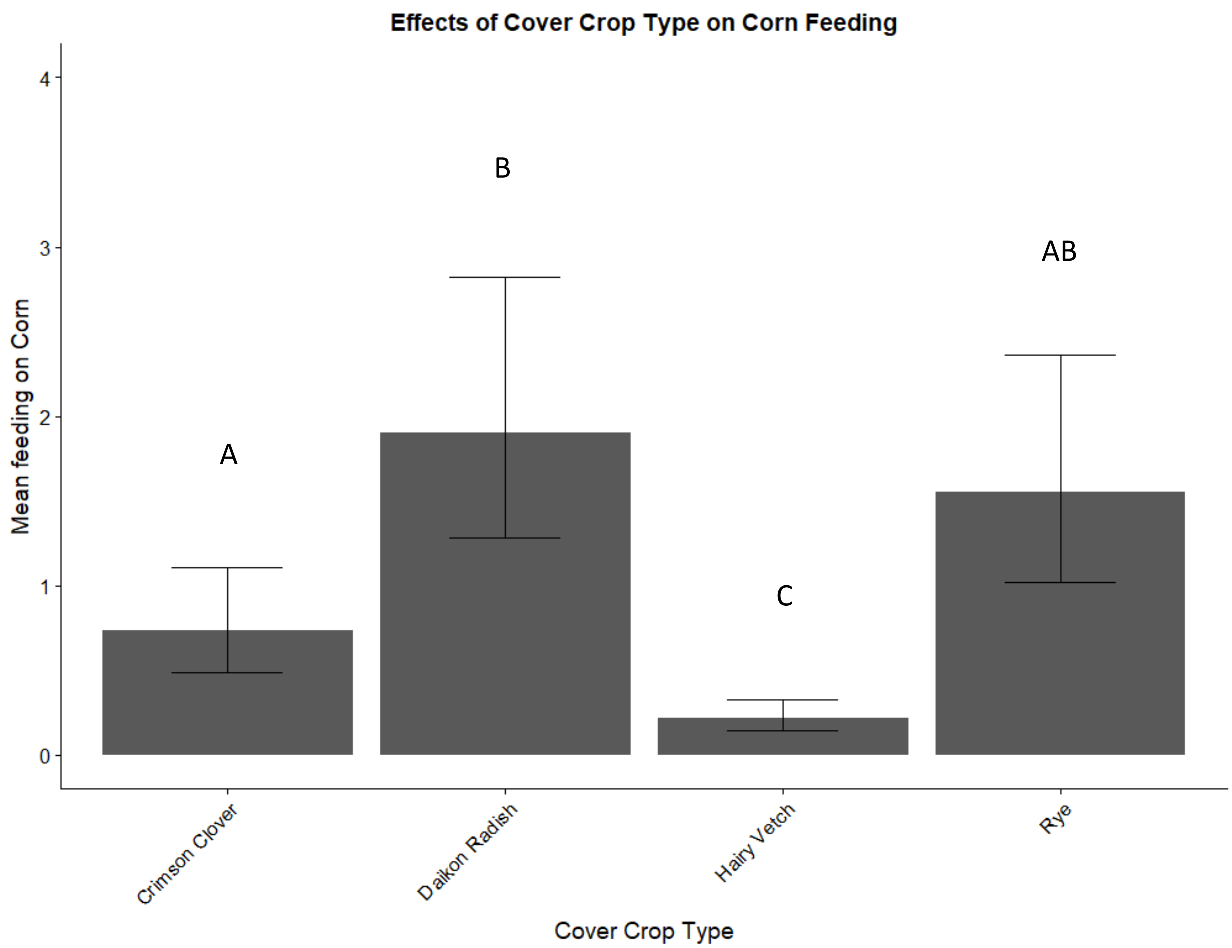
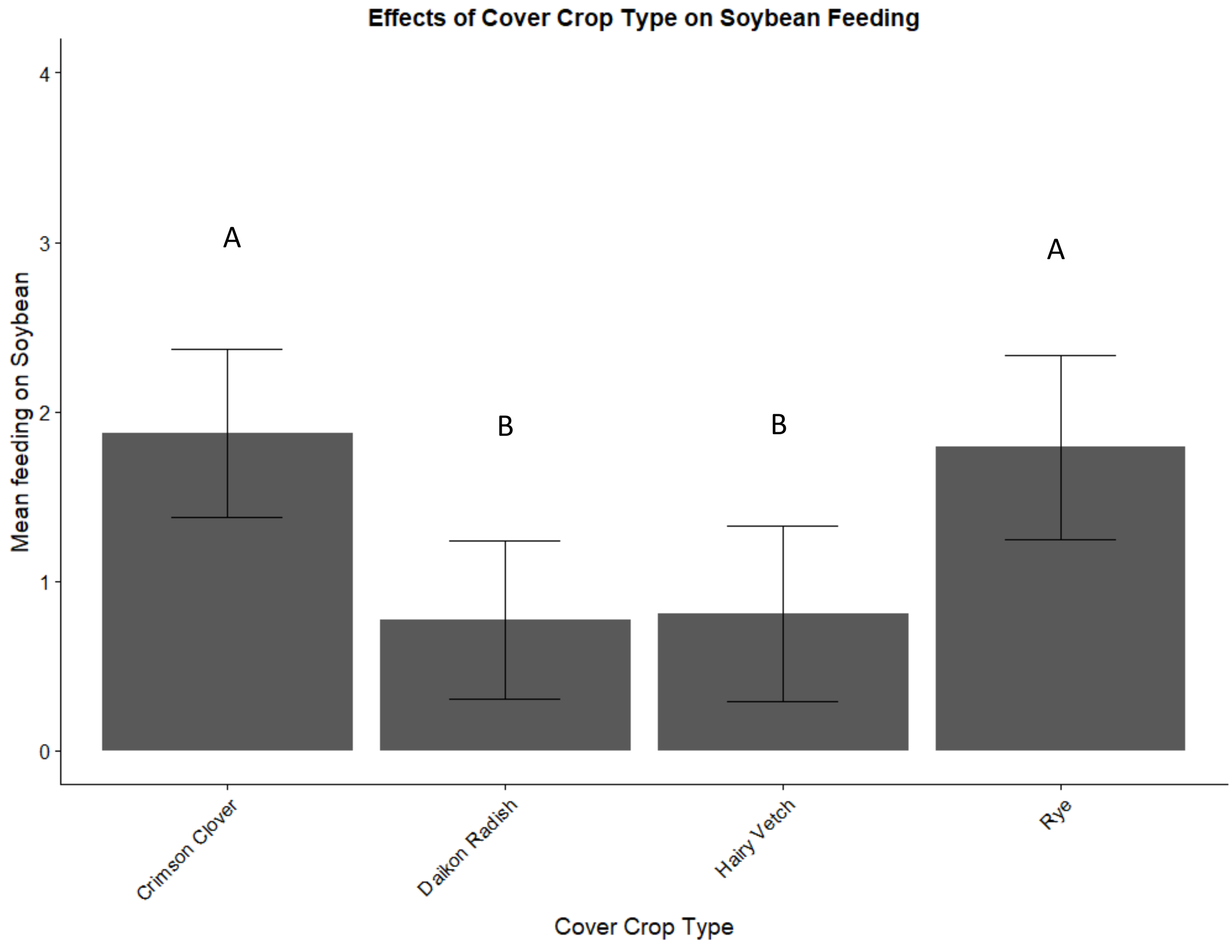


Figure 4. Mean consumption of maize tissue by adult *Deroceras laeve* when it was offered along with plant tissue from different cover crop species (crimson clover, daikon radish, hairy vetch, rye). Feeding score on a 1-4 scale [0=no feeding, 1=small signs of feeding (1-9% sample consumed), 2=moderate feeding (10-50% sample consumed), 3=heavy feeding (50-99% sample consumed), 4=food source entirely consumed].



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APPENDIX

Appendix 1

Scouting Protocol
Monitoring Fields for Slug Damage

Plant Injury Rating

- Scouting should begin as soon as plants emerge from the ground and continue until corn or soybean reach the V-5 growth stage. It will be more critical to evaluate soybeans as they emerge from the ground than corn.
- Scouting should be done weekly on each field.
- The field should be divided into four equal quadrants. The scout should quantify slug damage on one 10 ft row per quadrant (4 per field). The scouting report needs to specify the general location of each measurement site
- Count the total number of plants and record the growth stage of the plants. Rate plant injury on each plant on a 0-4 scale. Estimate the portion of plants with different injury ratings. For example half of the plants have an injury rating of 1, one quarter are at injury rating 2 and one quarter are at injury rating 4.

Rating Scale : 0= no damage ; 1= only one leaf showing damage (less than 25% defoliation) ; 2= all leaves showing moderate damage (25 – 50% defoliation) 3= all leaves consumed except one remaining intact (greater than 75% defoliation) 4= seedling completely cut off at the ground level.

Injury Rating 1: Note most recent emerged leaves are not damaged. Corn has been growing faster than slugs are consuming leaves.

Injury Rating 2: Unclear if corn is (or will) grow faster than slug feeding pressure. Cool cloudy weather will slow corn growth and slugs may still kill this plant.

Injury Rating 2: The early growth stage of this plant combined with the slug feeding makes it more critical to re-check this field quickly than the field above.

All Pictures on this Page are a Injury Rating 3. Slugs are clearly eating the plant as fast as it is growing.

If one in ten soybean cotyledons are showing this type of feeding injury then the field should be treated.

All Pictures on this Page area Injury Rating 4

This seedling Soybean Plant will not survive because the growing point is above the injured stem. Immediate slug control may be warranted to save other emerging plants.

All Pictures on this Page are an Injury Rating 4

These two corn seedlings recovered (with the assistance of a slug bait application followed by 2 or 3 nights of no rain).

Appendix 2: Slug field survey

Slug Field Survey

Field ID (preferably farmer's name, use numbers if multiples):

GPS coordinates:

Slug injury rating:

Plant injury %:

Plant stand targeted: **Plant stand actual:**

Crop (include variety or hybrid if known):

Growth stage of the plant:

Planting date:

Row cleaners used? Yes / No

Previous crop:

Cover crop (type, if any): **Burn down date:**

Tillage type: **Tillage date:**

Slug history (none, low, moderate, severe, unknown):

At plant or pre-plant fertilizer: **Fertilizer date:**

Weediness – whole field assessment (none, low, moderate, severe):

Pre-plant or at plant insecticide: **Application date:**

Molluscicide: **Application date:**

Soil classification (available online at http://websoilsurvey.nrcs.usda.gov):

Soil moisture level (dry, moderate, wet):

Estimate % ground cover:

General topography of the site (e.g., upland, rolling, river bottom):

Surrounding landscape (e.g. woodlands, crop fields, meadows):