Crambinae (Crambidae: Lepidoptera) of Ohio: Characterization, Host Associations and Revised Species Accounts

THESIS

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By

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Abstract

A review of the North American Crambinae sod webworm taxonomy, phylogenetic history, and biology is presented. Traditional analysis, combined with modern genetic analysis has changed and solidified the placement of these species. Previously cryptic and unidentifiable larvae were identified using genetic analysis of the mitochondrial CO1 gene and an evaluation of potential host plant associations is given. DNA sequencing is a useful tool that can be used to identify unknown sod webworm larvae, including the especially difficult to identify first and second instar larvae. Only *Parapediasia teterrella* larvae were recovered from the short-cut, golf course-type, creeping bentgrass (*Agrostis stolonifera*), as was a single *Agriphila ruricolella*. *Fissicrambus mutabilis* was obtained from lawn-height Kentucky bluegrass (*Poa pratensis*) and turf type tall fescue (*Festuca arundinacea*). Sod webworm adults were monitored with a standard blacklight trap between 2009 and 2013. Each year 14 species were recovered from the light trap. Species obtained from the managed turfgrass yielded only a fraction of the number of species attracted to the light trap. The sod webworm species *Euchromius ocellus* first appeared in late 2012. This is a first report for this species in Ohio.
Dedication

For my dear friend Donald L. Main who passed away the summer of 2013. Without whom I likely never would have found my way to where I am now.

You are greatly missed.
Acknowledgments

I would like to take this opportunity to express my deep thanks to those who have been a part of this process and made it possible for me to succeed. A heartfelt “Thank You” goes out to my committee for their time, effort, and support over these last few years. Thank you Dr. Gardner for being a part of this project. Dr. Andrew Michel I would like to thank for his willingness to spend time with me figuring out the nuances of, not just DNA analysis, but the at times irritating analysis software. To Dr. Steven Passoa I can never thank enough for seeming to always be there, ready and willing to answer sometimes the most random of questions about sod webworms and for his unending ability to reinvigorate my enthusiasm for these little creatures whenever it would begin to wane. Dr. David Shetlar I can never thank enough for his never ending support and patience on this long journey. He not only helped keep me on this path he also helped to teach me what it means to be a considerate, compassionate, and constantly questioning, scientist.

To my friends and support who have been there for me over these years I can never thank enough. A thorough breakdown of how they helped me would be a thesis in and of itself. I hope it is enough for now to at least say “Thank you all so very much”: Josh Bryant, Jennifer Andon, Barbara Bloetscher, Emily Linkous, Laura Watson, and Lauren Tyron.
And to my overlord cats who have taught me the value of prompt meal preparation, and late night purring. Fourteen years together and I’m still their loyal subject.
Vita

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Publications


Fields of Study

Major Field: Entomology
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Chapter 1: Introduction and Literature Review

1.1 Introduction

The Lepidoptera have long been a source for human fascination. Cultures across the world have incorporated them into their myths and legends (Kritsky 2000; Campbell 1991). Modern mythologies such as literature and cinema also weave these images into their narratives. But whether it is Bilbo Baggins looking with sadness upon the butterflies escaping Mirkwood (Tolkien 1937), a sphinx moth pupa symbolically silencing a homicide victim in The Silence of the Lambs (1991), or a black swallowtail alighting from Katniss Everdeen’s hand as the first Hunger Games victims are recognized (2012), entomological and specifically lepidopteran imagery continues to fascinate and strike a chord with humans in a rather unique way.

This fascination tends to be restricted to the charismatic macrolepidoptera. The moths and butterflies not blessed with large, flashy forewings are often overlooked except when they become a pest of economic importance. Though neglected, microlepidoptera make up a considerable proportion of the order Lepidoptera. Developing a more comprehensive understanding of their biology and ecology is essential if one is to appreciate the complexity and diversity that is the order Lepidoptera.
1.2 The Taxonomic History of Pyraloidea: Crambidae

The order Lepidoptera has gone through many changes since its introduction to the scientific literature in the 10th edition of Linnaeus’ seminal work *Systema Naturae* (1758). Multiple expansions and additions to the classification system, especially suborders, superfamilies, families and the like have been defined for this order. Historically, the Lepidoptera were divided into two suborders; Rhopalocera [butterflies] and Heterocera [moths], based on antennal morphology. Others used the suborders Jugatae and Frenatae based on the presence or absence of a frenulum on the hindwings. Additional attempts at sub-ordinal divisions established three suborders, Zeugloptera (mandibulate moths), and Monotrysia and Ditrysia based on number of female genital openings. More recent systematic treatments of the order have eliminated these subordinal designations in favor of four other suborders, clades and a number of superfamilies (van Nieukerken et al. 2011).

Within the clade Obtectomera (Lepidoptera with obtect pupae), the superfamily Pyraloidea is one of the largest lepidopteran superfamilies behind only Gelechiodea and Papilionoidea (van Nieukerken at al. 2011). Debate persists concerning the placement of the varying superfamilies, subfamilies and the families themselves. By 1980, the Pyraloidea consisted of eight families (Table 1). Today, it consists of only two, the Pyralidae and Crambidae (Solis 2007)
The presence of ventro-medial tympanal organs on the first and second abdominal segment has risen to be the key distinguishing feature of the Pyraloidea, a trait they share with members of Geometroidea who appear to have independently evolved this trait. This trait distinguishes pyralids from the Noctuidae that have tympana on their metathorax. These structures are possibly derived from chordotonal neuronal cells. These cells have been identified in related lepidopteran species (Actias luna: Saturniidae) and could indicate an evolutionary prototype for lepidopteran tympanal structures (Yack and Fullard, 1990). It is believed that tympanal organs of Lepidoptera co-evolved with the development of echolocation in bats (Fullard & Yack 1993). The presence of scales at the base of the proboscis is also a key trait that is shared with Gelechioidea, Tischerioidea and Choreutoidea (Regier et al 2012).

Fabré originally separated the Pyralidae and Crambidae with the placement of differing subfamilies within these two families continuing to be debated until recently (Solis 2007b; Landry 1995; Rieger et al. 2012). There has been little to no consistency in the literature as to the placement of the subfamily. An ongoing 1881 dialogue between CV Riley, and Lintner make multiple references to the pest Crambus vulgivagellus Clemens

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<tr>
<td>Pterophoridae</td>
<td>Pyralidae</td>
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<td>Thyrididae</td>
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<td>Hyblaeida</td>
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Table 1.1: Pyraloidea families, (Solis 2007b)
(now Agriphila vulgivagella) of the family Crambidae (Riley 1882). The family Crambidae has occasionally been synonymous with the now identified subfamily Crambinae (Grote 1880; Felt 1894). Stehr’s (2005) *Immature Insects* does not recognize Crambidae as a family and instead lists its few *Crambus* species as belonging to Pyralidae. This inconsistency is due to a mix of factors including differences in general, casual usage of the terms, those seeking more specific classifications due to morphological variation, and disagreement among Lepidoptera taxonomists as to the real-world importance – or lack thereof – for the distinction.

The subfamily Crambinae was initially defined by Ragonot (1890), revised by Hampson (1895), further edited by Forbes (1920), and most recently defined by Landry (1995). Munroe (1973a, 1973b, 1974, 1976a, 1976b) first proposed the less formal categories of crambiformes and pyraliformes. Landry (1995) provides a quality in depth review of the taxonomic history and nomenclature of the Crambinae.

Roesler (1973) (Figure 1.1B) was the first to suggest the Crambinae were a sister group to six other crambiform subfamilies recognizing an Acentropidae-Crambiadae complex (Landry 2012; Passoa 1988). Kuznetzov & Stekolnikov (1979) (Figure 1.1C) presented a phylogeny for the families and subfamilies of Palearctic Pyraloidea utilizing the male genitalia, concluding that the Crambinae a sister group to other crambiformes with Schoenobiinae and Nymphulinae being unrelated. Yoshiyasu (1985) (Figure 1.1A) also used the male genitalia to construct a cladogram as he doubted the validity of the characters utilized by Roesler (Passoa 1988). Nine subfamilies of the crambiform group
were examined with a monophyletic group including the Crambinae as distinct from the Pyralidae (Yoshiyasu 1985). Problems with each of the above proposals were noted by Passoa (1988) who constructed a new hypothesized cladogram incorporating traits noted by previous authors as well as new larval and pupal characters he identified (Figure 1.1D).

Landry (1995) revised the relationships within the Pyraloidea, opting for the Crambidae being demoted to the group heading of crambiformes and Pyralidae being the overarching family for them and their paraphyletic group pyraliformes. By examining 43 morphological characters, and data from the following listed sources, the below hypothesized phylogeny was created:

Figure 1.1G) Hasenfuss (1960); Minet (1982, 1983, 1985, 1991); Munroe (1972); Passoa (1988)

Figure 1.1H) Yoshiyasu (1985)
Figure 1.1: Important historical Pyraloidea cladograms demonstrating changes and disagreements over time.
As the understanding of the Pyraloidea changed over time, so did the number and placement of families (Table 1.2). Minet (1982, 1985) identified tympanic organ autapomorphies useful for identifying subfamilies. Solis (2007) agrees with earlier literature that the presence of a praecinctiorium protecting the abdominal tympanum of Crambidae is a key trait and sufficient to classify them as belonging to their own family. The Pyralidae lack a praecinctiorium (Figure 1.3).
Table 1.2: Changes in subfamilies within Crambidae in major primary literature over the last 30 years.

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<td>Musotiminae</td>
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<td><em>Pyraustinae</em></td>
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<td>Schoenobiinae</td>
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<td>Heliothelinae</td>
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<td>Unplaced subfamilies (not included in study)</td>
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<td>Wurthiinae*</td>
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<td>Cybalomiinae</td>
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<td>Heliothelinae</td>
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*Does not occur in Western Hemisphere

**International Commission for Zoological Nomenclature sustained Acentropinae as the senior valid name (Solis 1999)
Figure 1.2: Pyraloidea tympanal structure demonstrating presence (Crambidae) and absence (Pyralidae of praecinctorium (Munroe and Solis 1999).

Genetic analysis of 42 species of pyraloids brought some clarity as well as a bit of confusion to the phylogenetic tree (Reiger et al. 2012). Earlier morphologically based
phylogenies are now suggested to be inaccurate and in need of further in depth analysis. Their results support the two-family division but separate the Crambidae into two general clades, The PS (Pyraustinae, Spilomelinae, Wurthiinae) and the “non-PS Clade” the latter of which was basally divided into the the CAMMSS (Crambinae, Acentropinae, Musotiminae, Midilinae, Scopariinae, and Schoenobiinae) clade, and the OG (Odontiinae, Glaphyriinae) lineage. The CAMMSS clade was actually consistent with Yoshiyasu (1985) with the exception of Midilinae which is a New World taxon and not evaluated in his study. These groups were further labeled according to host plant and location associations. Figure 1.3 details the most recent phylogenetic relationship of the Pyraloidea.
Figure 1.3: Most recently revised Pyraloidea phylogeny using multiple genetic markers (Reiger et al. 2012).

The contribution of genetic analysis has not solidified this division with Landry dissenting from his coauthors in a 2012 revision and analysis of the phylogenetic relationship of the Pyraloidea (Rieger et al. 2012). The most recent, thorough work on the Nearctic members of subfamily Crambinae by Landry (1995) references them as being members of Pyralidae, rejecting the division of the two families. He states that the “sub-families cannot be separated by external diagnostic characters” suggesting that the presence of the praecinctiorium is a sufficient division for subfamily status but not family
(Landry 1995). This perspective has not changed and has continued through the publication by Reiger et al. (2012) believing the division is “subjective and that to a nonspecialist a crambiform is not always easily separated from a pyraliform, whereas a pyraloid can immediately be distinguished from other moths by virtue of the scaled proboscis and presence of abdominal tympanal organs” (Reiger et al. 2012).

The morphological traits used to distinguish between the two families are presented in Table 1.3. Table 1.3 was synthesized by Nus et al. (2013) and was derived from the works of (1972), Minet (1982), Maes (1985), and Munroe & Solis (1999).

For the purposes of this text, I accept to the consensus between morphological and genetic analysis and place the subfamily Crambinae within the family Crambidae.

Proper identification of adults and larvae has long been a major complication of this subfamily. Though many adults are strikingly distinct (ex: Urola nivala Drury, Chrysoteuchia topiaria Zeller, Pediasia trisecta Walker, etc.) many are similar in appearance making identification difficult. Felt (1894) stated that male genitalia were the most effective method of species identification. To this day, this continues to be one of the most reliable methods of morphological identification. Forewing coloration and patterning are fairly reliable identification methods for most species. However, due to rough living, dying, and/or handling, scales can be knocked off of the wings leaving the pattern difficult to discern. This is especially true of the Neodactria complex. There is
still much debate surrounding this complex with no clear morphological distinction between *N. caliginosella* and *N. luteolella*.

<table>
<thead>
<tr>
<th><strong>Pyralidae</strong></th>
<th><strong>Crambidae</strong></th>
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<tbody>
<tr>
<td>Forewing vein R5 stalked or fused with R3+4</td>
<td>Forewing vein R5 free</td>
</tr>
<tr>
<td>Forewing without oval sclerotization costad of base of vein A1+2</td>
<td>Forewing with oval sclerotization costad of base of vein A1+2</td>
</tr>
<tr>
<td>Bullae tympani closed cephalad</td>
<td>Bullae tympani open cephalad</td>
</tr>
<tr>
<td>Tympanum and conjunctivum in the same plane</td>
<td>Tympanum and conjunctivum lying at a blunt angle</td>
</tr>
<tr>
<td>Process tympani absent</td>
<td>Processus tympani present</td>
</tr>
<tr>
<td>Praecintorium absent</td>
<td>Praecintorium present</td>
</tr>
<tr>
<td>Accessory tympana absent</td>
<td>Accessory tympana present caudally of metacoxae</td>
</tr>
<tr>
<td>Male genitalia with uncus arms, (paired processes arising laterally from base of uncus)</td>
<td>Male genitalia without uncus arms</td>
</tr>
<tr>
<td>Segment A8 of larvae almost always with sclerotized ring around base of SD1</td>
<td>Segment A8 of larvae without sclerotized ring around base of SD1</td>
</tr>
</tbody>
</table>

Table 1.3: Morphological characters used for classification of the two Pyraloidea families (Crambidae, Pyralidae).

1.3 **Crambinae Collection & Identification**

The Crambidae are one of the most diverse groups in regard to their behavior and life history adaptations. Though most larvae feed on above ground plant parts as leaf rollers,
webbers, borers and miners, there are also a number of species that feed on roots and seeds. Wurthiinae species parasitically live in ant nests. Phycitinae species are predacious upon scales while others live within bee hives. Some larvae are aquatic to semi-aquatic (Acentropinae) (Mey 2008) while others are adapted to exceedingly dry environments (Phycitinae) with their larvae often feeding on stored products. With such diverse adaptations and habits it is even more important to have a solid morphological and genetic basis for determining group and individual identities.

Observations of sod webworm flight patterns indicate a habit of flying low to the ground, only rising a few feet to alight (Felt 1894). It is therefore important to keep this information in mind when selecting a blacklight for adult catches. Tomlinson, Jr. (1970) noted variation among catch height of three cranberry pests, one being the cranberry girdler, *C. topiaria*. Traps located 3 ft higher than the average tip height of cranberries captured significantly fewer girdlers than the trap at top of the cranberries. Banjeree (1967) noted males were far more likely to be attracted to light traps than females for nearly all of the species he observed. Pupal emergence studies indicate near equal emergence rates of the sexes (Banjeree and Decker 1966). Banjeree (1967) also noted variation in sex arrival times. Females would typically arrive within one to two hours post sunset. This pattern persisted throughout the season even as sunset shifted in time. However, the males continued to occur within 3 hours after midnight even as the season changed.
With some exceptions, the adults of the crambine sod webworms are relatively easy to identify. Visual identification of key characters of genitalia as well as forewing coloration and patterning are generally reliable indicators of adult identity. The same cannot be said for the larvae. Though there is some variability in appearance, crambine larvae are poorly described in the literature with the majority of works devoted to adult distinguishing traits (Passoa 1988; Solis and Maes 2002). Few of the larvae have been studied thoroughly. Key traits used for larval identification are only known for a minutiae of species and then only for the latter instars (Stehr 2005; Peterson 1962). Sod webworm larvae can vary in coloration depending upon species and instar. Beige, straw, brown, gray, pinkish-red or even a greenish color are common, punctuated by characteristic dark circular spots over their bodies (Bohart 1947; Felt 1894; Forbes 1905; Vittum 1999). The spots can be faint in early instars but become more defined during the fourth instar onward (Bohart 1947). Each succeeding instar sees the overall color and spots become more distinct with each increase in size (Bohart, 1947). They have dark, distinctive head-capsules (Bohart, 1947). Newly hatched larvae are quite miniscule (1-3mm long), have distinctive dark head capsules and notal shield with pale yellow bodies, somewhat dusky at the tip. The consumed, salmon-colored egg shell can be seen in the digestive track as well (Ainslie 1923a b, 1927, 1930; Bohart 1947) They will eventually grow to one to two centimeters in length, depending upon species (Ainslie 1922; Bohart 1947; Forbes 1905; Shetlar 1995). Females are slightly larger than males (Bohart 1947). Depending upon the species, crambine larvae may have six (Ainslie 1923a; Bohart 1947), seven (Ainslie 1923b), eight (Ainslie 1918, 1927), nine (Ainslie 1922a) to as many as ten instars. Ainslie (1918) reported one reared larvae reaching a twelfth instar. Pupation
seems to typically occur after the sixth or seventh instar for males and up to the eighth for females (Ainslie 1918; Bohart 1947).

Little is known about the habits of the Crambinae larvae. Passoa (1985) analyzed the economically important a fauna of Honduras noting morphological characters of the larvae and pupae as well as oviposition, larval feeding habits, expected life cycles, and host plant damage observed. Richmond and Shetlar (1999) noted larval habits of Parapediasia teterrella Zincken in various grasses. Detergent water flushes have proven to be a useful method of acquiring turf insects (Richmond and Shetlar 1999; personal exp).

Within this subfamily, six tribes are currently recognized in North America: Argyriiini, Chilonini, Crambini, Diptychophorini, Haimbachiini, and Prionapterygini (Klots 1970a). Landry’s (1995) revision of the North American Crambinae did not include the Chilonini and instead listed members found in that tribe as being unassigned members of the Haimbachini citing unreliable branching patterns and potentially inconsistent features of the tympanum. His analysis found Chilonini was not monophyletic (Landry 1995). Chilonini and Crambini include a number of pest species of significant economic importance (Ainsle 1922, 1927; Bohart 1947; Passoa 1985). Argyriini, which contains the striking U. nivala, often called the Snowy Urola Moth, has been implicated as a pest of Ligustrum (TAMU 2014) which is a common landscape ornamental, yet a pest plant in some locations. The pest status of these species is not restricted to the United States of
America. A recent publication identifies *P. teterrella* as an increasingly devastating pest in Shanghai of *Cynodon dactylon* L. (Bermudagrass) (Gao et al. 2013).

The first thorough accounting of crambine biodiversity in the state of Ohio was conducted by Hine in 1897. His efforts to list and survey the fauna resulted in the identification of 21 species. Subsequent observations and reports have increased the total. Utilizing black-light trap data from 1978-1989 for various locations across the state (exact dates differ by location), Niemczyk et al. (2000) constructed the first full-species log and correlated it with seasonal flight activity. Flight activity of seven univoltine and five multivoltine species were identified. Additionally, a record of the Crambinae moths represented in The Ohio State University insect collection was presented. One of these, *Crambus pusionellus* Zeller is believed to be a misidentification. The Crambinae listed in the Ohio Agricultural Research and Development Center (OARDC) lists 14. Solis (2007) also lists 14. The Ohio Lepidopterists (1994) published an accounting of 33 species. Both univoltine and multivoltine species have been identified with univoltine and bivoltine having the predominant representation. Adult presence varies with location and habitat.

Table 1.4 lists all the Crambinae sod webworms following a review of the pertinent literature (Hine 1876; Niemczyk et al. 2000; Solis 2008). From this review, the current estimation of Crambinae sod webworms is 37. This number does not include the sighting of a new species which will be discussed in Chapter 4. This figure includes evaluation of three Ohio museum collections at the OARDC Roy Rings Lepidoptera Collection in
Wooster, the Ohio Lepidopterist Society Collection in Columbus, and the Ohio State University Triplehorn Museum Collection in Columbus. The most relevant sources are noted in the table using the following abbreviations: OHL: Ohio Lepidopterist Society Checklist of Crambidae in Ohio; RDC: OARDC Collection; H: Hine 1876; N: Niemczyk et al., 2000; S: Solis 2008; MP: North America Moth Photographers Group; BNA: Butterflies and Moths of North America. If a species has been previously collected at the Waterman Farm light trap, this is noted with an ‘x’.

When considering the *Neodactria* sp. complex, it is important to consider the difficulty and lack of consensus that currently exists surrounding their true identification. *N. luteolella, N. caliginosella, N. murella* Dyar and *N. zeella* Fernald should be analyzed further to confirm their identities. NAMPG and BAMONA are two often frequented websites dedicated to the identification, analysis, and preservation of Lepidoptera in North America. These websites are generally highly trusted and are used and regulated by many lepidopterists. Still, identifications based solely on their presence on one or both of those websites but not in the primary literature should be met with a level of skepticism.
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Table 1.4: Crambinae of Ohio according to pertinent literature.
Of these 37 species, 16 are known to infest turfgrass in the United States. According to Vittum (1999) Five species are considered to be the primary pest species (C. topiaria, P. teterrella, F. mutabilis, Pediasia trisecta Walker and A. vulgivagella ) in Columbus, OH. These five species are commonly observed in the Columbus, Ohio region. In addition, Urola nivala is a commonly encountered crambine species present throughout the season. Seasonal occurrence of these six species varies throughout the year as some species are univoltine and several are multivoltine (Niemczyk et al. 2000; Heinrichs & Matheny 1970).

_N. caliginosella_ had been considered a pest of corn and tobacco for many years. The larvae of this species often burrow into stems at or near the soil surface and tunnel upwards and downwards through the stem, causing eventual death of the plant. The damage in tobacco ranged from minimal damage to nearly 100% loss of a field. Ainslie (1916) expressed concern over the extensive damage caused by this species, often forcing the replanting and resetting of thousands of acres of corn and tobacco in nearly every state east of the Mississippi. Damage was most pronounced in fields newly shifted from wild prairie to tobacco. Scott (1955) indicates an abundance of white heath aster, *Aster ericoides* L., in fields prior to and contiguous with tobacco plantings. This native plant may have served as host for the larvae. However, after several years of tobacco being the predominant field crop, _N. caliginosella_ ceased to be a major pest of the crop. Additionally, Scott reports Tennessee had no further injury of tobacco associated with _N. caliginosella_ after 1944. He noted a correlation with changes in agricultural practices as well as a marked increase in the abundance of beef and dairy cattle.
It is possible, given appropriate conditions the species in the other tribes could become pests of their respective host plants. Prior to planting corn in former pastureland, *A. vulgivagella* would not have been considered a potential pest. It was not until the appropriate conditions, monoculture of corn in former grasslands, that this species became a significant crop pest (Ainslie 1922; Riley 1882).

The tradition of referring to these moths as sod webworms or grass veneers may in fact be a misnomer. In addition to the above mentioned examples of *U. nivala* and *N. caliginosella* there are a number of species within this group which are known to feed on broadleaf plants other than grasses. Given the difficult nature of identifying the larvae of these species, it is quite probable that many reports of a particular species affecting a plant are not entirely accurate and were instead based on the assumption that, since the adult is present, the larvae must be of that species. Though this reasoning may have some anecdotal validity it has not been scientifically evaluated.

### 1.4 Turfgrass Use and Management in the Continental United States

As land use needs changed over the last century, so did the management practices. By the mid-20th century most agricultural fields were well established and no longer subject the former prairieland pest compliment. Many *Crambus* sp. caterpillars were no longer significant pests of newly created corn fields. The periodic booms of golf course construction also coincide with an increase in the publication of sod webworm research.
In North America three booms in turfgrass usage and expansion have occurred and subsequently peaked in 1930, 1970, and 2000 (Hueber 2012).

The first boom which began in the 1920’s occurred to accommodate an upper class that desired private golf courses and country clubs. The burgeoning middle class and an increase in public courses caused the boom in the 1960’s, peaking in 1970. Unlike the previous two booms the third boom of the 1990’s was in anticipation of the soon to retire “baby boomer” generation. Large planned and deed-restricted communities were built centered around courses. Over 40% of the courses created during the 1990’s were tied to master planned communities (Hueber 2012). With these changes in course associations, a shift in management style resulted. The economic resources of each location influences maintenance and pest management practices. Smaller public courses typically have fewer resources available to them.

Inspired by the great lawns of royals and lords in Britain, home lawns soon became a staple of the American Dream. Suburban communities began cropping up in the post-World War era and again in the 1970’s and 1980’s. As lawn and turfgrass culture in North America increased during the 1990s crambines have become one of the top five pests of turfgrass, especially of the short-cut turf of golf courses (Potter 1998; Niemczyk et al. 2000; Vittum 1999).

In high-cut turfgrass, such as lawns, grounds and sporting fields, crambines are primarily nuisance pests. The larvae rarely cause visible damage but the adults that take flight can
be distractions and cause distress to sensitive homeowners. However, during their larval stage, greens and tees of golf courses can be subjected to significant damage from bird predation. (Bohart 1947; Stirrett and Arnott 1932)

To address possible sod webworm problems many tactics have been employed over the years. Early efforts to control these pests in an agricultural setting were reliant upon fine tuning plowing and tilling times (Ainslie 1922, 1923a, 1927). As different species are abundant and more troublesome at different times, seemingly in succession, Felt (1894) commented these larvae “prey upon the grass as a succession of small armies” causing continued destruction throughout the year. The later in the season one was to plow, the less control would be achieved. However, if one plowed too early, their efforts would also be in vain. Thus, finding the optimum time in the autumn was imperative for control. Once large numbers of webworms had been identified these control methods were the only option available. Early accounts seem to indicate a fine line between no concern and overabundance.

1.5 Control Methods, History & Efficacy

Early chemical control methods varied in their logic and efficacy. Gilmore and Milan (1937) recommended nitrobenzene poisoned baits for *N. caliginosella*. Campbell and Stone (1937) report 100% success in killing *Crambus* larvae with a dichlorehyl-ether applied at one gallon per square yard of infested sod. North and Thompson (1933) recommend the use of lead arsenate at two pounds per 20 gallons of water for 1000 ft² of
turf. Bohart (1947) utilized multiple products in an effort to obtain webworm control: pyrethrum compounds, rotenone, ground tobacco, nicotinized powder, cuprous cyanide, dichloroethyl-ether, cryolite, as well as acid and lead arsenate, and calcium arsenate. The pyrethrums, rotenone, and dichlorethyl ether solutions were fairly successful in the short term however they would often need repeat applications throughout the season. His acid lead arsenate solution was highly successful and recommended at 5.0 pounds in 50 gallons of water sprayed over 1000 ft$^2$ of turf.

Though a foreign idea today, arsenicals have long been incorporated into pest control with the earliest reports dating around 700CE in China (Shepard 1939). Paris green’s (copper acetoarsenite pigment) first reported use came in 1867. It was used internationally for orchard pest control and even mosquito abatement (Peryea 1998; Peryea and Kammereck 1997; Gratwick 1965). Lead arsenate continued to be used throughout the United States for the first half of the twentieth century being nearly entirely replaced with the advent of DDT (Peryea FJ 1998). Where it did continue it was in much lower concentrations, and in conjunction with chlorinated hydrocarbons (Frank et al. 1976). Chlordane, Aldrin, and heptachlor were primary replacements for lead arsenate at this time as well (Dominick 1960). However, lead arsenates and chlorinated hydrocarbon use were officially banned by the Environmental Protection Agency (USEPA 1988, 2012).

With the loss of these insecticides control efforts shifted. Synthetic insecticides deemed less toxic as well as more environmentally friendly methods such as nematodes and fungi
were explored (Banjeree 1968). During this time, an increase in research examining the capabilities of turfgrass species and cultivars began. Recommendations for control now include the incorporation of resistant turfgrass types, especially those with endophytes.

By the 1940’s enough research had been conducted that interested parties were now able to provide more thorough and effective recommendations, though there were still many gaps in the knowledgebase. Bohart (1947) states that webworm species of California seemed to be restricted to bentgrass and bluegrass lawns, narrowing down possible culprits in other lawns or fields. Testing on Bermuda grass and Australian ryegrass lawns only yielded two or three *Crambus sp.* Both Bohart and Noble (1932) list turfgrass diagnostic characteristics such as “irregular brown patches” as opposed to circular ones, and uneven turf height near the brown patches as identifying characteristics of webworm damage. Felt (1894) also recommended a pyrethrum solution which would cause larvae in infested lawns to rise to the surface, allowing for accurate density counts.

Noble’s 1932 circular for the USDA identifies webworms as serious pests of golf courses for the first time. Prior to this, prairies, tobacco, and corn fields were considered to be the primary source of concern.

Though not to be considered primary sources of control, a number of natural enemies have been identified for webworms. Noble observed numerous blackbirds, flickers, robins and starlings in areas known to have high webworm populations (1932). Ainslie (1927) also noted crows were considered to be natural enemies as well. Parasitoids have
been also noted by numerous sources including Ainslie (1923a, 1927), Noble (1932), Stirrett and Arnott (1932), Crawford (1964) and Bohart (1947).

One of the most successful methods of obtaining long term control of pest species is the practice of slit-seeding endophytic grasses into non-endophytic turf. Endophytes are fungi which form a symbiotic relationship with another organism, in this case, grass. They colonize most of a plant’s tissues and will live within the cells, rarely emerging from the plant and then only with the fruiting bodies. They are found in all groups of fungi and each are believed to have evolved their endophytic capabilities independently. Current phylogenetic evidence points to endophytes have evolved from pathogenic fungi and vice versa. Why such a relationship would occur is still not wholly understood. The fungus gains access to nutrients and protection while residing within the host plant. However, the host plant is not well known for all cases. Some do see increased shoot and/or root growth, enhanced uptake of minerals and nutrients, as well as resistance to other plant pathogens and herbivores, including insects. Whether enhanced plant growth is a direct product of the relationship with the fungi or a byproduct of its protection is not yet clear. Generally, however, there is a net positive.

Ascomycota contains the majority of endophyte species with their closest relations being entomopathogenic and plant pathogenic species. The genera most associated with grasses are *Neotyphodium* and *Epichloë* (Family: Clavicipitaceae). These particular fungi produce toxic alkaloids which render the host plant unpalatable, even toxic, to herbivores. These alkaloids are not only harmful to insects but are of concern to those who manage
livestock and other animals as repeated consumption of some of these endophytic grasses can lead to poor growth, spontaneous abortion, and even death. The Neotyphodium and most of the Epichloë are asexual, however some of the Epichloë do have a sexual stage. Most endophytes are transmitted horizontally, i.e. from the outside environment. The endophytes which colonize grass however are propagated via vertical transmission, i.e. through the seeds of grasses. The Epichloë that have a sexual stage they have a heterothallic mating system and thus have the potential to transmit horizontally. However, it is believed that they rarely if ever do this and instead propagate asexually.

The inclusion of endophytic grasses has been part of the push to pursue a holistic approach when it comes to pest management. Integrated pest management (IPM) is a method of control which incorporates cultural, biological, and synthetic chemical control methods. Decreases in chemical applications can lead to more natural enemies and an overall increase in efficiency. However, one of the most important aspects of a successful IPM program is the accurate identification of the pests.

As noted earlier, pinpointing the best time to control sod webworms can be tricky, especially when using cultural/mechanical control methods such as plowing. Plowing too early or too late can be useless. Chemical treatments can be costly and if not properly timed ultimately useless. Part of this problem is likely due to the cryptic nature of these species. Understanding which species is present at which times will help eliminate the guesswork of earlier cultural control methods. Additionally, being able to separate these species into ones of concern (P. teterrella, A. vulgivagella, N caliginosella, etc.) versus
ones less likely to cause significant damage (*F. mutabilis, C. praefectellus*, etc.) is also important for drafting cost effective and efficient control recommendations.

### 1.6 Genetic Analysis of Lepidoptera & Cryptic Species

The utilization of molecular techniques for identification and analysis of entomological questions has advanced quite significantly in the last forty years. In the first edition of *Insect Molecular Genetics* by Hoy (1994), she questions why so few entomologists have engaged in molecular work. However, by the third edition (2013) she states that many techniques have become commonplace, referencing the 2011 proposal to genotype 5000 species of insects by 2016 (i5k 2014). These techniques have allowed scientists of many fields to develop a greater understanding of biological diversity, species identification, and a host of other areas.

Analysis using mitochondrial cytochrome oxidase 1 operates under the assumption that gaps occur at regular intervals between families, genera, and species. These gaps are believed to be separated by an order of magnitude, thus resulting in clear delineation between individuals. It must be remembered that these gaps may not be present in newly emerging species or closely related species. Creating a solid species tree based on mtDNA haplotypes should include multiple genes.

Species identification utilizing traditional morphological methods may not always be possible. Some species of skipper butterflies (*Hisperididae*) are known to be cryptic as
adults leading to significant underreporting of species richness. Herbert et al. (2004), Burns (2008) and others have conducted research in the Area de Conservación Guanacaste (ACG) in northwestern Costa Rica. When examining the species, *Astraptes fulgerator* Walch, genetic analysis of the mitochondrial cytochrome c oxidase I (CO1) gene revealed ten species where morphological examination indicated only one (Herbert et al. 2004).

Much like the crambine *Neodactria* complex, the *A. fulgerator* species complex of Costa Rica has been cause of distress for taxonomists. For most of its known history taxonomists have considered *A. fulgerator* a single species with varied and wide distribution. However, after the rearing of over 2500 wild caught caterpillars the simplicity of this supposed species identification became complicated. The morphologically distinctive traits of the caterpillars were not mirrored in the adults. Even analysis of the genitalia, one of the most surefire ways to accurately identify an individual, was of no use (Herbert et al. 2004).

Continued studies in the ACG show multiple examples of cryptic morphology of adults. This is often paired with morphological differences among larvae (Herbert et al. 2004; Burns et al. 2008; Janzen et al. 2011). With the increasing use of DNA barcoding it is important to remember that a blending of available data (morphology of all stages, ecological niches, behavior, etc.) is needed for making ultimate species determinations. Species genomic identities may only vary by a few nucleotides and therefore, be
genetically indistinguishable from each other depending upon the method and source of the code (Burns et al. 2007).

As is the case with the skippers mentioned above, the mitochondrial CO1 has been used for multiple purposes over the years but is especially useful when addressing cryptic species. In addition to Herbert et al. work (2004), Alexander et al. (2009), Burns et al. (2008), and Schulte II et al. (2003) many others have utilized the CO1 gene for phylogenetic and cryptic species analysis. Schulte II et al. (2003) used it in conjunction with other mitochondrial genes to determine phylogenetic relationships of lizard taxa. Using just the CO1 gene Alexander et al. (2009) analyzed the mayfly genus *Ephemera* (Ephemeroptera: Ephemeroellidae). Like the crambines and the skippers, this genus is morphologically indistinct and thus, needed genetic analysis to clarify species identities. Though they were able to do this for many specimens, there were some that remained unresolved by the end of their research. They caution that the CO1 may not be a useful barcode source for all groups. In their experience the utility of the CO1 was dependent upon sufficient genetic distance between selected species and at times there was not enough separation. Thus, intraspecific variation would overlap with interspecific divergence obscuring their true identities. Mitochondrial DNA haplotype trees may be useful in corroborating species trees, but hybridization resulting in mtDNA transfer of haplotypes could confound the data (Moore 1995).

1.7 Study Objectives
i. Characterize sod webworm species using adults through PCR techniques

ii. Identification of unknown sod webworm larvae using PCR to confirm usefulness of technique

iii. Match the identified adult sequences to sequences obtained from unidentified larval specimens. Successful pairing indicates the same species.

iv. When larvae have been successfully identified, determine if any host associations are indicated.

1.8 Hypotheses

1. Utilizing identified adults as source specimens, molecular techniques will identify unknown larvae.

2. Host habitats of the sod webworm larvae will have diverse species representation and richness.
Chapter 2: Crambine Sod Webworm Genetic Analysis and Identification

2.1 Introduction

Accurate identification of species is essential for biological analysis. Many species can easily be identified during their juvenile and adult stages. Even among species that exhibit a form of mimicry, morphological characters can help to separate them. However, there are a number of examples of species that have immatures that are not so clearly distinguishable requiring further genetic analysis.

Adult sod webworms (Crambinae: Crambidae: Lepidoptera) are generally considered easier to identify. With some exceptions, these species can be distinguished from one another by the color and patterning of their forewings as well as the structure of male genitalia. Genitalia being especially useful in the more cryptic species. Much of the taxonomic literature contains analysis of their behaviors, range, and economic impact (Felt 1894; Ainslie 1923; Scott 1955). Morphological and systematic studies are fewer in number (Matheny and Heinrichs 1972; Munroe and Solis 1999). Typically these analyses focus on the adult specimens. Passoa (1985), Hasenfus (1960), and Matheny and Heinrichs (1972) are examples of the few non-adult morphological studies presented. In these instances, traits such as head morphology, sclerite and pinacula structure, and chaetotaxy give key species features. However, these traits are only known for a select
few species and even then, only for the latter instars when they have grown sufficiently for careful analysis.

Reports of sod webworms in and near grasses date to the late 1800’s (Riley 1882). Modern turfgrass managers reporting adult presence while mowing, and during other turf use activities (Richmond and Shetlar, 1999). Several published studies describe black-light trap results on and near managed turf (Sorensen and Thompson 1984; Tomilson Jr 1970). However, when sod webworm larvae are recovered from these nearby areas they are rarely identified as anything other than “sod webworm.” Historically, adults collected from managed turf areas are often assumed to represent the larvae present in the grass. However, this assumption may prove false. There is evidence that not all of these species are grass feeders (Tunnock 1985, Roberts 1986; Texas 2014) and adult capture may simply reflect their presence in nearby ornamentals.

While evaluating pesticide efficacy, detergent flushes are often employed. Detergents apparently cause irritation of arthropod cuticle and thereby forcing many to surface, including second through sixth instar sod webworm larvae (pers. exp). However, given the limited information available and the amount of time required, species identification is rarely attempted.

Species identification utilizing traditional morphological methods may not always be possible. Some species of skipper butterflies (Hesperiidae) are known to be cryptic as adults, thereby leading to significant underreporting of species richness. Herbert et al. (2004), Burns (2008) and others have conducted research in the Area de Conservación
Guanacaste (ACG) in northwestern Costa Rica. When examining the species, *A. fulgerator*, genetic analysis of the mitochondrial cytochrome *c* oxidase I (CO1) revealed ten species where morphological examination indicated only one (Herbert et al. 2004). Continued studies in this same region show multiple examples of cryptic morphology of adults. This is often paired with morphological differences among larvae (Herbert et al. 2004; Burns et al. 2008; Janzen et al. 2011). With the increasing use of DNA barcoding it is important to remember that a blending of available data (morphology of all stages, ecological niches, behavior, etc.) is needed for making ultimate species determinations. Species genomic identities may only vary by a few nucleotides, and therefore be genetically indistinguishable from each other depending upon the method and source of the code (Burns et al. 2007; Olds et al. 2012).

For this study, I propose that using PCR methods similar to those of Herbert et al. (2004), and Burns et al. (2008), for species identification of adults and larval sod webworms. By first employing these methods to “finger print” the adults collected from a nearby light trap, accurate identification can be ensured. Larvae recovered from detergent flushes can then be subjected to the same techniques. With the confirmation that these methods are successful, these techniques can be applied to multiple turf habitats to examine species diversity and potential host associations. Therefore, I hypothesize: Sequencing of the mitochondrial CO1 segment of sod webworm adults can be used for adult identification confirmation, and then to identify unknown larvae.
2.2 Materials and Methods

Collecting: The Ohio State University Turfgrass Research Facility (TRF) (2710 North Star Rd, Columbus, OH 43221) is located in a semi-agricultural setting surrounded by residential suburban landscapes and adjacent to 40 acre wood lot. The TRF shares space with approximately 20 acres of corn, 50 acres dedicated to roaming cattle, in addition to the 40 acre wood lot. The TRF has a variety of common economically important turf types present. For the purposes of this research Kentucky bluegrass (*Poa pratensis* L.), creeping bentgrass (*Agrostis stolonifera* L.), and a turf type tall fescue (*Festuca sp.*) mix were selected. Larvae were sampled from these regions. Larvae were obtained using a soap-water flush technique utilizing lemon scented Joy® dish detergent at approximately 30mL detergent per one gallon of water. This soap had previously been determined to be the most effective product for obtaining larvae (D. Shetlar, per comm) and has been employed in previous studies (Richmond and Shetlar 1999). At the nearby Waterman Farm Research Facility a 15W black-light trap was used to collect adults. For both larvae and adults, sampling was conducted between April and October. Larvae were collected either bi-weekly (March through May; September through October) or weekly (June through August) depending upon the season. Adults were collected nightly. Adults caught near lights were also used when appropriate.

Genetic Analysis: DNA was obtained from the dry legs of adults. Larval DNA was obtained by removal of proximal-abdominal segments or terminal-abdominal segments. Initial samples were sourced from the terminal segments of the larvae. Per
recommendation by a committee member, this was switched to the mid-abdominal segments for later sequencing. Prior to removal of body part to be utilized for analysis, a photo of each was taken using a JVC-CMark Digital Camera model KY-F70B and processed using Auto-Montage stacking software.

Polymerase Chain Reaction (PCR) was conducted to amplify and isolate the COI gene of each specimen. A QuickExtract™ (company) kit was used to first clean and initiate amplification. The thermocycling protocol was: one 94°C cycle for two minutes followed by a 40X repeated cycle of 94°C for twenty seconds, 45°C for forty-five seconds and 72°C for one minutes. Upon completion of the 40th cycle, a ten minute run at 72°C finished out the process. The primers utilized were: 5’ TAA ACT TCA GGG TGA CCA AAA AAT CA -3’ and 5’ – TTT CTA CAA ATC ATA AAG ATA TTG G-3’. PCR products were electrophoresed in 1.0% TBE agarose gels, stained with ethidium bromide and visualized under UV light. PCR was conducted on freshly collected adults (light trap) and larvae (turf plots) as well as older archived specimens. Final sequence cycling and amplification was done by Functional Biosciences (505 South Rosa Road, Suite 238, Madison, WI 53719).

2.3 Results

Freshly collected larvae and adult specimens were used within a year of collection. The older, archived specimens date to the 1970’s. Both archived larval 80% ethyl alcohol (EtOH) and adult specimens (pinned and air dried) failed to produce usable DNA. There
are methods discussed in the literature which are effective for procuring older DNA, however these methods are time consuming and can be more costly and were not attempted. The potential results that would have been obtained from the older specimens would not necessarily outweigh the expense.

DNA sequences obtained from the adults and larvae were successfully correlated with each other and with NCBI (National Center for Biotechnology Information). The few sequences that fell below 97% match were inspected for sequence errors and gaps. These errors were corrected and adjusted as needed.

Freshly collected adults and larvae provided usable DNA for analysis. Larvae were generally preserved in 80% EtOH, however three specimens from 2012 were inadvertently preserved in KAAD. These samples were not able to be clearly identified. The archived larval specimens (dating from the 1970’s) preserved in KAAD (kerosene-acetic acid-dioxane solution) or alcohol could not be identified. In total four species of field collected sod webworm larvae were identified: *Parapediasia teterella* Zincken, *Fissicrambus mutabilis* Clemens, *Crambus praefectellus* Zincken and *Agriphila ruricolella* Zeller.

All of the sod webworm larvae obtained from the Kentucky bluegrass and tall fescue produced usable DNA. For the bentgrass, 13 caterpillars (9.7%) were unusable for 2011 samples, three specimens (2.88%) for 2012 samples, and one (3.23%) for 2013 samples.
Potential turf host associations are discussed in the following chapter (3).

2.4 Discussion:

DNA analysis has proved useful in many scenarios. Hypothesizing that we could use these methods to identify unknown larvae by comparing their sequences with those obtained from known adults is true and this was accomplished. Therefore this hypothesis is accepted at this time.

The type of preservative does potentially have an effect on the usefulness of the sample. Samples preserved in KAAD may not be useable. Those preserved in 80% EtOH had much lower failure rates. Older specimens, whether larval or adult, did not provide useable DNA. As mentioned previously, methods for extracting useable DNA from such samples are available. However, in this study, these techniques were not used. Though not the focus of this study it is important to note for future studies wishing to examine historical specimens, those methods may be needed.

Our protocol could easily be employed on other sod webworm species. Care must be taken to preserve key morphological characters. Photographic documentation proved useful in this effort and is recommended to be done prior to removal of source materials.
Chapter 3: Crambinae Host Associations

3.1: Introduction

As discussed more thoroughly in Chapter 1, previous reports indicate sod webworms are associated with managed turfgrass (Vittum 1999; Richmond and Shetlar 1999). These areas have diverse adult sod webworm populations when light traps are used and most appear to assume that sod webworm larvae obtained from the turf are equally diverse. Detergent flushing allows for easy recovery of sod webworm larvae from turfgrass. However, these larvae are rarely, if ever, identified passed the subfamily Crambinae and are classified simply as “sod webworm larvae.”

Light trap data indicates this group exhibits univoltine, bivoltine, and multivoltine seasonal habits (Niemczyk et al. 2000). Traditional insecticide treatments follow the assumption that larval infestations are just as diverse as observed adult presence, and therefore are present at potentially harmful amounts for the duration of the season. However, if only one of two univoltine species are present in a given turf type, this approach is not only wasteful it is potentially quite costly and could result in undue environmental degradation. With new management tools being developed (new pesticide chemistry, biobased insecticides, and biological controls) to control sod webworms in managed turf, questions of timing, frequency of application, and spectrum of activity are
even more important. Assuming that observed adult presence accurately reflects the larval activity in all turf types is no longer a logical approach.

Since it has been determined (Chapter 2) that sod webworm larvae can be genetically characterized and identified, we can now address questions about diversity and potential host preference and association.

A reevaluation of the Crambinae species in the state of Ohio has been conducted (Chapter 4) revealing a revised count of 38 species present. Fourteen of those have been recovered in the black light trap located by our research plots (Chapter 2, 4). Of those, six have been mentioned in the literature as potential grass pests (Ainslie 1923a, b) and four of those (Parapediasia teterrella Zincken, Pediasia trisecta Walker, Fissicrambus mutabilis Clemens, Neodactria caliginosella Clemens) are considered to be potentially serious pests rather than occasional grass feeders (Vittum 1999).

Following the current prevailing logic, I hypothesize that sod webworm larvae recovered from turfgrass will have a similar diversity profile to the adults collected from a nearby light trap, representing area diversity.

3.2: Materials and Methods

The three turfgrass types sampled were: creeping bentgrass (Agrostis stolonifera L.), Kentucky bluegrass (Poa pratensis L.), and turf type tall fescue (Festuca arundinacea Schreber). Creeping bentgrass was sampled 2011 – 2013. Attempts were made to
sample in the Kentucky bluegrass in 2011 and 2012 but no larvae were recovered during this time. This was believed to be an artifact of our sampling method and not reflective of true larval absence. The sampling methods were revised for 2013 and resulted in larvae being obtained. The fescue was only sampled during the final year, 2013.

The bentgrass plots are highly managed plots, kept at approximately 3-5mm height at least during the growing season through mowing three times a week. It does not receive regular insecticide treatment. The study area however, receives regular herbicide and fungicide treatments primarily for dollar spot. The Kentucky bluegrass and tall fescue plots are even less maintained with mowing occurring on average once per month and kept at a height of 4-6cm. It also does not receive regular insecticide treatments but does receive broadleaf weed control once per season. For all grasses, the areas surveyed were not subject to insecticide treatments.

In order to successfully obtain larvae from the Kentucky bluegrass and turf type tall fescue, the sampling method had to be altered. The highly managed bentgrass is kept at a height of approximately 3/16 - 1/8” (3-5mm) while the bluegrass and fescue are kept at a standard lawn height of approximately 2-3” (50-75mm). Literature indicates the largest of sod webworm larvae reach approximately 25mm. The larvae recovered from the bentgrass were typically 4th – 6th instars, approximately 10-22mm in length. Earlier instars (2nd – 3rd) are even smaller (approximately 5-10mm) and may be observed with very close inspection. However, these sizes were not observable in the Kentucky bluegrass and tall fescue without modifying the surface. A weed whacker was used to cut
the taller turf down to a shorter height. Such dramatic reduction in height can significantly damage these types of turfgrass for weeks to months. Therefore, sampling was moved around in these turf areas.

The detergent solution of approximately 30 mL Joy Ultra™ per one gallon of water was applied to the short cut areas and specimens that surfaced were collected. A number of spider (Aranea) and ant (Formicidae) species were observed but not collected. Earthworms (Megadrilacea) regularly surfaced as well but were also not collected.

Larvae were collected either bi-weekly (March through May; September through October) or weekly (June through August) depending upon the season. Larvae were preserved in an 80% EtOH solution. Chapter 2.2 describes the technical methods used for the PCR analysis.

The turf-type tall fescue plots were planted using endophytic seeds. However, the current endophytic levels were not assessed.

3.3: Results

Collection data for the creeping bentgrass are represented in Tables 3.1 – 3.3. These data include the number and diversity of sod webworm larvae, as well as other lepidopteran species recovered. Additionally, samples which did not yield useable DNA are recorded. Table 3.4 represents the Lepidoptera larvae recovered during 2013 from the Kentucky
Table 3.5 represents the Lepidoptera larvae recovered during 2013 from the Tall Fescue.

Tables 3.1 – 3.3 show the yearly larval abundance patterns of Lepidoptera larvae in the creeping bentgrass from 2011-2013. Tables 3.4 and 3.5 show the Lepidoptera larvae obtained from the Kentucky bluegrass and tall fescue, respectively. Tables 3.6 – 3.7 show the non-Lepidoptera arthropods obtained from creeping bentgrass, Kentucky bluegrass, and tall fescue respectively.

Tables 3.9 – 3.11 display the proportional representation of all taxa recovered in 2013 in bentgrass, Kentucky bluegrass, and tall fescue respectively.

Table 3.12 depicts the proportional representation of all Lepidoptera recovered between 2010 – 2013 in all turf types.

Table 3.13 displays all Crambinae recovered at all turf sampling locations (excluding off site recoveries) for all years.

Table 3.14 represents the relative proportion of all Crambinae recovered at all locations 2011-2013. Number of sampling attempts has been taken into account.
<table>
<thead>
<tr>
<th>2011</th>
<th>Parapediasia teterrella</th>
<th>Agrotis ipsilon</th>
<th>Spodoptera frugiperda</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 April</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>29 April</td>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 May</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12 May</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>15 July</td>
<td>21</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>25 Aug</td>
<td>27</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>2 Sept</td>
<td>55</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3. 1: Bentgrass 2011 Lepidoptera larvae collected.

<table>
<thead>
<tr>
<th>2012</th>
<th>Parapediasia teterrella</th>
<th>Agriphila rurocolella</th>
<th>Agrotis ipsilon</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 March</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30 March</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13 April</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 May</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>8 June</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>5 July</td>
<td>5</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>9 July</td>
<td>7</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>10 August</td>
<td>38</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>7 September</td>
<td>28</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>25 September</td>
<td>10</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 3. 2: Bentgrass 2012 Lepidoptera larvae collected.
### Table 3.3: Creeping Bentgrass 2013 Lepidoptera larvae collected.

<table>
<thead>
<tr>
<th>Date</th>
<th>Parapediasia teterrella</th>
<th>Crambus praefectellus</th>
<th>Agrotis ipsilon</th>
<th>Spodoptera frugiperda</th>
</tr>
</thead>
<tbody>
<tr>
<td>17 April</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>27 April</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 May</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15 May</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25 May</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>31 May</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>15 June</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12 August</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30 August</td>
<td>18</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 3.4: Kentucky bluegrass 2013 Lepidoptera larvae collected.

<table>
<thead>
<tr>
<th>Date</th>
<th>Fissicrambus mutabilis</th>
<th>Nomophila nearctica</th>
<th>Burrowing SWW</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 May</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>26 May</td>
<td>2</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>31 May</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>15 June</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 3.3: Creeping Bentgrass 2013 Lepidoptera larvae collected.

Table 3.4: Kentucky bluegrass 2013 Lepidoptera larvae collected.
<table>
<thead>
<tr>
<th>Date</th>
<th><em>Fissicrambus mutabilis</em></th>
<th><em>Agrotis ipsilon</em></th>
<th><em>Spodoptera ornithogalli</em></th>
<th>Burrowing SWW</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 May</td>
<td>0</td>
<td>2</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>10 May</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>26 May</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15 June</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>8 Aug</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>3</td>
<td>17</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 3. 5: Tall fescue 2013 Lepidoptera larvae collected.
<table>
<thead>
<tr>
<th></th>
<th>2013</th>
<th>Elateridae</th>
<th>Aphodius (Scarabeidae)</th>
<th>Formicidae</th>
<th>Drosophilidae</th>
<th>Carabidae</th>
<th>Staphylinidae</th>
<th>Tipulidae</th>
<th>Curculionidae</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>2 May</td>
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<td>15 May</td>
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<td>1</td>
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</tr>
<tr>
<td>31 May</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>4</td>
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<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>15 Jun</td>
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<td>0</td>
<td>0</td>
<td>2 (2sp)</td>
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<td>0</td>
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<td>2</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>30 Aug</td>
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<td>0</td>
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<td>1</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.6: Creeping Bentgrass 2013 All non-Lepidoptera Arthropods collected.
<table>
<thead>
<tr>
<th></th>
<th>Elateridae</th>
<th>Millipede</th>
<th>Formicidae</th>
<th>Carabidae</th>
<th>Staphylinidae</th>
<th>Tipulidae</th>
<th>Curculionidae</th>
<th>Phylophaga</th>
<th>Bluegrass BB</th>
<th>Other Scarab</th>
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<tbody>
<tr>
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<td>2 (2 sp)</td>
<td>4</td>
<td>0</td>
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<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
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</tr>
<tr>
<td>26 May</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>13</td>
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</tr>
<tr>
<td>15 June</td>
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<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
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</tr>
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<td>7</td>
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<td>0</td>
<td>1</td>
<td>1</td>
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</table>

Table 3. 7: Kentucky bluegrass 2013 All non-Lepidoptera Arthropods collected.

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<tr>
<th></th>
<th>Elateridae</th>
<th>Millipede</th>
<th>Formicidae</th>
<th>Carabidae</th>
<th>Staphylinidae</th>
<th>Tipulidae</th>
<th>Curculionidae</th>
<th>Bluegrass BB</th>
<th>Spotted Cucumber Beetle</th>
<th>Lampyridae</th>
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<tr>
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<tr>
<td>10 May</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>6</td>
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<td>15 June</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8 Aug</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>1</td>
<td>2</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>9</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3. 8: Tall Fescue 2013 All non-Lepidoptera Arthropods collected.
Table 3.9: Proportion of taxa in bentgrass. *P. teterrella* account for 15% (0.15) of all taxa recovered – 2013
Table 3.10: Propotion of Taxa - Kentucky bluegrass. *F. mutabilis* account for 6% (0.06) of all taxa recovered. – 2013
Table 3.11: Proportion of taxa in Tall fescue. *F. mutabilis* account for 9% (0.09) of all taxa recovered. – 2013
Table 3. 12: All Lepidoptera recovered, proportional to each other: 39% (0.39) *P. teterrella*, 5% (0.05) *F. mutabilis*, 3% (0.03) each *A. ruricolella* and *C. praefectellus*.
Table 3.13: Total Crambinae by location.
Table 3. 14: Proportion all Crambinae relative to each other, recovered from all sites.

Figures 3.1 – 3.4 display a variety of views of an A. *ruricolella* recovered from tall fescue in 2011. Note the absence of the terminal segments removed for genetic analysis.

Figures 3.5 – 3.7 are various views of a sod webworm hibernacula, the structure these creatures reside in during the winter months as well as occasionally during their summer life cycles.

Figures 3.8 – 3.9 are the eggs of *C. laqueatellus* in various stages of development.
Figure 3.10 is of a Crambinae species which did not yield usable DNA. It was collected in the Kentucky bluegrass.

Figure 3.11 is a lateral view of *F. mutabilis* collected from the tall fescue in 2013.

Figure 3.12 is of *P. teterrella*, the Bluegrass webworm. This species was obtained solely from creeping bentgrass.

The larva depicted in Figures 3.13 – 3.15 are of *C. praefectellus*. It was collected once in creeping bentgrass and in no other locations. The first two images were taken while it was still alive. The second is post segment removal.
Figure 3. 2: Agriphila ruricolella dorsal view

Figure 3. 3: Agriphila ruricolella - Ventral view of thoracic segments and mouthparts.
Figure 3. 4: *Agriphila ruricolella* - Close up lateral view.

Figure 3. 5: Hibernacula of sod webworm
Figure 3. 6: Hibernacula of sod webworm – dissected

Figure 3. 7: Close up of internal structure of hibernacula
Figure 3. 8: *Crambus laqueatellus* egg - Early in development.

Figure 3. 9: *Crambus laqueatellus* eggs - Various stages of development. Darker eggs are several days old.
Figure 3. 10: Lateral view of Crambinae larvae from Kentucky bluegrass which did not produce usable DNA for analysis.

Figure 3. 11: Lateral view of *Fissicrambus mutabilis* collected from tall fescue.
Figure 3. 12: Lateral view of *Parapediasia teterrella* collected from creeping bentgrass.

Figure 3. 13: *Crambus praefectellus* lateral view of medium-sized larva, live specimen.
Figure 3. 14: *Crambus praefectellus* dorsal view of medium-sized larva, live specimen

Figure 3. 15: *Crambus praefectellus* – Head and thoracic segments
Figure 3. 16: *Acrolophus popeanella*, lateral view of late instar, preserved specimen showing heavily sclerotized prothoracic collar and integument that appears velvet-like.

Figure 3. 17: *Acrolophus popeanella*, close-up of head and prothoracic collar.
Figures 3.16–3.18 depict a burrowing sod webworm (*Acrolophus popeanella*). *A. popeanella* is not a true sod webworm as it is not of the subfamily Crambinae. Though termed the “burrowing sod webworm” it is actually a member of the family Acrolophidae. These larvae were obtained in the Kentucky bluegrass and in the tall fescue but not in the creeping bentgrass.

Table 3.15 depicts an overall representation of expected larvae as predicted by the hypothesis contrasted with the observed data. *F. mutabilis* was recovered in low numbers in both of the higher cut turf types but the numbers are too low for statistical analysis. Similarly, the small number of *Agriphila ruricolella*, and *Crambus praefectellus* prevent conclusive statistical analysis. It does appear to have an association with these types
however no conclusive decision can be made regarding their true associations with these types.

<table>
<thead>
<tr>
<th>Species</th>
<th>Expected</th>
<th>Bentgrass</th>
<th>Kentucky Bluegrass</th>
<th>Tall Fescue</th>
<th>Other Location</th>
<th>Total Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Parapediasia teterrella</em></td>
<td>Y</td>
<td>269</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>269</td>
</tr>
<tr>
<td><em>Pediasia trisecta</em></td>
<td>Y</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Fissicrambus mutabilis</em></td>
<td>Y</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td><em>Neodactria caliginosella</em></td>
<td>Y</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Agriphila ruricolella</em></td>
<td>N</td>
<td>0</td>
<td>0</td>
<td>1</td>
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<td>2</td>
</tr>
<tr>
<td><em>Crambus praefectellus</em></td>
<td>N</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3. 15: Crambinae species that were predicted by the hypothesis and the numbers actually collected from the various turfgrass sites and other locations.

Even heterogeneity of the four pest species was expected in each of the three turf plots. This was not observed. Two of the species expected were not recovered. The other two species expected were recovered, however they were not universal in all plots. A standard ANOVA analysis is not possible as one of the primary variables is nominal. Thus, a Kruskal-Wallis test would typically be recommended, however the figures recorded were too low to provide meaningful results.

3.4: Discussion

With a revised Crambinae of Ohio list, including the newly identified species *E. ocellus*, 38 species are currently recognized as residents of Ohio. Mostly anecdotal evidence
exists detailing these species’ larval hosts with only a few select articles devoted to host preferences (Richmond and Shetlar 1999). This study helps to support and expand upon previous studies in this matter.

Endophytic grasses are traditionally recommended as a deterrent to insect herbivory. The symbiotic relationship between the endophytic fungi and the plant makes for a mutually beneficial relationship. Though there is still some question as to the full scope of these benefits, it has been observed that grass receives added resistance to herbivory while the fungus obtains vital nutrients and structural support from the grass (Breen 1994; Grewal et al. 1995). Alkaloids produced within the grass by the fungi have been shown to deter herbivory (Richmond and Shetlar 1999, 2000). Therefore, the expectation that no sod webworms will be found in the endophytic tall fescue seemed logical. However, this was not the case as larvae were recovered from the endophytic tall fescue plots.

Additionally, Williamson and Potter (1997) found that black cutworms did not thrive on Kentucky bluegrass and did not survive to pupation. However, when feeding upon endophyte-infected perennial ryegrass and tall fescue black cutworm larvae showed little to no ill effects. Similarly, Richmond and Shetlar (2001) found that the black cutworms did not favor either grass and would emigrate from polycultures containing large quantities of endophytic perennial ryegrass. However, as with Williamson and Potter’s work, the cutworms did not thrive on the Kentucky bluegrass and had linearly decreasing biomass as percent Kentucky bluegrass increased. Thus, we would not expect to find
cutworms in the Kentucky bluegrass creating decreased competition with other Lepidoptera, including the sod webworms.

The hypothesis that sod webworm larval diversity in each turf habitat would be just as diverse as the local adult population does not appear to be supported by our findings. Given the low numbers of *F. mutabilis*, *A. ruricolella*, and *C. praefectellus* definitive statistical analysis is not possible. *P. teterrella* represented over 99% of the sod webworms recovered from the creeping bentgrass from 2011 – 2013 which is decidedly not an even distribution of species in this turf. Sod webworm populations were not diverse in either a specific turf, nor overall. Thus, I reject this hypothesis.

*P. teterrella* accounted for over 78% of the total Lepidoptera recovered in the bentgrass. *F. mutabilis* was the only sod webworm larvae recovered from the Kentucky bluegrass and the predominant sod webworm species recovered in the tall fescue. The one *A. ruricolella* recovered from the fescue only in 2011 could simply be an incidental and an outlier, or may represent a possible fluctuation in host selection over time. More long term analysis could help to clarify this point.

In choice tests, *P. teterrella* are known to feed on Kentucky bluegrass as well as non-endophyte infected perennial ryegrass (Richmond and Shetlar 1999). Endophyte infected perennial ryegrass was shown to cause greater mortality when not given a food choice. Yet, when *F. mutabilis* presumably has the choice of food sources in its native
environment, it still occurred in tall fescue, an endophytic grass. This could demonstrate a tolerance of the endophyte content, a preference for a food source in the plots which is not the fescue, or perhaps even a lowered endophyte load in the grass, making it more palatable to this species. As the tall fescue had higher numbers of Lepidoptera compared to the Kentucky bluegrass it is unclear which factor or factors are really at play. Further analysis of these relationships is needed.

Sod webworms are known grass feeders, with a few exceptions. The species recovered are not too surprising. *A. ruricolella* are known borers of turfgrass and corn (*Zea mays*) and they are also reported to be detritivores of cereals (Robinson and Tolley 1982; Landry 1995; Zhang 1994). My results indicate they could potentially be associated with turf type tall fescue, an endophytic grass. In addition to the grass in this area, clovers and Canada thistle were observed. As a single larva was obtained from the fescue as well as in a thistle root it could be possible the caterpillar was feeding on the thistle in both instances. Further sampling is needed to clarify its association.

*Parapediasia teterrella* strongly associates with creeping bentgrass. Previous research indicates this species is able to feed on Kentucky bluegrass (Richmond and Shetlar 1999), however none were found in our plots. It is possible that due to their small size they were repeatedly overlooked in the other turf types. Though they remained the predominant Lepidoptera species collected in the bentgrass for all three years, there was a steady decrease over the three year period.
The third year decrease is likely due to a mix of delayed appearance and slightly fewer sampling dates. However, a trend of decreased abundance does appear to be happening. This could be due to the uncharacteristically warm winter which occurred during winter 2012-2013. It could also be due in part to the use of two herbicides and fungicides that were used on the plots. Neither of two products used mention potential non-target effects on arthropods. It is possible that these products do have some unintended effects that have not been studied. This must be kept in mind when formulating future projects. The delay in sod webworm appearance in the bentgrass is therefore the result of some other undetermined factor(s).

The turf-type tall fescue and Kentucky bluegrass both hosted *F. mutabilis*. *Avena sativa* L (common oat), *Dactylis glomerata* L. (orchardgrass), *Digitaria sanguinalis* L. (hairy crabgrass), *Hordeum vulgare* L. (barley), *Phelum pretense* L. (Timothy grass), *Secale cereal* L. (cereal rye), *Triticum sp* (wheat), and *Zea* species, specifically *Zea mays* L. (corn) have been reported hosts of this species, depending upon its geographic distribution (Ainslie 1923a; Solis 2008; Landry 1995). My results indicate Kentucky bluegrass is a host type and can potentially add turf type tall fescue to the list as well.

The prediction was there would be even representation of the four accepted Crambinae turf pests (*P. teterrella, P. trisecta, F. mutabilis, and N. caliginosella*) throughout all turfgrass types sampled. However, only two of these species were recovered in turfgrass.
N. caliginosella, and P. trisecta were not observed. P. teterrella had a clear association with the bentgrass, being found only there, and in significantly high numbers. F. mutabilis was recovered in low numbers in both of the higher cut turf types but the numbers are too low for statistical analysis. It does appear to have an association with these types however no conclusive decision can be made regarding their true associations with these types.

A further course of research should include determining the host finding and selection mechanisms employed by these groups. Do the adults lay eggs randomly through fields as have been suggested previously (Ainslie 1922)? Or do they have a more specific selection technique potentially utilizing tactile or chemical cues received from different grass types?

3.5 Non-Sod Webworm Analysis

Acrolophus popeanella has a known range extending from New Jersey, west to Ohio and south though Florida. It is known to feed on Trifolium pretense (red clover). The larvae of this species were collected in both Kentucky bluegrass and the tall fescue but not in the bentgrass plots. Though the bluegrass and fescue plots were predominated by these grass types, there were non-grassy weeds present, including red and white clovers (Trifolium repens). Canada thistle was another broadleaf plant which had invaded portions of these
fields, though never dominating a sample plot. Care was taken to avoid areas which were high in weeds and instead focus on regions predominantly bluegrass or fescue.

Black cutworms (*Agrotis ipsilon*) are a noted pest of many grasses (Sherrod et al. 1979; Williamson and Potter 1997). Williamson and Potter (1997) investigated the survivability and preferences of these caterpillars for different turfgrasses, including Kentucky bluegrass, creeping bentgrass, and endophytic and non endophytic perennial ryegrass and tall fescue. Their results indicate a strong preference for creeping bentgrass which is corroborated by my experience. No cutworms were recovered from the Kentucky bluegrass or fescue plots but were recovered from the bentgrass. A number of yellow striped armyworm (*Spodoptera ornithogalli* Guenée) larvae were recovered in the fescue. They were more abundant in the endophytic fescue than in the non-endophytic Kentucky bluegrass.

Elateridae were recovered from all three turf types. They were the second most commonly collected Coleoptera group in the bentgrass and Kentucky bluegrass. They were equal to bluegrass bill bugs *Sphenophorus parvulus* Gyllenhal in the tall fescue. The predominant Coleoptera in the bentgrass were black turfgrass atenius (*Ataenius spretulus* Haldeman) (Scarabaeidae). These beetles would surface quickly, before the sod webworms and continue after the webworms had entered their hibernacula for the winter. A similar looking scarab, *Aphodius lividus* Olivier was recovered from the bentgrass.
Chapter 4: Flight Activity of Crambine Sod Webworms in Columbus, OH with a Revised Account of Ohio Crambinae

4.1 Introduction

The work of Hine in 1897 was the first to attempt to catalog the Crambinae of Ohio. He identified 21 species. Niemczyk et al. (2000) collected twelve species and listed 19 archived in the C. A. Triplehorn Insect Collection at The Ohio State University (currently at 1315 Kinnear Rd, Columbus, OH 43212). One of these, *Crambus pusionellus* Zeller is believed to be a misidentification. The Crambinae listed in the Roy W. Rings Lepidoptera Collection at the Ohio Agricultural Research and Development Center (OARDC) includes 14 species. Solis (2008) also lists 14 from Ohio. The Ohio Lepidopterists (1994) published an accounting of 33 species.

There are now 38 identified species within the subfamily Crambinae known to occur in the State of Ohio with 15 known to occur with relative frequency in Columbus (Hine 1897; Niemczyk et al. 2000; personal exp.). Flight records for these species have been collected yearly between April and October by placing a black-light trap on the Waterman Farm complex of the Ohio State University (located at 2501 Carmack Rd, Columbus, OH 43210). This facility is located in a semi-agricultural setting surrounded by residential suburban landscapes and near 40 acre wood lot. The farm complex shares
space with approximately 20 acres of corn, 50 acres dedicated to roaming cattle, in
addition to the 40 acre wood lot and 20 acres of managed turfgrass as part of the Ohio
State University Turfgrass Research Facility. The light trap is placed outside in mid-
April with the first crambine sod webworms beginning to arrive in mid to late May. The
last sod webworms are collected in October and the trap is typically shut down in early
November. Data for May 2009 is currently not available.

Though commonly referred to as “sod webworms,” not all crambines are pests of grasses.
Some, such as have been implicated as both a grass pest and a pest of other plants such as
tobacco (Scott 1955; Ainslie 1923a, b). *Chrysoteuchia toparia* has historically been a
pest of cranberries, hence its common name the Cranberry Girdler (Scammel 1917;
Cockfield and Mahr 1994). In reference to turfgrass it is not clear if a species identified
as a pest of a grassy area has been actually been reared and confirmed in the adult form or
if it was simply assumed since the adult was found in that location. Given the difficulty
in rearing this group successfully from egg to adult, one cannot assume either scenario
(Riley 1882).

Efforts to obtain species richness data for an area are typically reliant upon blacklight
traps. Trap height and composition have been noted to produce potentially skewed
species, and sex results (Deay and Taylor 1954; Frost 1958; Tomlinson, Jr. 1970). Where
traps were kept at a height equal to cranberry vine tips or another three feet above the
vine tips, *Chrysoteuchia topiaria* were captured in the vine level traps at twice the level
of the above tip trap. For this species, the sexes were equally trapped at the two heights (Tomlinson, Jr. 1970).

Felt makes note of the 1889 and 1892 collections where an excess of males were trapped with one exception (1894). The exception was of *Crambus laqueatellus*, where females were more abundant. Also, only one female was found with an egg filled distended abdomen indicating the eggs had been dispatched before alighting and traveling towards the trap (Felt 1894).

Reports by Kamm (1970, 1974), and Crawford (1967, 1966) indicate that some crambine cycles are regulated by photoperiod. Larval development and subsequent adult emergence has been linked to this (Tolley and Robinson 1986). If this assumption is correct, I would expect to see relative consistency in dates of initial emergence.

However, some evidence suggests that development may be linked with temperature (Heinrichs and Matheny 1969; Banjeree 1969) or a combination of the two (Kamm 1970). If this is correct, I expect to see initial emergence dates being linked more closely with temperature, resulting in early or late emergence depending upon yearly temperature fluctuations.
4.2 Methods & Materials

A 15W black light trap was operated nightly from late April through October. This trap has the bottom approximately 1.5 m above the ground. The trap was usually emptied every morning and the contents were sorted. Crambines were identified, tallied, and recorded. Sex was not determined. The trap was often not emptied on Saturday, or Sundays. Collections made in 2009 and 2010 were made by Dr. Dave Shetlar as part of his continued analysis of turf pests in the region. Collections for 2011-2013 were made by the author.

To compile species list of crambine sod webworms found in the state of Ohio, literature of relevant published works were reviewed as well as observations from regional light trapping.

4.3 Results

A review of the literature and the collection of the new species *Euchromius ocellus* has placed the current estimated of Crambinae sod webworms at 38 species (Hine 1876; Niemczyk et al. 2000; Solis 2008). This figure includes evaluation of three Ohio entomological collections: OARDC Roy Rings Lepidoptera Collection in Wooster, the Ohio Lepidopterist Society Collection in Columbus, and the Ohio State University
Triplehorn Museum Collection in Columbus. Additionally, observations and records kept at two well respected and professionally managed websites was used: Butterflies and Moths of North America (www.BAMONA.com), and the Moth Photographers Group (http://mothphotographersgroup.msstate.edu/Plates.shtml). These websites are generally highly trusted and are used and monitored by many professional and top amateur lepidopterists. Still, identifications based solely on their presence on one or both of those websites but not in the primary literature should be met with a level of skepticism. Species observed during the 2011-2013 sampling seasons at Waterman Farm are noted.

When considering the Neodactria sp. complex, it is important to consider the difficulty and lack of consensus that currently exists surrounding it. N. luteolellus, N. caliginosellus, N. murellus, and N. zeellus are in need of further taxonomic analysis to confirm their identities. There is also potential similar complications in the Pediasia sp complex.
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<th>Light Trap</th>
</tr>
</thead>
<tbody>
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<td>x</td>
</tr>
<tr>
<td>2  Agriphila vulgivagella</td>
<td>Crambini</td>
<td>x</td>
</tr>
<tr>
<td>3  Arequipa turbatella</td>
<td>Crambini</td>
<td></td>
</tr>
<tr>
<td>4  Chrysoteuchia topiaria</td>
<td>Crambini</td>
<td>x</td>
</tr>
<tr>
<td>5  Crambus agitatellus</td>
<td>Crambini</td>
<td>x</td>
</tr>
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<td>Crambini</td>
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</tr>
<tr>
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<td>Crambini</td>
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</tr>
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<td>9  Crambus laqueatellus</td>
<td>Crambini</td>
<td>x</td>
</tr>
<tr>
<td>10 Crambus leachellus</td>
<td>Crambini</td>
<td></td>
</tr>
<tr>
<td>11 Crambus perlellus</td>
<td>Crambini</td>
<td></td>
</tr>
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<td>Crambini</td>
<td>x</td>
</tr>
<tr>
<td>13 Crambus fusionellus</td>
<td>Crambini</td>
<td></td>
</tr>
<tr>
<td>14 Crambus saltuellus</td>
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</tr>
<tr>
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<td>Crambini</td>
<td></td>
</tr>
<tr>
<td>16 Crambus watsonellus</td>
<td>Crambini</td>
<td></td>
</tr>
<tr>
<td>17 Euchromius ocellus</td>
<td>Crambini</td>
<td>x</td>
</tr>
<tr>
<td>18 Fissicrambus mutabilis</td>
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<td>x</td>
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<td>Crambini</td>
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<tr>
<td>20 Microcrambus copelandi</td>
<td>Crambini</td>
<td></td>
</tr>
<tr>
<td>21 Microcrambus elegans</td>
<td>Crambini</td>
<td>x</td>
</tr>
<tr>
<td>22 Microcrambus kimballi</td>
<td>Crambini</td>
<td></td>
</tr>
<tr>
<td>23 Neodactria caliginosellus</td>
<td>Crambini</td>
<td>x</td>
</tr>
<tr>
<td>24 Neodactria luteellus</td>
<td>Crambini</td>
<td>x</td>
</tr>
<tr>
<td>25 Neodactria murellus</td>
<td>Crambini</td>
<td></td>
</tr>
<tr>
<td>26 Neodactria zeellus</td>
<td>Crambini</td>
<td></td>
</tr>
<tr>
<td>27 Parapediasia decorrella</td>
<td>Crambini</td>
<td></td>
</tr>
<tr>
<td>28 Parapediasia teterrella</td>
<td>Crambini</td>
<td>x</td>
</tr>
<tr>
<td>29 Pediadia abnaki</td>
<td>Crambini</td>
<td></td>
</tr>
<tr>
<td>30 Pediadia trisecta</td>
<td>Crambini</td>
<td>x</td>
</tr>
<tr>
<td>31 Argyria auratella</td>
<td>Agryiini</td>
<td></td>
</tr>
<tr>
<td>32 Argyria critica</td>
<td>Agryiini</td>
<td></td>
</tr>
<tr>
<td>33 Urola nivalis</td>
<td>Agryiini</td>
<td>x</td>
</tr>
<tr>
<td>34 Chilo plejadellus</td>
<td>Chiloini</td>
<td></td>
</tr>
<tr>
<td>35 Eoreuma densella</td>
<td>Haimbachiini</td>
<td></td>
</tr>
<tr>
<td>36 Haimbachia placidella</td>
<td>Haimbachiini</td>
<td></td>
</tr>
<tr>
<td>37 Thopeutis forbesellus</td>
<td>Haimbachiini</td>
<td></td>
</tr>
<tr>
<td>38 Xabia panalopec</td>
<td>Haimbachiini</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: 1: Revised Crambinae of Ohio using literature and indication of whether they were recovered in the light trap operated during this study.

The species collected in decreasing order of relative frequency of capture using light trap data from 2009 – 2013 are listed in Table 4.2.
<table>
<thead>
<tr>
<th>Species</th>
<th>Number Collected</th>
<th>% of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Parapediasia teterrella</em></td>
<td>5918</td>
<td>39.2</td>
</tr>
<tr>
<td><em>Neodactria sp.</em></td>
<td>2717</td>
<td>18.0</td>
</tr>
<tr>
<td><em>Microcrambus elegans</em></td>
<td>1825</td>
<td>12.1</td>
</tr>
<tr>
<td><em>Fissicrambus mutabilis</em></td>
<td>1252</td>
<td>8.3</td>
</tr>
<tr>
<td><em>Pediaea trisecta</em></td>
<td>1055</td>
<td>7.0</td>
</tr>
<tr>
<td><em>Chrysoteuchia topiaria</em></td>
<td>682</td>
<td>4.5</td>
</tr>
<tr>
<td><em>Crambus agitatellus</em></td>
<td>642</td>
<td>4.3</td>
</tr>
<tr>
<td><em>Agriphila vulgivagella</em></td>
<td>396</td>
<td>2.6</td>
</tr>
<tr>
<td><em>Agriphila ruricolella</em></td>
<td>314</td>
<td>2.1</td>
</tr>
<tr>
<td><em>Urola nivala</em></td>
<td>154</td>
<td>1.0</td>
</tr>
<tr>
<td><em>Crambus laqueatellus</em></td>
<td>105</td>
<td>0.7</td>
</tr>
<tr>
<td><em>Crambus albellus</em></td>
<td>16</td>
<td>0.1</td>
</tr>
<tr>
<td><em>Euchromius ocellus</em></td>
<td>14</td>
<td>0.1</td>
</tr>
<tr>
<td><em>Crambus praefectellus</em></td>
<td>8</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Table 4. 2: Number of Crambinae species collected 2009-2013

Frequency distribution for each species was relatively consistent from year to year with variation appearing in abundance per period of time rather than by date. *A. ruricolella* for example was consistently found to first appear in August with greatest abundance in September. However the quantity collected during these two months varied from year to year. For this species total populations actually increased each year, nearly doubling in overall catch from 2011 to 2012.

Most species appear to fall into a clearly univoltine or bivoltine pattern. The species collected by Niemczyk et al. (2000) were all present. Three species, *C. albellus*, *U. nivala*, and *E. ocellus* were not discussed in their paper. *E. ocellus* had yet to be
identified in this state, *C. albellus* is difficult to identify and is still not clearly attributed to this state. *U. nivala* is not considered a turf pest, and while observed, it was not considered relevant to their study. All univoltine and bivoltine species identified below were also identified as such by Niemczyk et al. (2000).

According to Niemczyk et al. (2000), *P. trisecta*, and *C. praefectellus* are also bivoltine species. *P. trisecta* could be considered bivoltine but it did not appear to have discrete periods of absence of activity in the current study. *P. trisecta’s* presence is relatively consistent and varies from year to year. When examined over a longer period of time this may become clearer. When compared with the results of Niemczyk et al. (2000), the flight activity at the location nearest my trap (Milford Center) the results are similar. The southernmost and northernmost locations for *P. trisecta* in Niemczyk et al. (2000) appear to have more clearly defined peaks and valleys throughout the season. These differences are likely due to geographical and microclimatic variations in these regions.

*C. praefectellus* does not have a clear and consistent representation in Columbus, OH. When compared with the Milford Center, OH location the results are quite similar in their sparse representation. The species is most abundant at the Chemlawn Research Facility in Deleware, OH location. This is consistent with reports by D. Shetlar who collected *C. praefectellus* near his property in Deleware, OH (personal comm).
<table>
<thead>
<tr>
<th>Univoltine Species:</th>
<th>Bivoltine species:</th>
<th>Undetermined:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crambus laqueatellus</td>
<td>Parapediasia teterrella</td>
<td>Pediastia trisecta</td>
</tr>
<tr>
<td>Agriphila ruricolella</td>
<td>Fissicrambus mutabilis</td>
<td>Crambus albellus</td>
</tr>
<tr>
<td>Agriphila vulgivagella</td>
<td>Microcrambus elegans</td>
<td>Urola nivala</td>
</tr>
<tr>
<td>Euchromius ocellus</td>
<td></td>
<td>Crambus praefectellus</td>
</tr>
<tr>
<td>Chrysoteuchia topiaria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neodactria sp</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.3: Species life cycle patterns in Columbus, OH

*C. laqueatellus* (Figures 4.21 – 4.22) and *C. praefectellus* (Figures 4.23 – 4.24) are clearly univoltine. However, they may potentially have a late season second generation given appropriate conditions. It is still unclear what these conditions might be. More data from locations where these species are most abundant may help to elucidate the flight habits of these species.

*P. teterrella* (Figures 4.1 – 4.2) is the species with most abundant presence at the light trap. Yearly capture fluctuates significantly with overall catch more than doubling between 2009 and 2010; to being nearly cut in half the each year 2011 and 2012, then decreasing only slightly by the end of 2013. When examined on a monthly scale over the course of five years less variation appears with the exception of September of 2010 with a large spike in abundance.

The *Neodactria* species complex also has little variation when viewed yearly (Figure 4.3). This species complex appears to be increasing slightly as time progresses. Monthly
collection over the course of five years has little variation with a slight spike in July 2013 (Figure 4.4). It appears to be a univoltine complex.

*M. elegans* appears to more than double in abundance between 2009 and 2010 but then diminish by half between 2012 and 2013 (Figure 4.5). August consistently has the highest abundance (Figure 4.6). It appears to be ambiguously multivoltine in Columbus, OH.

*F. mutabilis* has significant variation in yearly abundance, nearly doubling in number in two years, and then subsequently dropping by almost 80% two years later (Figure 4.7). The yearly flight pattern consistent from year to year and is bivoltine (Figure 4.8)

*P. trisecta*, much like *F. mutabilis* has significant variation in overall abundance, doubling in one year then decreasing by 50% each year over the next three years (Figure 4.9). Its yearly cycle tends toward bivoltine but is not clearly so (Figure 4.10). Observations over a longer period of time will clarify this.

*C. topiaria* is very consistent in abundance fluctuating very little in overall abundance (Figure 4.11). It is also very clearly univoltine (4.12).

*C. agitatellus* fluctuates some in its yearly abundance but not significantly (Figure 4.13). It is clearly univoltine (Figure 4.14).
A. vulgivagella appears to have an abundance pattern of increase – decrease – repeat relationship (Figure 4.15). Observations over a longer period will clarify this. A. vulgivagella is a late season emerging, univoltine species (Figure 4.16).

Agriphila ruricolella appears to be steadily increasing in abundance (Figure 4.17). Further observation will clarify if this trend continues or drops when the species reaches an upper threshold and then repeats this trend. Like its cousin, A. vulgivagella this species is univoltine and late season emerging (Figure 4.18).

Urola nivala is relatively consistent in overall abundance with some fluctuation (Figure 4.19). It appears to be increasing. Like P trisecta this species appears to be bivoltine but it is not clearly defined just yet (Figure 4.20). Further observation is required to make a solid determination for this species in this location.

C. laqueatellus is an early season univoltine species (Figure 4.22). As discussed earlier, it is possible that this species, given appropriate conditions could have a late season cycle but this is not proposition is merely anecdotal at this time. Data for May 2009 was missing. This species was likely present but not recorded. Its yearly abundance appears to be steady with the potential to increase given appropriate conditions (Figure 4.21).
C. praefectellus is sporadic in its attendance and cannot be clearly identified as univoltine or bivoltine at this location (Figure 4.23 – 4.24)

Data for C. albellus does not establish any clear flight habits for this species. There is no clear pattern established between the five years. (Figure 4.25). However, given recent data obtained from DNA analysis some identifications of C. albellus may in fact be inaccurate. Analysis has revealed three previously collected specimens, tentatively identified as C. albellus were in fact A. ruricolella (2) and Cnaphalocrocis trapezalis Guenée (1). These individuals were small in size and seemed to be missing most of the scales from their wings. Data recorded for C. albellus reported below but must be looked upon with a strong level of skepticism at this point. This species has been personally observed in this region, however.

The appearance of E. ocellus was first documented in the summer of 2012. This species does not have any record for this state up until that time. It was not collected in 2013. (Figure 4.26)
Figure 4. 1: *Parapediasia teterrella* yearly collection data 2009-2013.

Figure 4. 2: *Parapediasia teterrella* monthly collection data 2009-2013.
Figure 4.3: *Neodactria* sp. yearly collections 2009-2013.

Figure 4.4: *Neodactria* sp. monthly collections 2009-2013.
Figure 4. 5: *Microcrambus elegans* yearly collection 2009-2013.

Figure 4. 6: *Microcrambus elegans* monthly collection 2009-2013.
Figure 4. 7: *Fissicrambus mutabilis* yearly collection 2009-2013.

Figure 4. 8: *Fissicrambus mutabilis* monthly collection 2009-2013
Figure 4.9: *Pediasia trisecta* yearly collection 2009-2013

Figure 4.10: *Pediasia trisecta* monthly collection 2009-2013
Figure 4. 11: *Chrysoteuchia topiaria* - yearly collection 2009-2013

Figure 4. 12: *Chrysoteuchia topiaria* monthly collection 2009 - 2013
Figure 4. 13: *Crambus agitatellus* yearly collection 2009-2013

Figure 4. 14: *Crambus agitatellus* monthly collection 2009-2013
Figure 4. 15: *Agriphila vulgivagella* yearly collection 2009-2013

![Yearly Collection Chart](chart1.png)

Figure 4. 16: *Agriphila vulgivagella* monthly collection 2009-2013

![Monthly Collection Chart](chart2.png)
Figure 4. 17: *Agriphila ruricolella* yearly collection 2009-2013

Figure 4. 18: *Agriphila ruricolella* monthly collection 2009-2013
Figure 4. 19: *Urola nivala* yearly collection 2009-2013

Figure 4. 20: *Urola nivala* monthly collection 2009-2013
Figure 4. 21: *Crambus laqueatellus* yearly collection 2009-2013

Figure 4. 22: *Crambus laqueatellus* monthly collection 2009-2013
Figure 4. 23: *Crambus praefectellus* yearly collection 2009-2013

Figure 4. 24: *Crambus praefectellus* monthly collection 2009-2013
Figure 4. 25: *Crambus albellus* collection data 2009-2013

Figure 4. 26: *Euchromius ocellus* monthly flight data 2012.
Table 4.4 lists the first dates of emergence as well as the range spanning earliest and latest dates for each species over the course of four or five years. *C. praefectellus* does not have sufficient data to allow for analysis. Data for May 2009 is currently unavailable thus the dates for species with first emergence occurring during or near this time have been omitted. This left *A. ruricolella* and *A. vulgivagella*. *A. ruricolella* and *A. vulgivagella* emerge most consistently with only a 2-day (former) and a 7-day range (latter). *P. teterrella* also had a fairly consistent first emergence with only a 5 day range. *M. elegans* had a limited 8-day range. Some species had moderate variation such as *U. nivala* (10 days), *C. agitatellus* (13 days), *C. laqueatellus* (12 days), and *F. mutabilis* (15 days). *P. trisecta* (20-days), *C. topiaria* (21-days), and the *Neodactria sp.* (32 days) had the most variability.

There does not appear to be any consistency in emergence ranges with univoltine or bivoltine species.
<table>
<thead>
<tr>
<th>Species</th>
<th>Date of First Emergence</th>
<th>Emerge Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2009</td>
<td>2010</td>
</tr>
<tr>
<td><em>Neodactria sp.</em></td>
<td>-</td>
<td>31-May</td>
</tr>
<tr>
<td><em>Parapediasia teterrella</em></td>
<td>-</td>
<td>25-May</td>
</tr>
<tr>
<td><em>Pediasia trisecta</em></td>
<td>-</td>
<td>21-May</td>
</tr>
<tr>
<td><em>Urola nivalis</em></td>
<td>-</td>
<td>31-May</td>
</tr>
<tr>
<td><em>Chrysoteuchia topiaria</em></td>
<td>-</td>
<td>14-Jun</td>
</tr>
<tr>
<td><em>Crambus agitatellus</em></td>
<td>-</td>
<td>1-Jun</td>
</tr>
<tr>
<td><em>Agriphila ruricoella</em></td>
<td>30-Aug</td>
<td>30-Aug</td>
</tr>
<tr>
<td><em>Agriphila vulgivagella</em></td>
<td>6-Sep</td>
<td>13-Sep</td>
</tr>
<tr>
<td><em>Crambus laqueatellus</em></td>
<td>-</td>
<td>12-May</td>
</tr>
<tr>
<td><em>Crambus praefectellus</em></td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td><em>Microcrambus elegans</em></td>
<td>-</td>
<td>1-Jun</td>
</tr>
<tr>
<td><em>Fissicrambus mutabilis</em></td>
<td>-</td>
<td>28-May</td>
</tr>
</tbody>
</table>

Table 4. 4: First dates of light trap capture for each sod webworm species 2009 – 2013.

4.4 Discussion

With the appearance of the new species *E. ocellus* in 2012, and with no return the following year, it is difficult to make any definitive conclusions about its habits based on the one season worth of data. The weather conditions for these years were drastically different and it is possible this played a factor in its appearance and then absence. The
highest month for collection was August with a drop in September and slight increase in October. This suggests a univoltine cycle in central Ohio. However, as other Lepidoptera are known to migrate into this region this could be an indication of a migratory species which ventured further north than its traditional range due to exceptionally dry conditions in the 2012 season rather than an established species undergoing its natural life cycle. More information is required to make a final determination.

My results are fairly consistent with those of Niemczyk et al. (2000) with the few exceptions noted above.
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