EVALUATING THE OCURRENCE, SEASONAL HISTORY, SPECIES COMPOSITION AND IMPACT OF *PHYLLOPHAGA* AND *CYCLOCEPHALA* GRUBS INFESTING BERMUDAGRASS (*CYNODON* SPP.) IN OKLAHOMA

By

JOSEPH P. DOSKOCIL

Master of Science in Entomology

Oklahoma State University

Stillwater, Oklahoma

2007

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 2007

EVALUATING THE OCURRENCE, SEASONAL HISTORY, SPECIES COMPOSITION AND IMPACT OF *PHYLLOPHAGA* AND *CYCLOCEPHALA* GRUBS INFESTING BERMUDAGRASS (*CYNODON* SPP.) IN OKLAHOMA

Thesis Approved:

Tom A. Royer

Nathan R. Walker

Greg Bell

James A. Reinert

A. Gordon Emslie

Dean of the Graduate College

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

In the United States, turfgrass occupies an estimated 10 million hectares and the area continues to increase with population growth (Waddington, 1992). Turfgrass maintenance was a $25 billion industry for professional lawn turfgrass management and homeowner lawn care in the mid 90's (Gibb et al., 1995). The turfgrass industry in Oklahoma is the third largest agricultural commodity behind wheat and cattle production with $86 million spent on turfgrass maintenance a year (Hartnell, 1978).

A number of arthropod pests damage turfgrasses. They include foliar chewing insects such as immature Lepidoptera (caterpillars), piercing sucking insects such as Hemiptera (true bugs), and root feeding insects such as immature Scarabaeidae (white grubs) (Watschke et al., 1995; Vittum et al., 1999). *Phyllophaga* is a large genus of beetles in the family Scarabaeidae with more than 150 known species in North America. Collectively *Phyllophaga* spp. are known as May or June beetles because the adults typically fly during these two months of the year. Adults are polyphagous, feeding on shade and forest trees. Oak trees are preferred by many species but hickory, walnut, elm, and birch are also common hosts. Some adult species also feed on grass blades and on a diverse number of forbs (Luginbill and Painter, 1953).

Much less is known about the feeding habits of *Phyllophaga* larvae, but at least 25 species are known to feed on turfgrasses in the United States (Luginbill and Painter,

1953). Larvae of *Phyllophaga* feed primarily on living roots and other underground plant parts and supplement their diet with organic matter (Ritcher, 1966; Crocker et al., 1995).

Relatively few studies have been conducted on the biology and damage potential of the known turf-infesting *Phyllophaga* species. Published treatment recommendations for white grubs are based on general observations and generally not confirmed by field studies (Crutchfield and Potter, 1995). In fact, there is little published data upon which economic levels for white grubs in ornamental turfgrass can be based (Potter, 1982). Industry and extension recommendations for economic thresholds for white grubs vary from 1 (Bowen, 1980), 2-5 (Bruneau, 1993), to 8-10 (Baxendale and Gaussoin, 1992) per 0.1 m^2. Research has evaluated the damage potential of Japanese beetle, *Popillia japonica* (Newman), and southern masked chafer, *Cyclocephala lurida* (Bland), in several cool season grasses (Crutchfield and Potter, 1995), but there is little data for white grubs and warm season turfgrasses.

The larvae of *Cyclocephala* spp., also called masked chafers, are widely considered to be the most injurious pests in turfgrass across the United States (Potter, 1998). Mature *Phyllophaga* larvae are two to three times larger than mature C*yclocephala* larvae; hence they are potentially capable of causing more damage per individual grub. The damage potential of *Phyllophaga* spp. and *Cyclocephala* spp. for bermudagrass in Oklahoma has not been assessed and new data concerning this problem is needed to develop effective thresholds for their management.

If damage thresholds were determined, then the damaging species of *Phyllophaga* would need to be identified and their life histories characterized. It is difficult to identify

Phyllophaga adults and larvae to species. The adults may be recognized by external characteristics, but the morphology of the genitalia is the most accurate basis for identification (Luginbill and Painter, 1953). Identification of larvae is based on morphological characters, such as teeth patterns on the mandibles, sensory spots on the terminal antennal segment, size of the thoracic and abdominal spiracles, and setal patterns on the head and terminal abdominal segment (Ritcher, 1966). An alternative method for larval identification is to rear the larvae to adulthood, which can be tedious, time consuming and an inconsistent process. A more reliable method for larval identification is needed that allows for reliable characterization of the population of damaging species.

In the past decade, great strides have been made in the area of DNA extraction and sequencing. These tools offer a method to identify species that are difficult to resolve with morphology-based identification systems. Emmerson and Wallis, (1995) used mitochondrial cytochrome oxidase II (COII) to determine the phylogenetic relationships in *Prodontria* spp., a genus of beetles closely related to *Phyllophaga*, thus demonstrating that phylogenetic analysis can be used to identify adult scarab beetles to species. More recently Herbert et al. (2003) proposed that the mitochondrial gene cytochrome *c* oxidase I (COI) can serve as a "barcode system" for a global bio-identification system. These techniques could provide a method for identifying difficult genera such as *Phyllophaga* that is more reliable than using morphologically inconsistent characters.

Objectives

The objectives of this research were:

1. Determine the species composition and seasonal occurrence of adult *Phyllophaga* spp. associated with turfgrass environments in Oklahoma.
2. Determine the damage potential of *Phyllophaga* and *Cyclocephala* larvae on bermudagrass.
3. Determine those species of larval *Phyllophaga* which infest turfgrass in Oklahoma.

CHAPTER II

REVIEW OF LITERATURE

Of all the plants, grasses have been of greatest use to the human race (Hitchcock, 1950). To the grasses belong the family Gramineae, which contain cereal grains, sugarcane, and the bamboos. Grasses not only furnish the principal source of nutrition to the world's human population and domesticated animals but are also used extensively for ornamental and environmental applications such as in parks and gardens (Vittum et al., 1999).

Bermudagrass

Cynodon dactylon (L.) and *Cynodon transvaalensis* (L.), are native to tropical, semi-tropical regions of Southeast Africa (Taliaferro et al., 2004). The tropical race has pan-tropical distribution. Plants are short in stature, <20 cm, and produce a loose turf. They are adapted to the leached, acid soils common to the tropics and to the seasonal extremes of high and low rainfall that produce intermediate periods of water saturation to drought conditions, respectfully. The temperate and tropical races are similar in appearance, differing most dramatically in adaptation characteristics. The temperate race is much more cold tolerant and is found in cooler climates. Plants typically form a more dense turf and are less tolerant of seasonally water saturated soils or soils low in pH or fertility. They are also more susceptible to disease (Taliaferro et al., 2004).

Cynodon dactylon is commonly referred to as bermudagrass because it was first introduced into the United States from the Bermuda Islands by Henry Ellis in 1751. It was established in Savannah, GA and quickly became one of the most important grass species in the Southern United States (Burton, 1951). Now it is widely distributed in the southern half of the United States from Virginia to Florida and westward to Arizona and California (Stefferud, 1948). Bermudagrass is economically important because of its distribution, prevalence, and multiple uses. This genus is widely variable, ranging from small, fine-textured hybrid and improved lines used for turf, to large, robust forms that produce significant biomass and are used for forage production (USDA, 2000).

Since its introduction into Oklahoma, bermudagrass has gained recognition as an asset to the region both agriculturally, economically, and for conservation purposes (Elder, 1953). A survey of the turfgrass industry in Oklahoma in 1976, estimated that the maintenance and establishment cost of all turfgrasses in the state was over $86 million. A conservative estimate of the value of turfgrass in the state of Oklahoma was made at over one billion dollars making turfgrass the third largest agricultural commodity in the state behind wheat and cattle respectively (Hartnell, 1978).

Bermudagrass as Turf

Many cultivars of fine-textured bermudagrasses, either *C. dactylon* or hybrids of *C. dactylon* x *C. transvaalensis*, are grown on lawns and used for athletic applications throughout tropical and warm temperate regions of the world. In such regions, turf type bermudagrass is a foundation of the turfgrass industry. The development of turf-type bermudagrass cultivars began by the early 1900s, but its incidental use for this purpose

probably began much earlier. During the early 1900s, bermudagrass seed from Australia and Arizona was widely planted for turf in the southern United States (Taliaferro et al., 2004).

A formal breeding program for turf type bermudagrass was initiated in 1946 by researchers in the USDA-ARS Coastal Plains Experiment Station, Tifton, GA. This early breeding program led to the development of clonally propagated triploid F_1 hybrid cultivars of crosses between *C. dactylon* x *C. transvaalensis*. In 1956 and 1960, two hybrid lines 'Tifgreen' and 'Tifway', were developed and released, respectively and quickly became industry standards for turf type bermudagrass (G.W. Burton, 1991). Each cultivar was selected based on visual quality which integrates density, texture, uniformity, color, growth habitat, and smoothness (Martin et al., 2001). Since then, bermudagrass with superior turf characteristics have been collected by turf enthusiast in South Africa and have led to the development of numerous commercial cultivars, including 'Bradley', 'Magennis, and 'Royal Cape' among others (Taliaferro et al., 2004).

Insect Pests of Turfgrasses

The turfgrass environment provides a suitable habitat for numerous vertebrate and invertebrate animals that can be beneficial, damaging or have no effect on the turfgrass. The turfgrass ecosystem consists of perennial plant cover with distinct zones or regions that are occupied by various organisms. Pests in the upper or foliar zone of turfgrass are often conspicuous and fairly easy to control because of their exposure. Foliar chewing insect pests of bermudagrass include armyworms and cutworms (Lepidoptera: Noctuidae) and webworms (Lepidoptera: Pyralidae). Other types of foliar pests cause damage with

sucking or rasping mouth parts and include; mites (Acari), thrips (Thysanoptera), aphids (Hemiptera: Aphidoidea), and mealybugs (Hemiptera: Pseudococcidae).

Pests in the stem/thatch zone can evade detection until the damage they cause begins to appear. The thatch layer is preferred by sap feeding arthropods including chinch bugs (Hemiptera: Lygaeidae), spittlebugs (Hemiptera: Cercopidae), mealybugs (Hemiptera: Pseudococcidae), and scales (Hemiptera) and several families of chewing Lepidoptera larvae. Arthropods in the soil zone can evade detection until their damage to turf has resulted in significant injury (Watschke et al., 1995; Vittum et al., 1999).

Insects that inhabit the soil zone are some of the most devastating pests because they can damage roots, crowns, rhizomes and stolons. Plants can tolerate light to moderate damage to these structures but when significant damage occurs the plant often dies. White grubs (Coleoptera: Scarabaeidae), billbugs (Coleoptera: Curculionoidea), mole crickets (Orthoptera: Gryllotalpidae), and ground pearls (Hemiptera: Margarodidae) are the most common and damaging pests in this group. White grubs can consume much of the root system and as a result the turf can easily be lifted or removed (Watschke et al., 1995; Vittum et al., 1999) and are important pests of both cool-season and warm-season turfgrasses throughout the United States (Vittum et al., 1999).

The White Grub Complex

The white grub complex includes beetles in the genera *Ataenius*, *Continis*, *Cyclocephala*, *Euetheola*, *Exomala*, *Maladera*, *Phyllophaga*, *Popillia*, and *Rhizotrogus*. The more destructive native genera include *Phyllophaga* spp. (May/June beetles) (Luginbill and Painter, 1953), *Cyclocephala* spp. (masked chafers) (Potter, 1981),

Continis nitida (Linnaeus) (Green June beetles) (Hellman, 1995), *Ataenius spretulus* (Haldeman) (black turfgrass ataenius) (Niemczyk and Wegner, 1979), and *Euetheola rugiceps* (LeConte) (sugarcane beetle) can be found across most of North America. *Popillia japonica* (Newman) (Japanese beetle) was introduced from Japan and is found primarily east of the Mississippi River (Fleming, 1972); however, it continues to spread to states west of the river. *Rhizotrogus majalis* (Razoumowsky) (European chafer) introduced from Europe (Gambrell et al., 1942), *Maladera castanea* (Arrow) (Asiatic garden beetle) introduced from Japan (Hallock, 1938), *Anomala oreintalis* (Waterhouse) (Oriental beetle) introduced from Japan, are at present restricted to the Northeastern United States (Britton, 1925).

Northern masked chafers are common pests of cool-season turfgrasses from New England west to Illinois, where as southern masked chafers are typically pests of turfgrasses in transition zones and in southern bermudagrass areas from southern Pennsylvania west to Nebraska and south (Potter, 1981). In Kansas several species were collected including; *C. borealis* Arrow, *C. lurida* Bland, *C. hirta* LeConte, *C. longula* LeConte, *C. melanocephala* F., and *C. pasadenae* Casey (Bauernfeind, 2001). In a survey of *Phyllophaga spp.* from Stillwater, OK in 2004, *C. lurida* was the only species identified (Royer and Walker, unpublished data).

White grubs are perennial pests of all cool-season and warm-season turfgrasses. Larvae of *Phyllophaga* and *Cyclocephala* eat organic matter and the roots of plants. The first symptoms of damage often appear as drought stress and are the result of a pruned root system. Heavily infested areas first appear off-color or gray green then wilt rapidly if soil moisture is limited. Tunneling by the larvae cause the turf to feel spongy

underfoot and continued feeding can cause turf to die in large, irregular shaped patches. Populations may not be high enough to cause observable injury but predatory mammals such as skunks, raccoons, possums, armadillos, moles and birds can often cause collateral damage to the turfgrass stand as they search for larvae (Vittum et al., 1999).

Intensely managed turfgrasses, like those on golf courses and lawns, are favored by mated females of *Phyllophaga* and *Cyclocephala* because of the stable environment they provide. Lawns and golf courses are ideal locations for oviposition because they provide dense vegetation and adequate soil moisture which prevent egg and larval desiccation. Turfgrasses also provide an ideal environment for developing larvae with a plentiful food source consisting of roots and organic matter (Ritcher, 1966).

White Grub Damage Thresholds

There is considerable inconsistency regarding threshold densities at which white grubs cause economic damage to turfgrass. Most information on damage thresholds are based on general observations not supported by field studies (Crutchfield and Potter, 1995). In fact, there is little published data upon which economic levels of white grubs in ornamental turfgrass can be based (Potter, 1982). The limited available data are inconsistent, observing different combinations of turfgrass damaged by different genera of white grubs. Densities of 11 or more *P. japonica* larvae per 0.1 m^2 were noted to cause significant damage to drought stressed turf (Fleming, 1962). Tashiro (1987) suggested that economic thresholds for *Cyclocephala* spp. may be significantly higher than for other white grubs of similar size because the former are believed to feed proportionately more on decaying organic matter. Neiswander (1938) however observed

that 11-16 larvae per 0.1 m^2 of *C. borealis* caused severe damage to Ohio lawns. Industry and extension recommendations for economic thresholds of white grubs widely vary from 1 to 10 per 0.1 m^2 (Bowen, 1980; Bruneau, 1993; Baxendale and Gaussoin, 1992).

Control of White Grubs

Cultural practices can impact white grub population densities (Potter et al., 1996). For example, withholding irrigation and raising mowing height during peak flights of beetles can reduce the severity of grub infestations (Potter et al., 1996). White grub eggs require moist soil to hatch and eggs and young larvae are very susceptible to desiccation. Another approach involves fertilization and irrigation to encourage growth of turfgrass that permits the plants to compensate for damaged roots through new root growth (Potter et al., 1996).

Several natural predators, parasites, and diseases have been identified that influence white grub populations. Endoparasitic tachinid flies (Diptera: Tachinidae) have been found in 0.5% of specimens of *P. anxia* (Poprawski, 1994), and parasitic wasps, specifically *Myzine* spp. and *Tiphia* spp, (Hymenoptera: Tiphiidae), commonly parasitize white grubs (Richer, 1940; Rogers and Potter, 2004). These parasitoids were shown to effectively reduce white grub populations in certain areas of the eastern United States. However, it may take two to three years for adequate population reductions to occur and the wasps do not discriminate between healthy grubs and those which are already infected by nematodes or disease. This trait can create a potential for interference between biological control agents (Rogers and Potter, 2004).

Several strains of the bacterium, *Bacillus popilliae* (Dutky), *Pseudomonas aeruginosa* (Schroeter), *Serratia marcescens* (Bizio), *Micrococcus nigrofasciens* (Northrup), and *Bacillus cereus* (Frankland and Frankland), have been found to infect white grubs (Poprawski and Yule, 1990). The bacterium is acquired by feeding, and multiplies in the hemolymph causing the body fluids to turn milky white before grub death, hence the name "Milky Disease"(Wheeler, 1946). The influence of milky disease is considered minor. Only 7.5% of grubs force fed bacteria died of milky disease in laboratory studies (Poprawski and Yule, 1990). The same study conducted over a four-year period, showed that only 3% of grubs examined in the field died from bacterial infections.

Entomopathogenic nematodes in the genera *Steinernema* and *Heterorabtitis* have been shown to be effective against white grubs. Field trials generally resulted in less than 50% control, although *Heterorhabditis heliothidis* (Khan, Brooks, Hirschmann) strains have achieved control of 80% or better (Waschke et al., 1995). Studies comparing the performance of the chemical insecticides to strains of *Steinernema kushidai* (Mamiya) showed that this nematode provided better control of Japanese beetle in field tests over an extended period due to the nematode's persistence in the soil (Koppenhoefer et al., 2000). At present, available strains of nematodes are not effective from one season to the next against *Phyllophaga* and *Cyclocephala* species. This may be due to the relatively high defecation rate, which could expel nematodes, anaerobic hindguts, and sieve plates over the spiracular opening, which make penetration difficult and they may remove nematodes by grooming (Villani et al., 1999).

Since white grub problems are typically sporadic, preventative applications of pesticides for control of anticipated populations are not recommended but may be warranted in areas where perennial infestations occur. Registered insecticides are effective at killing 75-90% of the target population (Zenger and Gibb, 2001). Timing of application is critical for maximum grub control. Ideally, pesticide should be applied shortly after beetle flights, when eggs have hatched and grubs are small and actively feeding so the majority of the target population is exposed to the active ingredient (Zenger and Gibb, 2001). If a preventative application cannot be made in May or June, a curative application can be applied in September if populations reach damaging levels. Third instar larvae are more difficult to kill because their body weight is 70-80 times more than newly hatched larvae and this may account for why fall and spring time applications of insecticides are often ineffective (Waschke et al., 1995).

Phyllophaga

Twenty-five species of *Phyllophaga* have been found present in turfgrasses (Luginbill and Painter 1953). Identification of each to species is difficult and requires training, experience, and published keys (Watschke et al., 1995). Those species reportedly associated with turfgrasses east of the Rockie Mountains are *P. hirticula* (Koch), *P. crenulata* (Froelich), *P. tristis* (F.), and *P. ephilida* (Say) (Teetes et al., 1976). *Phyllophaga crinita* (Burmeister) is an important pest in Texas and Oklahoma (Teetes et al, 1976), and can be also found from Louisiana to Georgia (Watschke et al., 1995). Also found in Texas are *P. congrua* (LeConte), *P. crassissima* (Blanchard), *P. rubiginosa* (LeConte), and *P. hirtiventris* (Horn) (Crocker et al., 1999). *P. latifrons* (LeConte) is

found commonly in Florida and only a few species like *P. anxia* (LeConte) and *P. fervida* (Fabricius) are found throughout North America (Watschke et al., 1995). In 2004, a preliminary study conducted in Stillwater, Oklahoma found *P. congrua, P. bipartita* (Horn), *P. crassissma, P. submucida* (LeConte), *P. hirtiventris, P. rubignosa, P. profunda* (Blanchard), *P. praetermissa* (Horn), *P. vehemens* (Horn), *P. crenulata, P. rugosa* (Melsheimer), *P. calceata* (LeConte), *P. sylvatica* (Sanderson), and *P. implicita* (Horn) in light traps. (Royer and Walker, unpublished data). A summary of all of the *Phyllophaga* species and their attraction to pheromones was published by Robbins et al., 2006.

All *Phyllophaga* species are holometabolous, which is characterized by an egg, three larval instars, a pupa, and an adult. The generation time for different species ranges from one to four years depending upon geographic location. Development is probably dependant upon soil temperature which varies with latitude and altitude. Some *Phyllophaga* spp. in the south develop in a single year (Rolston and Barlow, 1980), while the majority of *Phyllophaga* spp. take two years to develop (Fattig 1944; Miner 1952) at high altitudes in the mountains or in northern parts of the United States they can take up to 3 years to reach maturity (Kard and Hain, 1988).

The life-cycle begins with a reproductive flight triggered by certain environmental conditions, usually rainfall and increased soil temperature (Crocker et al., 1995). Soil temperatures could dictate vertical larval movement and development because of the poikilothermic nature of their metabolism (Villani and Wright, 1988). Larvae migrate deeper in the soil during the winter months to stay below the frost line and migrate closer to the soil surface in the spring as the soil temperatures increase and allow for increased

metabolism and feeding (Speers and Schmiege 1961; Kard and Hain 1988, 1990). The development of larvae, pupae, and adults before emergence are dependent upon soil temperature which dictates the pace of their metabolism. Adequate soil moisture is necessary for emergence and for the survival of eggs and first instar larvae once they hatch. Gaylor and Frankie (1979) demonstrated that both too little moisture and excessive moisture was less than ideal for oviposition of *P. crinita.* They suggested that the lack of adult flight activity during droughty conditions may be a behavioral mechanism which insures that few adults try to oviposit during unfavorable conditions.

Adults can emerge as early as April in Southern states and more typically in May to June in the rest of the United States. Hence the common name May and June bugs (Hammond, 1940; Ritcher, 1940). Males are the more active fliers and usually the dominant sex found in light trap samples (Frankie et al., 1973). A few species of *Phyllophaga* are active during the day, but the most do not appear before dusk (Ritcher, 1966). Feeding and reproduction occur throughout the nocturnal hours until dawn. Although adults are strongly attracted to light sources at night, most species hide under plant litter on the soil during the day (Crocker et al., 1995).

For many species, the male locates a receptive female by detecting a species-specific pheromone that she emits (Robbins et al., 2006). Mating can occur on plants, the surface of the ground, or in the soil. A mating pair generally remains coupled for a prolonged period and for some species the female feeds on foliage during copulation (Crocker et al., 1995). For root feeding species the female lays up to 50 eggs, 9 to 10 days after mating, individually packed into balls of soil over a 1-3 week period (Hammond, 1940; Ritcher, 1940). The egg, first two instars, and pupa are relatively short

in duration for most *Phyllophaga* species with the majority of the life-cycle spent as a third instar larva (Crocker et al., 1995).

Larvae of *Phyllophaga* feed primarily on living roots and other underground plant parts and supplement there diet with organic matter. Before pupating, a third instar larva ceases feeding and empties its gut, resulting in a shrunken raster (Crocker et al., 1995). *Phyllophaga* species with annual life-cycles pupate in February and March, while species with two, three, and four year life-cycles pupate in late summer and over-winter as adults in the soil (Watschke et al., 1995).

Larvae of *Phyllophaga* may be distinguished from other scarab larvae by several characteristics. The head does not have eye spots. The frons has a transverse pair of posterior frontal setae or a single posterior frontal seta on each side. The epipharynx has a distinct epizygum and zygum. The haptomerum has a group of six to 21 heli. Plegmatia are present and rather long. The haptolachus often has numerous microsensilla. Maxilla posses a row of ten or more rather short truncate teeth. The anal slit is V- or Y-shaped, with the stem of the Y being much shorter than the arms and the anal lobe is usually divided by a sagittal cleft, sometimes divided by a sagittal grove. The claws of the meta-thoracic legs are very small (Ritcher, 1966.) Full-grown third instar larvae range in length from 1.9 cm to 3.18 cm (¾ inch to 1 ¼ inches) (Hammond, 1940).

Adult *Phyllophaga* species are similar in general appearance. Their exoskeleton is pigmented in various shades of brown, support different amounts of body pubescence from heavily pubescent to nearly glabrous. Body size range in length from 0.83 cm to 2.54 cm (1/3 inch to 1 inch) and body shape is parallel sided to oval (Luginbill and Painter, 1953). Characteristics for classification of adults include the morphology of their

clypeus, antenna, tibial spurs and tarsal claws. Although these morphological characteristics can be used to determine the identity of the adult species, the internal genitalia are the most significant characteristic, particularly that of the male (Crocker et al., 1995). The most detailed key for the identification of adult *Phyllophaga* to species is found in "May beetles of the United States and Canada" (Luginbill and Painter, 1953).

Phylogenetics

The current morphology-based systems for identification rely on the expertise of a dwindling population of highly skilled taxonomists. According to Hebert et al. (2003), morphology-based systems exhibit four flaws: (1) phenotypic and genetic variability in characters used for species recognition can lead to incorrect identification, (2) morphologically cryptic taxa are often over looked, (3) most morphological keys are effective for only a particular life-stage or gender, in which many individuals or life stages cannot be identified and (4) the use of keys demands a lofty level of expertise which often results in misidentification when used by a novice. These problems inherent in morphological taxonomy are enough to merit a different approach to systematic identification.

Mitochondrial cytochrome oxidase II (COII) was used to determine the phylogenetic relationships and species identification in *Prodontria,* a genus of beetles closely related to *Phyllophaga* (Emerson and Wallis, 1995). Additional studies have shown that the mitochondrial gene cytochrome *c* oxidase I (COI) can serve as a core for a global bio-identification system. In this system, DNA sequences of the COI gene are used as "barcodes" for taxonomic identification (Hebert et al., 2003). Major flaws in the

rationale and methodology the bar-coding life concept have been pointed out since its inception. A major criticism was that abandonment of morphological techniques in favor of a limited molecular system would impede the progress of understanding biodiversity (Will and Rubinoff, 2004). While the sole use of the COI gene for identification is insufficient, when it is coupled with morphologically based systems it may adequately fill some of the short comings of such a system (Will and Rubinoff, 2004). This has been successfully accomplished with *Philodytes umbrinus* (Motschulsky), a predacious water beetle, where COI was used to link an unknown larval specimen to an adult specimen which was identified using morphological characteristics (Miller et al., 2005). This same method can be applied to the identification of other genera such as *Phyllophaga* where a life-stage is very difficult to identify using traditional morphological characters.

CHAPTER III

MATERIALS AND METHODS

Seasonal occurrence and composition of *Phyllophaga* spp. associated with turfgrass dominated environments in Oklahoma

A single universal black light trap with a 12 watt U-shaped bulb, powered by AC 120v 60Hz current (Bio Quip, Rancho Dominguez, CA) was placed 1.2 m above the ground near a fairway at each of seven golf courses across Oklahoma (Figure 1) during the spring and summer of 2005. Each trap contained a 40-gm strip of Insect Guard™ (Professional Pest Control Products, Columbus, GA) insecticide (active ingredient 18% dichlorvos, 2, 2-dichlorovinyl dimethyl phosphate) used to kill trapped insects. During the spring and summer of 2006, eight locations were selected for trapping with the majority of them being duplicates from 2005 (Figure 1, Table 1). GPS coordinates for all eight locations were recorded with a portable GPS navigator (Garmin International, Olathe, KS) (Table 1). Cooperators were asked to operate traps one night per week until the first *Phyllophaga* beetles were observed; thereafter traps were operated three nights each week. Traps were operated from 28 March 2005 to 17 July 2005 and 23 March 2006 to 1 September 2006. Cooperators were asked to collect the contents of the traps the following morning, place it in a plastic bag and store in a refrigerator or freezer until samples could be gathered for subsequent identification. *Phyllophaga* beetles were

identified to species (Luginbill and Painter 1953) and all other Scarabaeidae collected were identified to genus using museum specimens from the K. C. Emerson Entomology Museum at Oklahoma State University (Department of Entomology and Plant Pathology, Stillwater, OK). Identification of beetles was confirmed by Don Arnold, museum curator.

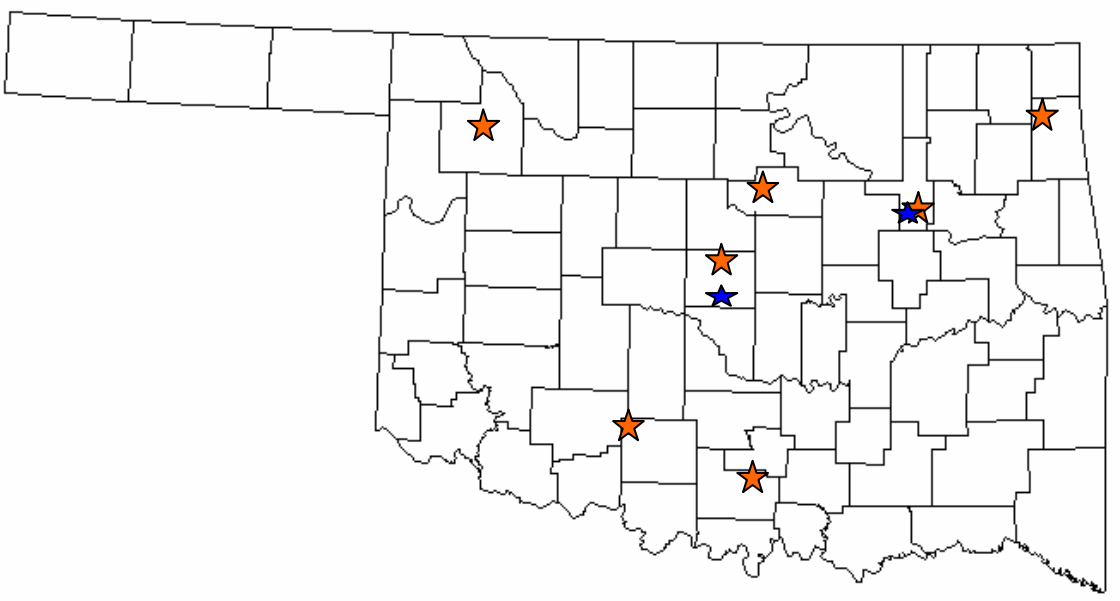

Figure 1. Map of black light trapping locations used to catch *Phyllophaga* spp. adults**.** Orange stars denote the location of golf courses where traps were placed in 2005. Blue stars denote additional locations monitored in 2006.

Table 1. Black light locations for trapping of *Phyllophaga* spp. adults with the corresponding, GPS coordinates, elevation, and date of trap initiation in 2005 and 2006.

Location	Coordinates	Elevation	Trap Initiation 2005	2006
Ardmore, OK	N 36 34.097 W 094 50.972	244.75 m	28 March 2005	23 March 2006
Duncan, OK	N 36 01.672 W 095 59.419	193.5 m	28 March 2005	23 March 2006
North Oklahoma City, OK	N 36 10.808 W 097 03.895	302.9 m	05 April 2005	23 March 2006
South Oklahoma City, OK	N 35 35.526 W 097 36.058	339.85 m	N/A	23 March 2006
Woodward OK	N 35 28.143 W 097 34.839	372.1 m	11 April 2005	30 March 2006
Stillwater, OK	N 34 13.012 W 097 08.181	262.7 m	12 April 2005	10 April 2006
Jenks, OK	N 34 30.458 W 098 03.633	332.8 m	12 April 2005	06 April 2006
Afton, OK	N 36 27.111 W 099 18.672	565.7 m	09 April 2005	06 April 2006

Determination of *Phyllophaga* and *Cyclocephala* damage thresholds

2005 Plots

Plots (1 m^2) at the Oklahoma State University Plant Pathology Research Farm in Stillwater, OK were used to evaluate the impact of different white grub larvae populations on U-3 bermudagrass. In 2005, the established U-3 (common) bermudagrass was removed from each plot using a sod cutter (Ryan, Johnson Creek, WI). Nine micro-plots, consisting of polyvinylchloride (PVC) pipe (25.4 cm diameter x 25.4 cm deep) were inserted into the clay soil base of each plot and filled with a sand soil mix. A 5 cm portion of the PVC pipe remained above the soil surface to prevent migration of the larvae. A piece of the U-3 bermudagrass sod approximately 15 cm x 15 cm was placed inside each micro-plot. The entire stand, 6.1 m x 12.2 m, was initially fertilized with 0.45 kg (1 pound) with a single application of 12-2-24 (NPK). Subsequent fertilization of the stand occurred every two weeks with 0.225 kg (0.5 pounds), 10-2-08 (NPK) Nature Safe (Griffin Industries, Cold Spring, KY) fine grade, and micro-plots were mowed using a weed eater every two weeks for the following three months. The stand was watered twice daily for the first week and then every other day for the remainder of the study.

On 11 August 2005, micro-plots were infested with either *Phyllophaga* spp., *Cyclocephala* spp. or a combination of both (Table 2). Each replication consisted of two adjacent 1 m^2 plots containing a total of 17 micro-plots. Within each replication, two non-infested micro-plots, one in each 1 m^2 plot, served as a control treatment. A completely randomized block design with five replications was used.

Table 2. Infestation rates of *Phyllophaga* spp. or *Cyclocephala* spp. larvae in 2005 to determine damage thresholds. Each row is an infestation rate for each individual micro-plot.

Number of larvae/plot	
Phyllophaga	*Cyclocephala*
0	3
0	6
0	9
1	0
1	3
1	6
1	9
2	0
2	3
2	6
2	9
3	0
3	3
3	6
3	9

On 9 August 2005, cores were taken from each micro-plot with a 5.71 cm soil probe to preliminarily evaluate turfgrass root weight and length. Loose soil was shaken vigorously from the roots and crown and the remaining soil was washed away under running water. Average root length was recorded to the nearest 1 mm and the roots and stolons were removed from the crown, dried at 50^{o}C for 24 h, and weighed to 1 mg.

On 9 August 2005, 238 *Phyllophaga* spp. and 78 *Cyclocephala* spp. larvae were collected from the Turf Research Center Oklahoma State University in Stillwater, OK. A sod cutter was used to cut the sod and exposed larvae were collected by hand from the soil. Each *Phyllophaga* larva was placed in an individual cell of a plastic 3.81 cm x 3.81 cm x 7.62 cm seed germination carton well and covered with soil. *Cyclocephala* larvae

were placed three per cell because of their smaller size and less tendency for cannibalism. The larvae were held overnight in a cool chamber at the Noble Research Center at Oklahoma State University and their identity was confirmed to genus. On 10 August 2005, 300 additional *Cyclocephala* spp. larvae were collected from Cimarron Trails Golf Course in Perkins, OK, and held over night in a cool chamber. Sites for larval collection were selected for their close proximity to the research plots and based on knowledge of previous infestations of white grubs. On 11 August 2005, the first two replications were infested in the morning and the third infested in early afternoon. To infest plots, the contents of the carton wells were separated using a sieve and the larvae were placed on the surface of bermudagrass in each respective micro-plot. After ten minutes, if a larva was not successful in burrowing into the soil they were removed and replace with another larva (Crutchfield and Potter 1995). The last two replications were infested the following morning on 12 August 2005.

A wire mesh fence supported with T-posts was constructed around the plots to exclude predation of larva by mammals. Management of the micro-plots continued as described previously for the duration of the study. In addition, soil temperature, UV intensity, and air temperature were recorded from the Oklahoma Mesonet website and canopy temperature for each micro-plot was taken weekly starting 9 September 2005, until the conclusion of the study on 20 October 2005. Canopy temperatures were acquired using an infrared thermometer model 08406 (Cole Parmer,Vernon Hills, IL). The thermometer was held one meter above each micro-plot and allowed to equilibrate before recording. At the conclusion of the study, turfgrass quality was visually rated on a scale of 1 to 5: 1 = poor quality turfgrass and 5 = excellent, highly desirable turfgrass.

Core samples were taken from each micro-plot with a 5.71 cm (2.25 inch) soil probe to evaluate root length, root and stolon weights as previously described. Bermudagrass was removed and the sand/soil mix was excavated from each micro-plot to determine the number of living larvae present. Replications one through three were evaluated on 20 October 2005 and the last two replications were excavated and evaluated the following day. Data was subjected to ANOVA (SAS Institute, Cary, NC) and means separated using Duncan's Multiple Range Test to determine the interaction between micro-plot treatment and larval mortality.

2006 Plots

Micro-plots established in 2005 at the Oklahoma State University Plant Pathology Research Farm in Stillwater, OK were used in studies conducted in 2006. The plots were managed the same in 2006 as they were in 2005. However, the experimental design was altered so that populations of *Phyllophaga* spp. and *Cyclocephala* spp. larvae could be evaluated separately. Infestation rates for each treatment are provided in Table 3.

Table 3. Infestation rates of *Phyllophaga* spp. or *Cyclocephala* spp. larvae in 2006 to determine damage thresholds. Each row is an infestation rate for each individual micro-plot.

Number of larvae/plot	
Phyllophaga	*Cyclocephala*
0	3
0	6
0	12
0	18
2	0
4	0
6	0
8	0

On 24 August 2005, 125 *Phyllophaga* spp. and 225 *Cyclocephala* spp. larvae were collected from the Oklahoma State University Turf Research Center in Stillwater, OK. Each *Phyllophaga* larva was placed in an individual cell of a plastic 3.81 cm x 3.81 cm x 7.62 cm seed germination carton well and covered with soil. The cartons were then kept in an ice chest until transported to the lab at the Noble Research Center. In the lab, the ice chest lids remained partially open to allow air flow and held at room temperature. All plots were infested at twilight on the day they were collected. To infest plots, the contents of the carton wells were separated using a sieve and the larva were placed on the bermudagrass in each micro-plot. If a larva was not successful in burrowing into the soil after ten minutes, they were removed, and replace with another larva (Crutchfield and Potter, 1995).

After infestation, micro-plots were managed, rated and excavated as previously described. Replications one and two were evaluated and excavated on 20 October 2006

and the remaining three replications the following day. Within each replication an uninfested micro-plot served as the control treatment. The experiment was arranged in a completely randomized block design with five replications and data was analyzed as in 2005.

DNA based identification of *Phyllophaga* spp. larvae

For DNA analysis, adult *Phyllophaga* specimens were utilized from those collected with light traps from golf courses in 2005 and 2006. A minimum of five adult representatives, both male and female, were used for each of the 20 species collected in Oklahoma. Additional specimens of, *P. bipartita, P. corrosa* (LeConte), *P. crenulata, P.fusca* (Froelich), *P. implicata, P. hirticula, P. hornii* (Smith), *P. rugosa, P. tristis,* and *P. vehemens* were obtained from Dr. Robert Bauernfeind at Kansas State University, Manhattan, KS. Each beetle was identified to species using keys provided by Luginbill and Painter (1953), then placed individually into a small plastic bag and stored at -15º C until use.

Prior to DNA extraction, each beetle was thawed and any foreign insect, mite or plant material was removed from the specimen using a fine paint brush and forceps. After cleaning, half of a leg from the pro- or meso-thorax was excised using sterile scissors and forceps and placed in a 2 mL eppendorf tube. Liquid nitrogen was poured into the eppendorf tube, immediately allowed to volatilize, and 150 µL of polyethylene glycol 200 (Chomczynski and Rymaszewski, 2006) was added to the tube. The sample was ground using a plastic disposable pestle and placed in a thermal vortexer (Eppendorf, Westbury, NY) at 1000 RPM, 72 ˚C, for 30 minutes.

The primers MelHCO (5'-AACWTTATACTTYCTMTTYGG-3') and MelLCO (5'-AAAAAATCARAATAGRTGTTGG-3') modified from Folmer et al. (1994) were used to amplify the cytochrome *c* oxidase subunit 1 (COI) region. DNA was amplified in 25 micro-liter PCR reactions that consisted of 12 µL Green GoTaq (Promega Corporation, Madison, WI), 7 µL nuclease free water, 3 µL MelHCO, 2 µL MelLCO, and 1 µL genomic DNA template. Unequal amounts of each primer were used due to the greater degeneracy of MelHCO. Amplification was performed on a PTC-200 thermocycler (MJ Research, Waltham, MA) using an initial denaturation step of 4 minutes at 95 ˚C, followed by 40 cycles of 45 sec at 94˚C, 45 sec at 39.7˚C, ramping 1˚C/sec to 72˚C, 1 minute at 72˚C, and terminating with a final 7 minutes extension at 72˚C. Amplified products were confirmed visually by 1% agarose sodium borate gel electrophoresis (Brody and Kern, 2004) run at 200 volts for 15 minutes. DNA on the gel was stained with 0.2 µg/ml ethidium bromide and visualized with ultraviolet light. Confirmed amplified products were purified using an Ultra Clean PCR Clean-up kit (Mo Bio, Carlsbad, CA).

The purified PCR products were submitted for automated sequencing at the Recombinant DNA/Protein Resource Facility at Oklahoma State University on an ABI Model 3700 DNA Analyzer (Applied Biosystems, Foster City, CA) using both forward and reverse primers separately. Sequence contigs for each specimen were assembled using Chromas Pro (Technelysium Pty., Queensland, Australia) and manually adjusted. Variable termini were trimmed resulting in final sequences of 633 base pairs. All sequences will be deposited in GenBank (Appendix 2). GenBank is an annotated collection of all publicly available nucleotide sequences and their protein translations.

This database is produced at National Center for Biotechnology Information (NCBI) as part of the International Nucleotide Sequence Database Collaboration. Multiple sequence alignment and phylogenetic analyses were conducted using Clustal X version 1.83 (Thompson et al., 1997) and a single, neighbor-joining tree (positions with gaps excluded) was constructed using *Ataenius imbricatus* (Melsheimer) as an out group to root the resulting tree. Consensus sequences were generated using BioEdit© (Ibis Therapeutics, Carlsbad, CA).

Immature *Phyllophaga* spp. were collected from sod farms and golf courses from nine different locations through out Oklahoma (Table 4). Specimens were stored individually in containers, the head capsule of each individual measured, and stored at -15˚C.

Table 4. Collection sites for larval *Phyllophaga* used for species determination through DNA sequencing of COI.

City	Coordinates		Elevation
Bixby, OK	N 35 57.394	W 095 51.591	181.9 m
Claremore, OK	N 36 22.476	W 095 40.150	177.1 m
Durant, OK	N 33 44.095	W 096 22.409	173.1 m
Elk City, OK	N 35 25.067	W 099 20.221	606.2 m
Haskell, OK	N 35 47.369	W 095 37.030	167.0 m
Oklahoma City, OK	N 35 28.079	W 097 34.511	364.8 m
Stillwater, OK	N 36 07.222	W 097 06.128	269.1 m
Woodward, OK	N 36 27.111	W 099 18.672	565.7 m
Yale, OK	N 36 04.324	W 096 42.308	248.1 m

Prior to DNA extraction, each larva was thawed and any soil or foreign material was removed using a fine paint brush and forceps. The quantity of tissue collected for analysis from each specimen varied according to instar. A single leg from third instar larvae, two legs from second instar larvae, and the entire head capsule from first instar larval specimens were excised using sterile scissors and forceps. The DNA was

extracted, amplified, confirmed, and purified as previously described. Larval DNA was sequenced as previously described; however, only the forward primer MelLCO was used.

The nucleotide sequence was manually adjusted for each specimen using Chromas Pro® (Technelysium Pty., Ltd., Queensland, Australia), and the length of most sequences trimmed to 561 base pairs. Larvae were identified using Clustal X version 1.83 (Thompson et al., 1997) to align larval sequences with species-specific consensus sequences derived from identified adults, resulting in a neighbor-joining tree. Larvae grouping with adult sequences with bootstrap support (>50) were tentatively identified as the same species as the adult.

CHAPTER IV

RESULTS

Species Composition

The first capture of beetles in 2005 occurred on 14 April at Stillwater, OK with the majority of locations having captures of *Phyllophaga* spp. the following week. Twelve species of *Phyllophaga* were identified from the 2,709 adults collected across the state (Table 5). The five most frequently caught species were; *P. crassissima*> *P. glabricula* > *P. crinita*> *P. praetermissa* and > *P. congrua*. Regionally, the predominant species were *P. glabricula* in the west and northeast, *P. crassissima* in the central and *P. crinita* in central and south Oklahoma. *Cyclocephala* spp. and *Polyphylla* spp. were other scarab genera commonly found in the traps.

Table 5. 2005 black light trap data, the total number of adult individuals from each species of *Phyllophaga* captured in a location, the totals for each species, and total number of beetles by location.

Phyllophaga species	Afton	Broken Arrow	Stillwater (East)	Stillwater (West)	Oklahoma City	Ardmore	Duncan	Woodward	**Totals**
bipartita	0	0	30	7	0	0	0	0	37
congrua	0	0	0	15	17	127	0	0	159
crassissima	168	22	427	12	130	211	0	18	988
crenulata	0	0	0	2	1	0	0	1	4
crinita	0	0	0	0	183	326	17	0	526
ephilida	50	0	0	0	0	0	0	0	50
glabricula	636	0	34	0	42	0	0	33	745
hirtiventris	0	0	7	0	4	0	0	0	11
implicita	0	0	0	0	0	0	1	1	2
praetermissa	0	0	173	0	0	0	0	0	173
profunda	0	0	0	1	0	0	0	0	1
rubiginosa	0	0	5	3	0	1	4	0	13
Total # of Beetles	854	22	676	40	377	665	22	53	2709

In 2006, the first beetles were captured on 7 April in Duncan, OK with the majority of locations recording captures of *Phyllophaga* spp. the following week. Twenty species of *Phyllophaga* were identified from the 6,277 adults collected (Table 6). All 12 species collected in 2005 were also collected in 2006 along with eight additional species. The five species that comprised the majority collected in 2006 were; *P. crinita* > *P. submucida* > *P. crassissima* > *P. congrua* and > *P. torta*. Regionally, the predominant species were *P. crassissima* in the northeast, *P. submucida* in the west and northeast, *P. crinita* in central and south, and *P. torta* and *P. congrua* in south Oklahoma. Other scarab genera collected were *Cyclocephala* spp., *Polyphylla* spp., and *Pelidnota* spp.

Table 6. 2006 black light trap data, the total number of adult individuals of *Cyclocephela*, *Polyphylla*, and *Pelidnota* and each species of *Phyllophaga* captured in a location. The totals for each genus, *Phyllophaga* species, and total number of beetles by location.

Phyllophaga Species	Afton	Jenks	Stillwater (East)	Oklahoma City (North)	Oklahoma City (South)	Ardmore	Duncan	Woodward	**Total**
affabilis	0	0	1	0	2	0	0	2	5
bipartita	0	6	14	7	7	0	7	0	41
calceata	0	0	0	0	0	0	27	0	27
congrua	28	2	3	6	9	553	2	0	603
corrosa	0	0	0	0	0	0	5	1	6
crassissima	198	210	70	83	174	99	61	8	903
crenulate	0	1	0	0	0	0	0	1	2
crinita	0	0	0	246	1397	1152	375	0	3170
ephilida	62	12	0	0	0	0	0	3	77
futilis	0	0	0	0	0	0	0	1	1
glabricula	0	0	0	34	0	0	0	0	34
hirtiventris	0	1	2	0	0	0	2	0	5
implicita	0	0	0	0	0	0	1	1	2
praetermissa	0	0	33	0	0	0	0	0	33
profunda	0	0	1	0	0	3	6	0	10
prunina	0	0	0	0	0	0	4	0	4
rubiginosa	0	0	0	0	2	18	41	0	61
submucida	401	105	101	0	0	0	230	234	1071
sylvatica	0	1	0	0	0	0	1	0	2
torta	0	0	0	0	51	0	169	0	220
Total # of Beetles	**689**	**338**	**225**	**376**	**1642**	**1825**	**931**	**251**	**6277**
Cyclocephela Species	49	332	154	74	395	118	332	197	1651
Polyphylla Species	0	127	0	0	0	0	0	553	680
Pelidnota Species	0	0	0	0	0	0	0	52	52

Thirteen species of *Phyllophaga* had flight periods that occurred in May or June. Seven species were collected from July thru September. In both years, distinct flight periods were evident for individual species (Figure 2 and 3). Captures of *P. congrua, P.crassissima, P. crinita, P. submucida,* and *P. torta* occurred over a three month period in the years that they were collected.

The results of this study suggest that each species of *Phyllophaga* emerges and flies during distinct periods. Some species such as; *P. corrosa, P. glabricula, P. hirtiventris, P. prunina,* and *P. praetermissa* flew over a 2- or 3- week period suggesting that these species may have specific conditions for emergence. Other species; *P. congrua, P. crassissima, P. crinita, P. submucida,* and *P. torta* flew during a three month period, a longer flight period, suggesting that these species are less sensitive to environmental factors.

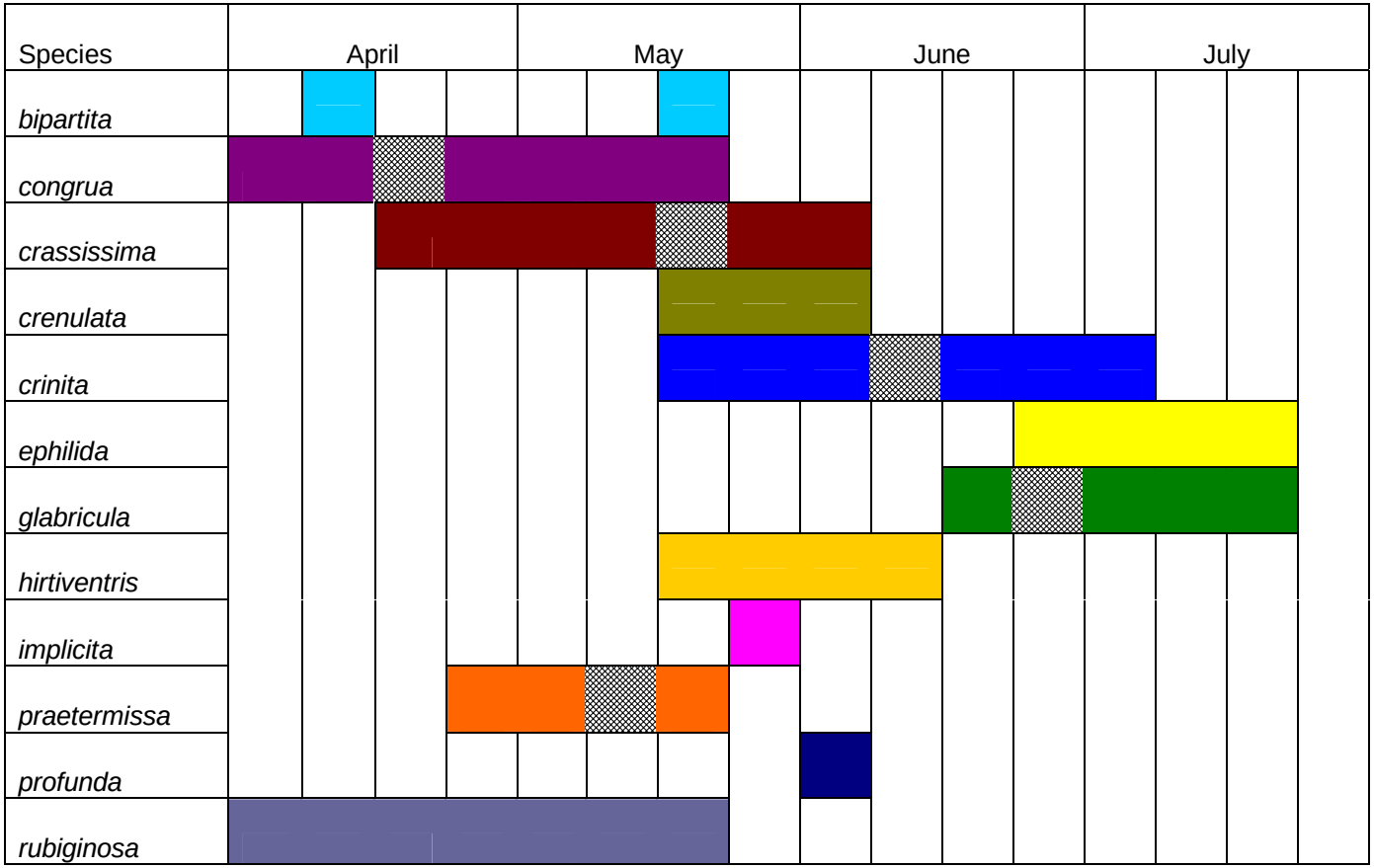

Figure 2. 2005 flight period for adult *Phyllophaga* spp. collected from the seven black light trap locations across Oklahoma. (The checkered areas denote the peak flight of adults for the five most abundant species.)

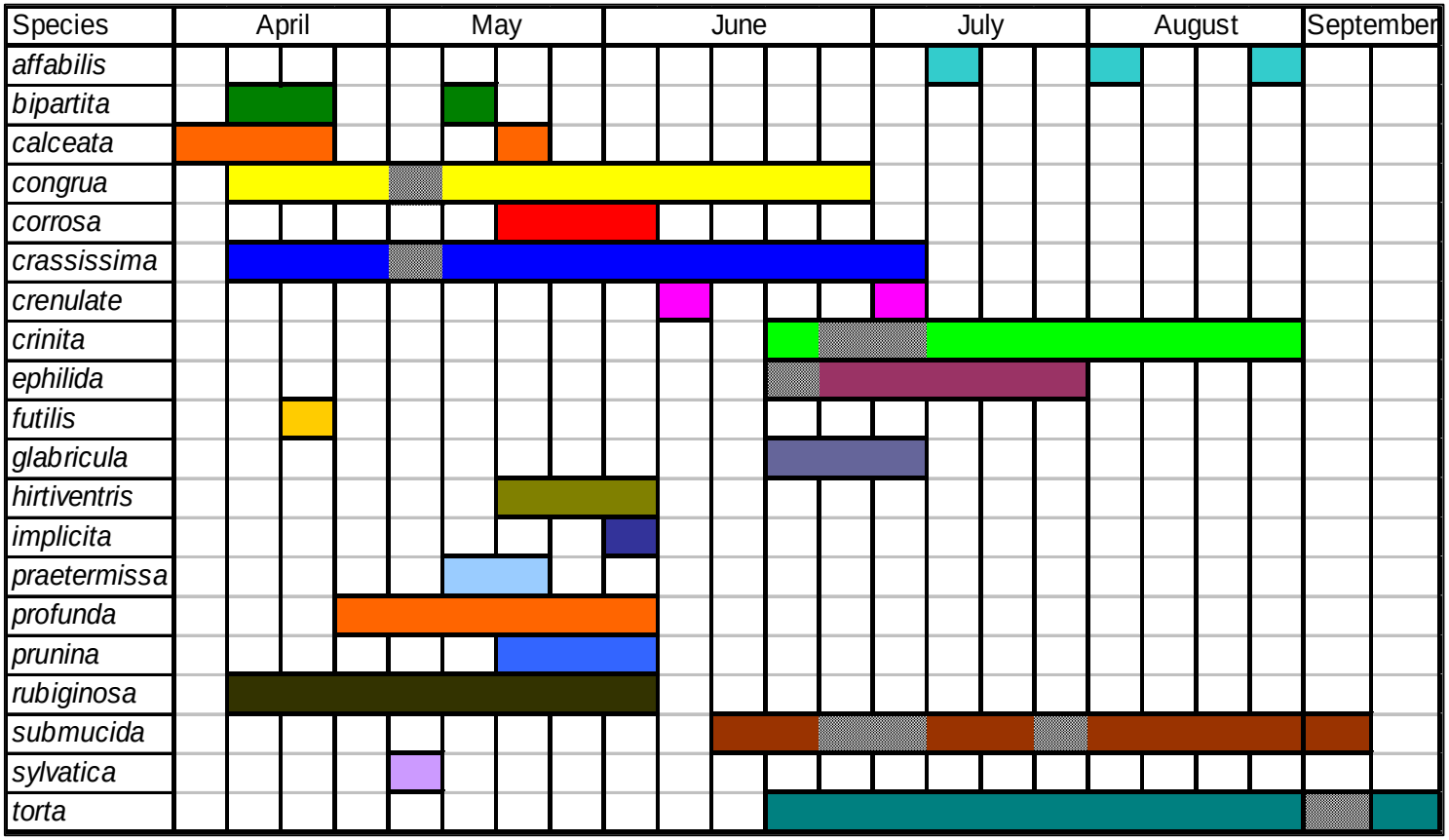

Figure 3. 2006 flight period for adult *Phyllophaga* spp. collected from eight black light trap locations across Oklahoma. (The checkered areas denote the peak flight of adults for the six most abundant species.)

Determination of *Phyllophaga* and *Cyclocephala* Damage Thresholds

In 2005, root length and root and stolon weight data was collected prior to infestation and at the conclusion of the study (Appendix 1). No trends were found in the data set. The absence of *Phyllophaga* spp. and *Cyclocephala* spp. larvae at the conclusion of the study was high (Table 7). Presence of *Cyclocephala* spp. was higher in those plots infested with 6 or 9 *Cyclocephala* spp. with the omission of *Phyllophaga* spp. from the plot than in those plots infested with 3 C*yclocephala* spp. in the omission of *Phyllophaga* spp. ($P<0.0257$) (Table 8). This may be due to the omission of *Phyllophaga* spp. larvae which are considered more aggressive in nature and have a higher tendency for cannibalism. There was no significant treatment effect on the presence of *Phyllophaga* spp. ($P<0.0257$) (Table 8). The absence of larvae at the conclusion of the study in 2005 did not make it possible to establish a damage threshold.

Table 7. Total number of *Phyllophaga* spp. and *Cyclocephala* spp. larvae used for damage threshold studies in 2005 and 2006, larval presences and the respective absentee rates for each genus for each study.

2005 Threshold Study Absenteeism		
Infestation	*Phyllophaga* spp.	*Cyclocephala* spp.
Initial	120	375
Final	29	128
Absentee rate	0.76	0.66
2006 Threshold Study Absenteeism		
Infestation	*Phyllophaga* spp.	*Cyclocephala* spp.
Initial	100	195
Final	3	39
Absentee rate	0.97	0.8

Table 8. Statistical analysis of *Cyclocephala* spp. larval presence in the damage threshold micro-plots by treatment level in 2005 (five replications).

Treatment #	*Phyllophaga*	*Cyclocephala*	Mean
1	0	0	0.00 d[y]
2	0	3	0.40 cd
3	0	6	3.60 a
4	0	9	3.20 ab
6	1	3	1.20 abcd
7	1	6	1.80 abcd
8	1	9	3.00 ab
10	2	3	1.00 bcd
11	2	6	2.20 abcd
12	2	9	2.60 abc
14	3	3	1.20 abcd
15	3	6	2.40 abcd
16	3	9	3.00 ab
Duncan's	NS[x]	2.22	
Pr>F	P > 0.1319	P > 0.0257	

[x] NS = Not Significant

[y] Means followed by the same letter are not significantly different ($P \leq 0.05$) According to Duncan's Test.

With the high absentee rates of the originally designed threshold study in 2005, a different approach was taken in 2006. To reduce variability in results, the project design was simplified with the objective of determining at which density of *Phyllophaga* spp. and *Cyclocephala* spp. individually cause severe damage to bermudagrass.

In 2006, core samples were taken for the first two replications of the experiment prior to the excavation of the replication. Core samples were not taken from the remaining replications since few to none of the larvae were found in the micro-plots from

the first two replications. Instead the remaining replications were only excavated and the number of living larvae present determined.

More *Cyclocephala* spp. larvae were found in plots with treatments of 8 and 4 *Phyllophaga* spp. larvae than for all other treatments, except the treatment of 6 *Phyllophaga* spp. ($P<0.0001$) (Table 9). There was no significant treatment effect on the presence of *Phyllophaga* spp. ($P<0.0001$) (Table 9). Several instances occurred where larvae from one genus or the other was found in a micro-plot to which it was not introduced. This was most common with *Cyclocephala* spp. and less common for *Phyllophaga* spp. larvae. Larval displacement was observed in 2.2% of the micro-plots in the 2005 study and was more frequent in 2006 at 35.5%.

Table 9. Statistical analysis of *Cyclocephala* spp. larval presence in the damage threshold micro-plots by treatment level in 2006 (five replications).

Treatment #	*Phyllophaga*	*Cyclocephala*	Mean
1	0	0	0.00 c[y]
2	2	0	0.00 c
3	4	0	2.60 a
4	6	0	1.60 ab
5	8	0	2.80 a
6	0	3	0.20 bc
7	0	6	0.20 bc
8	0	12	0.20 bc
9	0	18	0.20 bc
Duncan's	NS[x]	5.96	
Pr>F	P > 0.3625	P > 0.0001	

[x] NS = Not Significant

[y] Means followed by the same letter are not significantly different ($P \leq 0.05$) According to Duncan's Test.

Phyllophaga Larval Identification

The number of adults per species that yielded high quality sequences varied and was dependant upon their relative abundance (Table 10). *Ataenius* spp. was used as the out-group for the adult phylogenetic tree. This genus of beetle was chosen as the out-group because it is native to Oklahoma and it is common in turfgrass. Phylogenetic analyses distinguished all species into well supported clades using a neighbor joining tree analysis with the exception of *P. glabricula* and *P. submucida* (Figure 4). These two species, while significantly different morphologically as adults, were not genetically diverse enough at the COI loci to distinguish between them using a neighbor joining tree. Further analysis using a molecular clock function was capable of distinguishing *P. glabricula* from *P. submucida*. This type of analysis is more precise and looks at the proteins produced from the genetic code to determine if there is a difference between very similar sequences. Once each species was distinguishable, adult consensus sequences were generated for each using BioEdit© (Ibis Therapeutics, Carlsbad, CA).

Table 10. The total number of adult *Phyllophaga* specimens extracted and sequenced for each species.

Phyllophaga spp.	# extracted	# sequenced
P. affabilis	10	6
P. bipartita	20	7
P. calceata	15	10
P. congrua	17	8
P. corrosa	12	6
P. crassissima	10	6
P. crenulata	10	4
P. crinita	20	10
P. ephilida	14	12
P. fusca	9	3
P. futilis	10	1
P. glabricula	13	10
P. hirtiventris	10	6
P. implicita	10	5
P. praetarmissa	9	4
P. profunda	11	4
P. prunina	10	5
P. rubiginosa	21	12
P. submucida	20	10
P. torta	8	3
Total	259	132

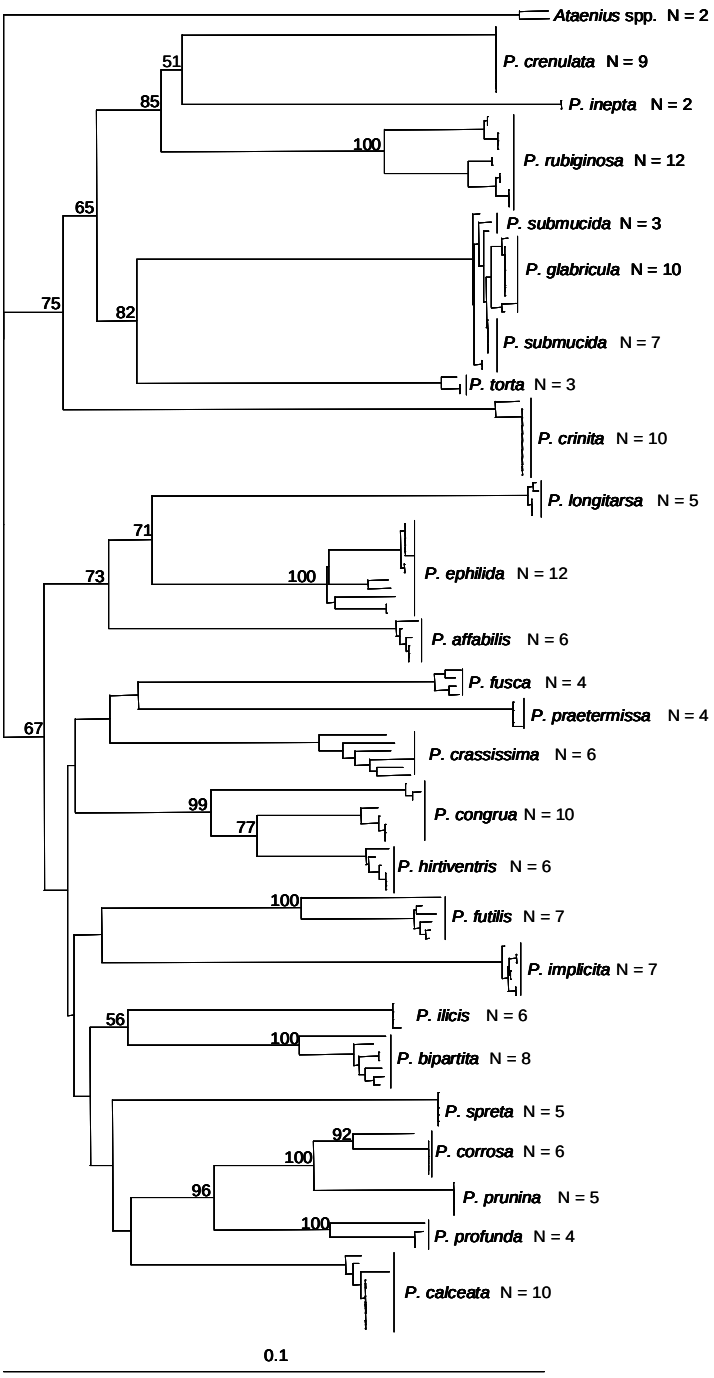

Figure 4. Bootstrapped neighbor joining tree of adult *Phyllophaga* COI sequences with nodes ≥ 50% supported. *Ataenius* spp. out group. N value is equal to the number of individuals sequenced from each species.

The number of larvae that yielded high quality sequences varied and was less than that of the adult beetles (Table 11). The COI DNA from 123 larvae was successfully sequenced and grouped into 10 unique groups (Figure 5). When larval DNA sequences were analyzed with the consensus sequences from the adult *Phyllophaga* spp., nine of the 10 larvae groups matched with adults (Figure 6). The one cluster which failed to group with any of the adult *Phyllophaga* species was comprised of unique larval samples collected from Woodward, OK. The larvae were submitted to the Oklahoma State University Plant Disease and Insect Diagnostic laboratory and were identified as *Strigoderma* spp.

Table 11. Total number of larval COI sequences which matched adult consensus sequence for each species. The percentage of each species represents from the total number of larval specimens collected.

Phyllophaga spp.	# sequenced	% of Grubs Collected
P. bipartita	17	13.8%
P. calceata	15	12.2%
P. congrua	18	14.6%
P. crassissima	25	20.3%
P. crinita	19	15.4%
P. ephilida	3	2.4%
P. submucida	11	8.9%
P. hirtiventris	9	7.3%
P. torta	1	0.8%
Strigoderma spp.	5	4.1%
Total	123	

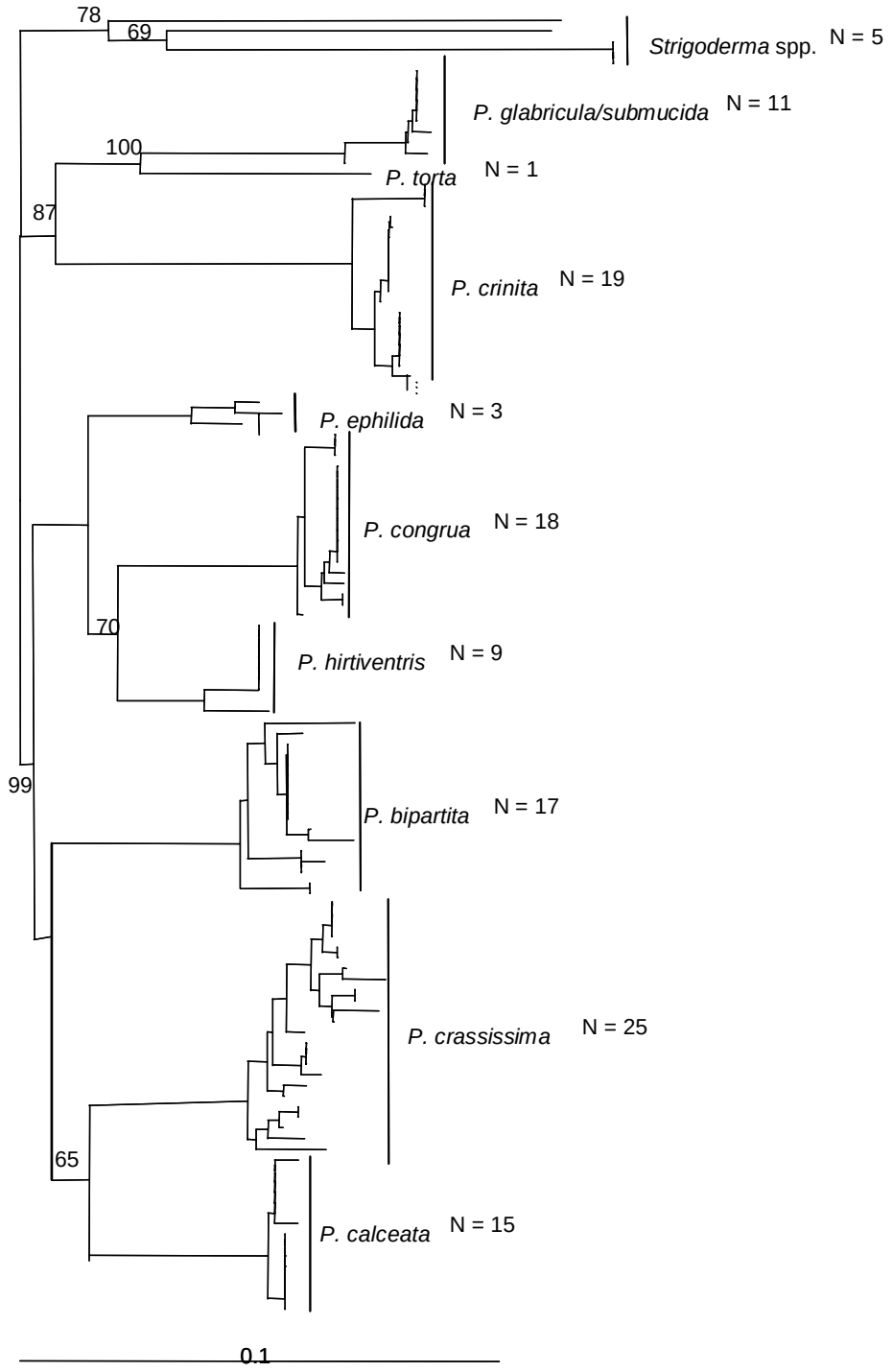

Figure 5. Bootstrapped neighbor joining tree of *Phyllophaga* larvae COI sequences with nodes ≥ 50% supported. *Strigoderma* spp. out group. N value is equal to the number of larvae found to match the sequence of the adult from that species.

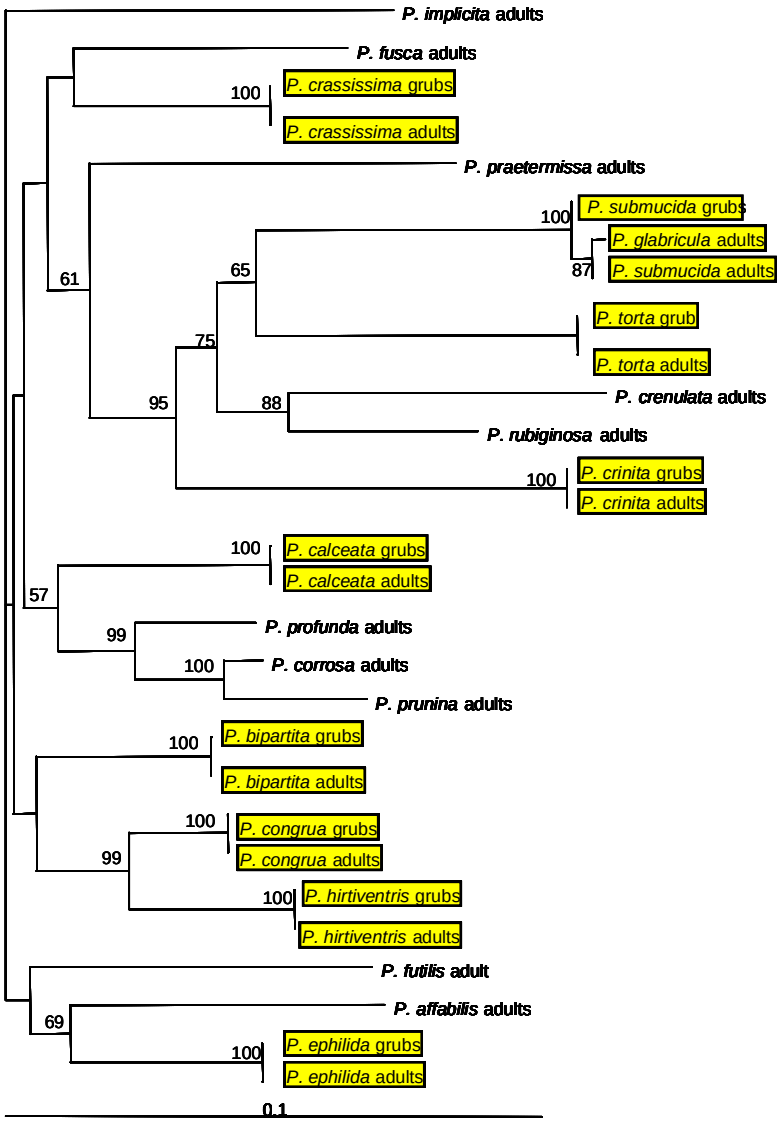

Figure 6. Neighbor joining tree of COI consensus sequences from *Phyllophaga* spp. adults and larvae with nodes ≥ 50% supported. Species that had larval sequences match adult sequences are highlighted.

Twenty species of adult *Phyllophaga* were collected in blacklight traps associated with golf courses, nine of which were positively identified as larvae present in turfgrass. Six of the nine species of larvae; *P. bipartita, P. calceata, P. crassissima, P. hirtiventris, P. submucida,* and *P. torta* have not previously been associated with turfgrass systems. In addition to the *Phyllophaga* spp., five specimens of *Strigoderma* spp., 4.1% of all larvae were collected and mistakenly identified as *Phyllophaga* spp. This mistake can easily be made as the raster pattern for these two genera are very similar. These larvae were used as the out-group for the larval tree.

Larval species presence by location are listed in Table 12. *P. crassissima* and *P. calceata* were the most widely distributed species and were found at all seven locations throughout Oklahoma (Table 12). *P. congrua, P. ephilida,* and *P. hirtiventris* were only found in the northeastern portion of Oklahoma (Table 12). Bixby, Claremore and Durant had the most diversity of species by location. Larval samples, in most cases, were collected from sod farms in the same region as the golf course where the black light trap was located. The variation in location is due to the ease of collecting larval specimens at sod farms where the grass was removed versus enticing golf course superintendents to cut up their fairways.

Table 12. *Phyllophaga* spp. with matching larval sequences and the larval species present in each larval collection location.

	Location								
Phyllophaga spp.	Bixby	Claremore	Durant	Elk City	Haskell	Oklahoma City	Stillwater	Woodward	Yale
P. bipartita	x		x				x		x
P. calceata	x		x			x		x	x
P. congrua	x	x					x		
P. crassissima	x	x	x	x			x	x	
P. crinita			x	x				x	
P. ephilida		x			x				
P. submucida	x		x						x
P. hirtiventris	x	x			x				
P. torta						x			

CHAPTER V

DISCUSSION

Damage thresholds for *Phyllophaga* spp. and *Cyclocephala* spp. in bermudagrass were not determined. The hardiness of the larvae was less than anticipated. Their absenteeism in 2005 may be attributed to the long delay between collection and infestation. Exposure to UV radiation and high temperatures during the infestation time period may also have reduced the over all fitness. Modifications were made in 2006 to the experimental design and infestation methods in an effort to reduce the exposure of larvae to the environment. However, an even higher incidence of absenteeism was observed for both genera. With such a high percentage of larvae not recovered in the 2006 study, the possibility exists that the larvae did not perish in the micro-plots but instead may have migrated from the plots.

A sand based soil mix was chosen for this study for its ease in excavation of the plots at the termination of the study. Sand is more abrasive than silt or clay soils and contains far less organic matter. It is known that *Phyllophaga* spp. and *Cyclocephala* spp. in general feed on decaying organic matter (Ritcher, 1966). The larvae may have found living conditions inadequate and since the micro-plots were not covered, they could have crawled out to find a more hospitable environment. Although larvae of *Phyllophaga* spp. and *Cyclocephala* spp. are not generally believed to be mobile like

there relative the Green June beetle, *Cotinis nitida* (Hellman, 1995), not enough is known about the larval behavior to determine if or how often surface movement occurs.

The results of the species composition study suggest that there is great variability among the emergence and subsequent egg deposition for *Phyllophaga* species in Oklahoma. This trait may make it challenging to properly time a single insecticide treatment for acceptable control of white grubs in turfgrass. The variability in flight activity could result in the common practice of applying insecticides by calendar date to be less effective. If the *Phyllophaga* species complex infesting a stand of turfgrass is diverse, the adults may not have flight activity in May or June which has come to characterize this genus. An insecticide application in May or June may be too late to control the larvae of beetles which have oviposited in April (*P. congrua, P. calceata* and *P. bipartita*) and may be too early to control larvae of beetles that oviposit in August and September (*P. submucida* and *P. torta*).

Knowing that a single well timed application of insecticide may not control all *Phyllophaga* spp. grubs, determining which species are inhabitants of turfgrass may be of the utmost importance. Although 20 different species of *Phyllophaga* were found in turfgrass environments, not all were found in turfgrass. The blacklight trap used for collection is a non-discriminate attractor of beetles and other insects within sight of it. Beetles could have flown from fields or natural habitats surrounding the golf course. This makes it imperative that larvae must be recovered and identified that reside in turfggrass.

Through phylogenetics, we were able to link the unknown turf-residing immature life-stage of *Phyllophaga* to its identifiable adult stage. The use of phylogenetic analysis

of the COI region of *Phyllophaga* as a tool for identification of a previously unidentifiable life-stage has provided a reliable, time and cost effective method to determine species. The process of rearing *Phyllophaga* larvae to adults for identification can take many months and usually is fraught with significant levels of natural mortality.

Once a species has been identified as turf inhabiting by larval identification of collected grubs, blacklight trapping should be sufficient to confirm the pest species presence in a given location. While larval samples did not confirm the presence of certain species like *P. crinita* at locations in Oklahoma City, adults of this species have been collected there in large numbers by black light trapping. *P. crinita* larvae are most likely to be feeding on turfgrasses in the Oklahoma City metropolitan area, since this species is confirmed as a turfgrass pest from other areas of Oklahoma and neighboring states.

The *Phyllophaga* species complex that affects Oklahoma turfgrasses has been further characterized as a result of this study. The identification of turf damaging larvae can be used to identify the flight times of the adult stage. With the additional pest species identified, the presence or absence of the adults could be confirmed by blacklight trapping. This knowledge may give the turfgrass industry the information needed to make proper and environmentally conscientious decisions regarding the use of insecticides. If an insecticide application is needed, proper timing of the application may result in more effective control of white grub turfgrass pests and provide for more environmentally sound pest management on turfgrass areas.

REFERENCES

Bauernfeind, R. J. 2001. Distribution of *Cyclocephala* spp. (Coleoptera: Scarabaeidae) in Kansas. Envirn. Entomol. 30: 899-902.

Baxendale, F.P. and R.E. Gaussoin (ed.). **1992.** Integrated management guide for Nebraska turfgrass. Univ. Nebraska. Bull. EC92-1557-S.

Bowen, W.E. (ed.). **1980.** Turfgrass Pests. Univ. California. Publ. 4053.

Britton, W.E. 1925. A new pest of lawns. Connecticut Agr. Exp. Stn. Bull. Immed. Inform. 52: 25-28.

Brody, J.R. and S.E. Kern. 2004. History and principles of conductive media for standard DNA electrophoresis. Analytical Biochemistry, 333: 1-13.

Bruneau, A.H. (ed.). **1993.** Turfgrass pest management manual. A guide to major turfgrass pests and turfgrasses. North Carolina Coop. Ext. Serv. Bull. AG-348.

Burton, G.W. 1951. Intra-and inter-specific hybrids in bermudagrass. J. Hered. 42:153-156.

Burton, G.W. 1991. A history of turf research at Tifton. Green Section Rec. 29 (3):12-14. U.S. Golf Assoc, Far Hills, NJ.

Chomczynski, P. and M. Rymaszewski. 2006. Alkaline polyethylene glycol-based method for direct PCR from bacteria, eukaryotic tissue samples, and whole blood. BioTechniques 40: 454-458.

Crocker, R.L., Nailon Jr., W.T., and J.A. Reinert. 1995. May and June beetles, pp. 72-75, *In:* Brandendurg, R.L. and M.G. Villani (eds), Handbook of Turfgrass Insect Pests. Entomol. Soc. Am. Lanham, MD.

Crocker, R.L., Nailon Jr., W.T., Matis, J.H., and Woodruff, R.E. 1999. Temporal pattern of ovipositional readiness in spring species of *Phyllophaga* (Coleoptera: Scarabaeidae) in North Central Texas. Ann. Entomol. Soc. Am. 92: 47-52.

Crutchfield, B.A. and D.A. Potter. 1995. Damage relationships of Japanese beetle and southern masked chafer (Coleoptera: Scarabaeidae) grubs in cool-season turfgrasses. J. Econ. Entomol. 88: 1049-1056.

Elder, W.C. 1953. Bermuda-grass (*Cynodon dactylon*). Oklahoma Agr. Exp. Stn. Forage Crop L-14.

Emerson, B.C. and G.P. Wallis. 1995. Phylogenetic relationships of the *Prodontria* (Coleoptera; Scarabaeidae; Subfamily Melolonthinae), derived from sequence variation in the mitochondrial cytochrome oxidase II gene. Mol. Phylogenetics Evol. 4:433-447.

Fattig, P. W. 1944. *Phyllophaga* or May beetles of Georgia. Emory Univ. Mississippi. Bull. 2: 1-32.

Fleming, W.E. 1962. The Japanese beetle in the United States. U.S. Dept. Agr. Handb. No. 236: 30 pp.

Fleming, W.E. 1972. Biology of the Japanese beetle. U.S. Dept. Agr. Tech. Bull. No. 1449: 129 pp.

Frankie, G.W., H.A. Turney, and P.J. Hamman. 1973. White grubs in Texas turfgrass. Texas Agr. Ext. Service L-1131: 3 pp.

Gambrell, F.L., S.C. Mendel, and E.H. Smith. 1942. A destructive European insect new to the United States. J. Econ. Entomol. 35: 289.

Gaylor, M.J. and G. W. Frankie. 1979. The relationship of rainfall to adult flight activity; and of soil moisture to oviposition behavior and egg and first instar survival in *Phyllophaga crinita*. Environ. Entomol. 8: 591-594.

Gibb, T.J., S. Kellam, F. Whitford, C.F. Sadof, and C.S. Throssell. 1995. Pesticide use and pest management practices by the Indiana lawn care industry – 1992. Purdue Agric. Res. Prog. Bull. 715.

Hallock, H.C. 1938. Notes on the biology and control of the Asiatic garden beetle. J. Econ. Entomol. 29: 348-356.

Hammond, G.H. 1940. White grubs and their control in eastern Canada. Dom. Canadian Dept. Agr. Pub. No. 668: 18 pp.

Hartnell, T. 1978. 1978 Oklahoma turfgrass survey. Oklahoma Agr. Exp. Stat. Misc. Pub. 105:1-16.

Hebert, P.D., Cywinska, A., Ball, S.L., and J.R. deWaard. 2003. Biological identifications through DNA barcodes. Proc. Royal Soc. London. 270; 313-321.

Hellmen, L. 1995. Green June beetle, pp. 57-59. *In:* Brandenburg, R.L. and M.G. Villani (eds), Handbook of turfgrass insect pests. Entomol. Soc. Am. Lanham, MD.

Hitchcock, A.S. 1950. Manual of the Grasses of the United States 2nd Ed. U.S. Gov. Printing Office, Washington D.C. 504 pp.

Kard, B. M. R. and F. P. Hain. 1988. Influence of ground covers on white grub (Coleoptera: Scarabaeidae) populations and their feeding damage to roots of Fraser fir Christmas trees in the Southern Appalachians. Environ. Entomol. 17: 63-66.

Kard, B. M. R. and F. P. Hain. 1990. Flight pattern and white grub population densities of three beetle species (Coleoptera: Scarabaeidae) in the mountains of northwest North Carolina. J. Entomol. Sci. 25: 34-43.

Koppenhoefer, A.M., Wilson, M., Brown, I., Kaya, H.K., and Gaugler. 2000. Biological control agents for white grubs (Coleoptera: Scarabaeidae) in anticipation of the establishment of the Japanese beetle in California. J. Econ. Entomol. 93: 71-80.

Luginbill Sr., P. and H.R. Painter. 1953. May beetles of the United States and Canada. U.S. Dep. Agric. Tech. Bul. No. 1060. Washington, D.C.

Martin, D.L., G.E. Bell, J.H. Baird, C.M. Taliaferro, N.A. Tisserat, R.M. Kuzmic, D.D. Dobson, and J.A. Anderson. 2001. Spring dead spot resistance and quality of seeded bermudagrasses under different mowing heights. Crop Sci. 41: 451-456.

Miller, K.B., Y. Alaries, G.W. Wolfe, and M.F. Whiting. 2005. Association of insect life stages using DNA sequences: the larvae of *Philodytes umbrinus* (Motschulsky) (Coleoptera: Dytiscidae). Systematic Entomol. 30: 499-509.

Miner, F.D. 1952. Biology of the prairie white grub *Phyllophaga crassissima*. Arkansas Agr. Exp. Stn. Bull. 521: 1-75.

Neiswander, C.R. 1938. The annual white grub, *Ochrosidia villosa* Burm., in Ohio lawns. J. Econ. Entomol. 31: 340-344.

Niemczyk, H.D., and G.S. Wegner. 1979. Life history and control of the black turfgrass ataenius. Ohio Rep. 64: 85-88.

Poprawski, T.J. 1994. Insect parasites and predators of *Phyllophaga anxia* (LeConte) (Col., Scarabaeidae) in Quebec, Canada. J. Appl. Entomol. 117: 1-9.

Poprawski, T.J. and W. N. Yule. 1990. Bacterial pathogens of *Phyllophaga* spp. (Col., Scarabaeidae) in southern Quebec, Canada. J. Appl. Entomol. 109: 414-422.

Potter, D.A. 1981. Biology and management of masked chafer bugs. Am. Lawn Appl. (July/Aug.): 2-6.

Potter, D.A. 1982. Influence of feeding by grubs of the southern masked chafer on quality and yield of kentucky bluegrass. J. Econ. Entomol. 75: 21-24.

Potter, D.A., Powell, A.J., Spicer, P.G., and D.W. Williams. 1996. Cultural practices affect root-feeding white grubs (Coleoptera: Scarabaeidae) in turfgrass. J. Econ. Entomol. 89: 156-164.

Potter, D.A. 1998. Destructive Turfgrass Insects. Biology, Diagnosis and Control. Ann Arbor Press, Chelsea, MI.

Ritcher, P.O. 1940. Kentucky white grubs. Kentucky Agr. Exp. Stn. Bull. 401: 71-157.

Ritcher, P.O. 1966. White Grubs and Their Allies. Oregon State Univ. Press. pp. 75-134.

Robbins, P.S., S.R. Alm, C.D. Armstrong, A.L. Averill, T.C. Baker, R.J. Baurenfiend, F.P. Baxendale, S.K. Braman, R.L. Brandenburg, D.B. Cash, G.J. Couch, R.S. Cowles, R.L. Crocker, Z.D. DeLamar, T.G. Dittl, S.M. Fitzpatrick, K.L. Flanders, T. Forgatsch, T.J. Gibb, B.D. Gill, D.O. Gilrein, C.S. Gorsuch, A.M. Hammond, P.D. Hastings, D.W. Held, P.R. Heller, R.T. Hiskes, J.L. Holliman, W.G. Hudson, M.G. Klein, V.L. Krischik, D.J. Lee, C.E. Linn, Jr., N.J. Luce, K.E. MacKenzie, C.M. Mannion, S. Polavarapu, D.A. Potter, W.L. Roelofs, B.M. Royals, G.A. Salsbury, N.M. Schiff, D.J. Shetlar, M. Skinner, B.L. Sparks, J.A. Sutschek, T.P. Sutschek, S.R. Swier, M.M. Sylvia, N.J. Vickers, P.J. Vittum, R. Weidman. D.C. Weber, R.C. Williamson, and M.G. Villani. 2006. Trapping *Phyllophaga* spp.

(Coleoptera: Scarabaeidae: Melolonthinae) in the United States and Canada using sex attractants. J. Insect Sci. Vol. 6, No. 39, pp. 1536-2442.

Rogers, M.E. and D.A. Potter. 2004. Biology of *Tiphia pygidialis* (Hymenoptera: Tiphiidae), a parasitoid of masked chafer (Coleoptera: Scarabaeidae) grubs, with notes on the seasonal occurrence of *Tiphia vernalis* in Kentucky. J. Environ. Entomol. 33: 520-527.

Rolston, L. H. and T. Barlow. 1980. Insecticide control of a white grub *Phyllophaga ephilida* Say (Coleoptera: Scarabaeidae), on Sweet Potato. J. Georgia. Entomol. Soc. 15: 445-449.

Speers, C. F. and D. C. Schmiege. 1961. White grubs in forest tree nurseries and plantations. US Dep. Agric., Forest Serv. Forest Pest Leafl. 63: 1-4.

Stefferud, A. 1948. Grass Year Book of Agriculture 1948. U.S. Gov. Printing Office. Washington D.C. pp. 663-664.

Taliaferro, C.M., Rouquette Jr., F.M. and P., Mislevy. 2004. Warm Season (C_4) Grasses, Bermudagrass and Stargrass. Agron. Monog. No. 45. Am. Soc. Agron., Madison, WI. pp. 1-59.

Tashiro, H. 1987. Turfgrass Insects of the United States and Canada. Cornell Univ. Press, Ithaca, NY.

Teetes, G.L., Wade, L.J., McIntyre, R.C., and C.A. Schaefer. 1976. Distribution and seasonal biology of *Phyllophaga crinitia* in the Texas high plains. J. Econ. Entomol. 69: 59-63.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. and D. G. Higgins. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24: 4876-4882.

U.S. Department of Agriculture. 2000. Bermudagrass *Cynodon dactylon* (L.) Pers. Plant Fact Sheet. U.S. Dep. Agric., Beltsville, MD.

Villani, M.G., and R.J. Wright. 1988. Use of radiography in behavioral studies of turfgrass-infesting scarab grub species (Coleoptera: Scarabaeidae) Bull. Entomol. Soc. Am. 34: 132-144.

Villani, M.G., L.L. Allee, A. Diaz, and P.R. Robbins. 1999. Adaptive strategies of edaphic arthropods. Annu. Rev. Entomol. 44: 233-256.

Vittum, P.J., Villani, M., and H. Tashiro. 1999. Turfgrass Insects of the United States and Canada. 2nd Edit. Cornell Univ. Press, Ithaca, NY.

Waddington, D.V., 1992. Turfgrass. American Society of Agronomy, Madison, WI.

Watschke, T.L., Dernoeden, P.H., and D.J. Shetlar. 1995. Advances in Turfgrass Science Managing Turfgrass Pests. Lewis Publ. Ann Arbor, MI. pp. 171-302.

Will, K.W. and D. Rubinoff. 2004. Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. Cladistics. 20: 47-55.

Zenger, J.T. and T.J. Gibb. 2001. Impact of four insecticides on Japanese beetle (Coleoptera: Scarabaeidae) egg predators and white grubs in turfgrass. J. Econ. Entomol. 94: 145-149.

APPENDIX I

Tables of white grub threshold data, organized by treatment, 2005.

Treatment
Phyl - 0 Cyclo - 0

	Collected		Visual Rating	Average (cm)			Grams			Grams		
Plot #	Phyllophaga	Cyclocephala	5-1	Root Length	Final	Difference	Root Weight	Final	Difference	Stolon Weight	Final	Difference
101	0	0	4	6.5	8.2	1.7	0.945	0.783	-0.162	1.888	0.702	-1.186
117	0	0	4	5.2	11.4	6.2	0.269	0.596	0.327	0.202	0.171	-0.031
201	0	0	4	10.5	7.7	-2.8	1.007	0.177	-0.830	0.663	1.260	0.597
212	0	0	4	6.9	10.9	4.0	0.938	0.617	-0.321	1.008	0.250	-0.758
303	0	0	3	10.8	7.9	-2.9	0.745	0.286	-0.459	1.054	0.259	-0.795
314	0	0	4	14.5	10.3	-4.2	0.482	0.408	-0.074	0.120	0.183	0.063
410	0	0	4	13.7	8.0	-5.7	0.440	0.381	-0.059	0.137	0.404	0.267
412	0	0	4	6.2	10.1	3.9	0.183	0.288	0.105	0.095	0.055	-0.040
509	0	0	4	8.1	15.1	7.0	0.674	0.199	-0.475	1.061	0.181	-0.880
516	0	0	3	7.9	10.1	2.2	0.236	0.246	0.010	0.363	0.341	-0.022

Treatment
Phyl - 1 Cyclo - 0

	Collected		Visual Rating	Average (cm)			Grams			Grams		
Plot #	Phyllophaga	Cyclocephala	5-1	Root Length	Final	Difference	Root Weight	Final	Difference	Stolon Weight	Final	Difference
118	0	0	4	9.6	9.8	0.2	0.771	0.307	-0.464	0.752	0.093	-0.659
210	0	0	4	10.6	9.4	-1.2	1.000	0.767	-0.233	1.174	0.505	-0.669
306	1	0	2	8.3	5.8	-2.5	0.251	0.219	-0.032	0.068	0.085	0.017
403	0	0	3	6.0	8.0	2.0	0.225	0.329	0.104	0.393	1.308	0.915
513	0	0	3	8.2	4.3	-3.9	0.904	0.132	-0.772	2.225	0.180	-2.045

Treatment

Phyl - 2 Cyclo - 0

	Collected		Visual Rating	Average (cm)			Grams			Grams		
Plot #	Phyllophaga	Cyclocephala	5-1	Root Length	Final	Difference	Root Weight	Final	Difference	Stolon Weight	Final	Difference
105	0	0	3	8.0	6.9	-1.1	0.372	0.372	0.000	0.511	0.196	-0.315
207	1	0	4	7.1	5.4	-1.7	0.174	0.198	0.024	0.057	0.027	-0.030
318	1	0	4	11.2	9.7	-1.5	0.601	0.734	0.133	5.320	0.382	-4.938
417	0	0	5	8.6	5.1	-3.5	0.780	0.170	-0.610	0.630	0.145	-0.485
518	0	0	4	10.4	11.9	1.5	0.265	0.435	0.170	0.490	0.189	-0.301

Treatment

Phyl - 3 Cyclo - 0

	Collected		Visual Rating	Average (cm)			Grams			Grams		
Plot #	Phyllophaga	Cyclocephala	5-1	Root Length	Final	Difference	Root Weight	Final	Difference	Stolon Weight	Final	Difference
107	0	0	3	6.0	12.1	6.1	0.454	0.210	-0.244	0.444	0.000	-0.444
208	1	0	3	10.0	3.5	-6.5	0.488	0.253	-0.235	0.328	0.000	-0.328
307	1	0	3	7.8	9.4	1.6	0.360	0.455	0.095	0.189	0.502	0.313
418	0	0	4	7.0	1.6	-5.4	0.342	0.027	-0.315	0.180	0.121	-0.059
501	0	0	3	8.5	9.2	0.7	0.562	0.353	-0.209	0.562	0.768	0.206

Treatment

Phyl - 1 Cyclo - 3

	Collected		Visual Rating	Average (cm)			Grams			Grams		
Plot #	Phyllophaga	Cyclocephala	5-1	Root Length	Final	Difference	Root Weight	Final	Difference	Stolon Weight	Final	Difference
109	0	0	5	5.0	5.8	0.8	0.658	0.454	-0.204	0.797	0.309	-0.488
205	1	2	4	6.2	8.6	2.4	0.368	0.475	0.107	0.212	0.634	0.422
309	1	2	4	6.4	12.1	5.7	1.040	0.501	-0.539	1.138	1.495	0.357
414	0	1	4	6.4	7.9	1.5	0.240	0.323	0.083	0.254	0.367	0.113
506	0	1	4	9.2	7.0	-2.2	0.294	0.155	-0.139	0.163	0.268	0.105

Treatment

Phyl - 1 Cyclo - 6

	Collected		Visual Rating	Average (cm)			Grams			Grams		
Plot #	Phyllophaga	Cyclocephala	5-1	Root Length	Final	Difference	Root Weight	Final	Difference	Stolon Weight	Final	Difference
108	0	0	3	6.0	4.4	-1.6	0.410	0.161	-0.249	0.467	0.164	-0.303
202	0	2	4	8.4	9.6	1.2	0.878	0.275	-0.603	0.480	0.020	-0.460
305	0	0	3	9.4	9.9	0.5	0.816	0.355	-0.461	0.551	0.662	0.111
415	0	4	4	6.5	4.5	-2.0	0.235	0.163	-0.072	0.259	0.000	-0.259
504	0	3	4	7.3	13.4	6.1	0.797	0.226	-0.571	1.106	0.193	-0.913

Treatment

Phyl - 1 Cyclo - 9

	Collected		Visual Rating	Average (cm)			Grams			Grams		
Plot #	Phyllophaga	Cyclocephala	5-1	Root Length	Final	Difference	Root Weight	Final	Difference	Stolon Weight	Final	Difference
114	0	4	4	7.4	6.9	-0.5	0.715	0.205	-0.510	0.474	0.285	-0.189
218	1	4	4	9.4	9.2	-0.2	0.914	0.285	-0.629	0.509	0.551	0.042
301	0	3	2	9.1	3.3	-5.8	1.096	0.361	-0.735	1.216	0.320	-0.896
407	0	3	3	9.9	7.1	-2.8	0.722	0.211	-0.511	0.282	0.102	-0.180
503	0	1	4	9.2	7.9	-1.3	0.166	0.289	0.123	0.084	0.020	-0.064

Treatment

Phyl - 2 Cyclo - 3

	Collected		Visual Rating	Average (cm)			Grams			Grams		
Plot #	Phyllophaga	Cyclocephala	5-1	Root Length	Final	Difference	Root Weight	Final	Difference	Stolon Weight	Final	Difference
111	0	1	5	10.0	7.4	-2.6	0.728	0.710	-0.018	0.934	0.153	-0.781
209	2	1	3	11.5	5.9	-5.6	0.504	0.262	-0.242	0.442	0.682	0.240
304	1	0	1	7.9	5.0	-2.9	0.069	1.352	1.283	0.032	0.034	0.002
401	0	1	3	7.3	10.0	2.7	0.226	0.199	-0.027	0.446	0.346	-0.100
517	0	2	4	10.0	12.5	2.5	0.345	0.227	-0.118	0.580	0.357	-0.223

Treatment

Phyl - 2 Cyclo - 6

	Collected		Visual Rating	Average (cm)			Grams			Grams		
Plot #	Phyllophaga	Cyclocephala	5-1	Root Length	Final	Difference	Root Weight	Final	Difference	Stolon Weight	Final	Difference
113	0	1	4	8.7	8.1	-0.6	0.255	0.562	0.307	0.077	0.260	0.183
214	0	2	3	8.1	7.4	-0.7	0.696	0.184	-0.512	1.181	0.083	-1.098
310	1	1	3	7.0	13.6	6.6	0.558	0.772	0.214	0.170	0.334	0.164
408	0	5	3	8.1	9.5	1.4	0.396	0.578	0.182	0.389	0.350	-0.039
505	0	2	4	10.5	11.0	0.5	0.577	0.108	-0.469	0.434	0.321	-0.113

Treatment

Phyl - 2 Cyclo - 9

	Collected		Visual Rating	Average (cm)			Grams			Grams		
Plot #	Phyllophaga	Cyclocephala	5-1	Root Length	Final	Difference	Root Weight	Final	Difference	Stolon Weight	Final	Difference
106	2	4	3	8.0	7.2	-0.8	0.620	0.437	-0.183	0.880	0.024	-0.856
215	1	2	4	9.0	6.5	-2.5	1.510	0.307	-1.203	0.628	0.646	0.018
313	0	2	2	8.8	15.4	6.6	0.515	0.870	0.355	0.683	0.503	-0.180
416	0	1	4	7.4	7.6	0.2	0.560	0.288	-0.272	0.186	0.320	0.134
510	0	4	4	8.9	6.8	-2.1	0.562	0.265	-0.297	0.730	0.020	-0.710

Treatment

Phyl - 3 Cyclo - 3

	Collected		Visual Rating	Average (cm)			Grams			Grams		
Plot #	Phyllophaga	Cyclocephala	5-1	Root Length	Final	Difference	Root Weight	Final	Difference	Stolon Weight	Final	Difference
110	2	2	3	7.0	5.6	-1.4	0.912	0.223	-0.689	1.486	0.197	-1.289
206	0	1	4	7.3	8.4	1.1	0.271	0.466	0.195	0.273	0.670	0.397
317	1	3	4	7.2	6.7	-0.5	0.534	0.226	-0.308	0.519	0.048	-0.471
402	1	0	3	8.6	6.8	-1.8	0.804	0.384	-0.420	1.013	1.066	0.053
507	0	0	4	8.0	8.4	0.4	0.276	0.570	0.294	0.141	0.030	-0.111

Treatment

Phyl - 3 Cyclo - 6

	Collected		Visual Rating	Average (cm)			Grams			Grams		
Plot #	Phyllophaga	Cyclocephala	5-1	Root Length	Final	Difference	Root Weight	Final	Difference	Stolon Weight	Final	Difference
103	3	4	2	7.5	7.3	-0.2	0.345	0.325	-0.020	0.564	0.250	-0.314
211	1	0	4	7.1	8.1	1.0	0.392	0.346	-0.046	0.984	0.027	-0.957
312	1	0	4	7.5	8.9	1.4	0.661	0.445	-0.216	0.249	0.417	0.168
405	0	8	4	7.9	8.4	0.5	0.339	0.311	-0.028	0.638	0.572	-0.066
511	0	0	4	8.1	6.0	-2.1	0.581	0.128	-0.453	0.580	0.090	-0.490

Treatment

Phyl - 3 Cyclo - 9

	Collected		Visual Rating	Average (cm)			Grams			Grams		
Plot #	Phyllophaga	Cyclocephala	5-1	Root Length	Final	Difference	Root Weight	Final	Difference	Stolon Weight	Final	Difference
112	2	6	2	6.5	6.3	-0.2	0.634	0.160	-0.474	0.660	0.109	-0.551
203	1	1	5	9.4	9.4	0.0	1.286	0.484	-0.802	1.192	1.005	-0.187
315	1	3	3	11.9	15.4	3.5	0.711	0.330	-0.381	0.280	0.189	-0.091
406	0	4	3	9.0	8.8	-0.2	1.231	0.248	-0.983	0.958	0.112	-0.846
515	0	1	4	9.6	11.9	2.3	0.733	0.299	-0.434	1.748	0.000	-1.748

Treatment

Phyl - 0 Cyclo - 3

	Collected		Visual Rating	Average (cm)			Grams			Grams		
Plot #	Phyllophaga	Cyclocephala	5-1	Root Length	Final	Difference	Root Weight	Final	Difference	Stolon Weight	Final	Difference
104	0	0	4	7.0	8.7	1.7	0.630	0.723	0.093	1.048	0.573	-0.475
213	0	0	4	6.9	10.4	3.5	0.545	0.504	-0.041	0.995	0.906	-0.089
302	0	0	3	4.6	5.6	1.0	0.233	0.366	0.133	0.182	1.046	0.864
409	0	1	3	8.0	10.1	2.1	0.260	0.304	0.044	0.606	0.247	-0.359
502	0	1	4	7.9	13.9	6.0	0.933	0.413	-0.520	0.577	0.372	-0.205

Treatment

Phyl - 0 Cyclo - 6

	Collected		Visual Rating	Average (cm)			Grams			Grams		
Plot #	Phyllophaga	Cyclocephala	5-1	Root Length	Final	Difference	Root Weight	Final	Difference	Stolon Weight	Final	Difference
116	0	3	3	13.9	11.6	-2.3	0.434	0.883	0.449	0.413	0.435	0.022
204	0	3	4	7.1	7.8	0.7	0.314	0.176	-0.138	0.268	0.318	0.050
311	0	2	4	8.0	10.7	2.7	0.742	0.374	-0.368	1.079	0.526	-0.553
413	0	6	4	6.9	9.8	2.9	0.429	0.364	-0.065	0.226	0.524	0.298
512	0	4	4	7.2	4.4	-2.8	0.285	0.139	-0.146	0.240	0.091	-0.149

Treatment

Phyl - 0 Cyclo - 9

	Collected		Visual Rating	Average (cm)			Grams			Grams		
Plot #	Phyllophaga	Cyclocephala	5-1	Root Length	Final	Difference	Root Weight	Final	Difference	Stolon Weight	Final	Difference
115	0	5	5	8.6	6.3	-2.3	2.195	0.311	-1.884	2.311	0.193	-2.118
217	0	0	4	8.2	11.9	3.7	0.636	0.160	-0.476	0.357	0.078	-0.279
316	0	1	4	8.3	13.7	5.4	0.309	0.236	-0.073	0.646	0.275	-0.371
411	1	3	4	7.7	9.2	1.5	0.573	0.273	-0.300	0.532	0.522	-0.010
514	0	7	4	7.6	8.8	1.2	0.398	0.273	-0.125	0.173	0.056	-0.117

VITA

Joseph P. Doskocil

Candidate for the Degree of

Master of Science

Thesis: EVALUATING THE OCURRENCE, SEASONAL HISTORY, SPECIES COMPOSITION AND IMPACT OF *PHYLLOPHAGA* AND *CYCLOCEPHALA* GRUBS INFESTING BERMUDAGRASS (*CYNODON DACTYLON*) IN OKLAHOMA

Major Field: Entomology

Biographical:

Personal Data: Born in Dallas, Texas on 1 December 1981 to Joe and Christine Doskocil

Education: Graduated from Maypearl High School, Maypearl, Texas in May 2000. Received Bachelor of Science degree, double major in Agronomy and Entomology from Texas A&M University, College Station, Texas in December 2004. Received Master of Science degree in Entomology from Oklahoma State University, Stillwater, Oklahoma in May 2007.

Experience: Internship with Texas Cooperative Extension in Brazos County, Texas May 2004 to December 2004. Research/Teaching Assistant, Dept. of Entomology and Plant Pathology, Oklahoma State University, Stillwater, Oklahoma, January 2005 to May 2007.

Professional Memberships: Entomological Society of America, 2003 to present.

Name: Joseph P. Doskocil Date of Degree: May, 2007

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: EVALUATING THE OCURRENCE, SEASONAL HISTORY, SPECIES COMPOSITION AND IMPACT OF *PHYLLOPHAGA* AND *CYCLOCEPHALA* GRUBS INFESTING BERMUDAGRASS (*CYNODON DACTYLON*) IN OKLAHOMA

Pages in Study: 65 Candidate for the Degree of Master of Science

Major Field: Entomology

Scope and Method of Study:

Findings and Conclusions:

ADVISER'S APPROVAL: Tom A. Royer