

THE SEASONAL OCCURRENCE, SOIL DISTRIBUTION AND
FLIGHT CHARACTERISTICS OF *CURCULIO SAYI*
(COLEOPTERA: CURCULIONIDAE) IN MID-MISSOURI

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The undersigned, appointed by the Dean of the Graduate School, have examined the
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FLIGHT CHARACTERISTICS OF *CURCULIO SAYI*
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And hereby certify that in their opinion it is worthy of acceptance.

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CHAPTER 1

Literature Review

A. Host Plant

1. Chestnut

The chestnut tree (*Castanea* spp.) is a deciduous plant that belongs to the family *Fagaceae* (or beech family) and is native to warm temperate regions of the Northern Hemisphere (Garcia-Carbonell et al. 2002, Higaki 2005). Due to the wide variation in tree grafting and genetic recombination of chestnut trees as well as variability due to environmental conditions, there is not an accurate time table that applies to all chestnut tree development. Generally, chestnut trees take eight to ten years to reach the reproductive stage where commercial nuts are produced (Davelos and Jarosz 2004, Bounous and Marinoni 2005). However, cross breeding to produce a chestnut tree capable of nut production in as little as six years is possible (Ken Hunt [University of Missouri]). After reaching sexual maturity, chestnut flowers (or catkins) begin blooming at the end of May in Mid-Missouri and continue through most of June, though some cultivars do not finish flowering until early July (Ken Hunt [University of Missouri], Soltész et al. 2003). Variation in time and duration of florescence occurs among the many cultivars, and some chestnut trees produce a second flowering during the summer that creates a second set of burs and fruit.

The fruit, or cupule, of the chestnut tree is encompassed by a spiny covering known as the bur that surrounds the developing nuts. At maturity the bur will split open, releasing the nuts that then fall to the ground. The nut or fruit from a chestnut tree are distinct based on their geographical origin. For example, the American chestnut

(*Castanea dentata*) tends to produce nuts that are smaller in size (~6g), and densely covered in fine pubescence or hair over 1/3 to 2/3 of its surface area, whereas the Chinese (*Castanea mollissima*) and European chestnut (*Castanea sativa*) produce much larger fruit (up to 30g) with relatively few hairs (present perhaps only at the tip of the nut). Chinquapin (*Castanea pumila*), though closely resembling chestnuts, will only have one fruit or nut per bur, while chestnut burs contain 2-3 fruit. Chinquapin nuts, though covered in hair like its chestnut cousin, also tend to be much smaller than the American chestnut fruit, and the chinquapin nut has a distinct, pointed tip (Wells and Payne 1975, Miller 2003).

The natural range of the American chestnut tree extended from the lower parts of Canada to Georgia and from the Atlantic seaboard to Indiana. It was the dominant tree along the Appalachian Mountains (Anagnostakis 2005). Prior to 1904, an estimated 25-30% of timber acreage in the Eastern United States was American chestnut (Davelos and Jarosz 2004, Anagnostakis 2005). This high density of American chestnut across the Eastern United States could produce a large volume of nuts for wildlife and human consumption each year. Native Americans, early settlers and more modern street vendors used this abundant crop for human consumption. Many literature examples refer to the classical phrase of “roasting chestnuts” (Johnson 1956, McEowen 2005, Hunt et al. 2006). However, the high density of the American chestnut over the eastern United States would also prove to be a factor in the demise of the tree, as a disease could quickly spread over the native range due to the proximity of the host trees (Anagnostakis 1987).

2. Chestnut blight

In 1904, Herman W. Merkle at the Bronx Zoological Gardens in New York, first documented an infection on many chestnut trees (Anagnostikas 1987). This infection, now referred to as chestnut blight, is typically identified by its bright orange coloration and is caused by the fungus *Cryphonectria parasitica* that can be spread in two forms. The first is a dry, disc-like form (ascospores) and can be spread by the wind large distances. The second is a smaller, sticky form (pycnidia) that is spread by fruiting bodies after rain fall and has a relatively shorter range of dispersal. This fungus causes large swollen or sunken orange colored cankers on the trunks or limbs of the chestnut tree (Anagnostikas 1987). After infection, the leaves above the point of infection die, followed by the limbs (Anagnostikas 1987, Kepenecki et al. 2004, Guidone et al. 2007). Within a few years the entire tree is dead above the point of initial infection. The roots and root collar appear to be resistant, and sprouting shoots from dead stumps is common, though the blight often reappears before the new tree sprouts reach sexual maturity and nut production (Johnson 1956, Davelos and Jarosz 2004). The blight is believed to have been transported via nursery stock from China, and between 1904 and 1950 this disease spread to all corners of the native range of American chestnut. The blight also attacks several close relatives to the American chestnut including three species of chinquapin (Allegheny chinquapin, Alder leaved chinquapin, Ozark chinquapin) (Johnson 1956, Anagnostakis 1987, Hunt et al. 2006).

After the blight killed the vast majority of the three native *Castanea* trees, there was most likely a sharp decline in chestnut weevil (*Curculio* spp.) numbers as well (Johnson 1956, Menu 1993a,b). However, within a few years of bearing nuts, new

chestnut tree orchards seem to be in danger of weevil infestation (Johnson 1956). The only explanations posed to explain this phenomena are that chestnut weevil are capable of surviving on chinquapins and on chestnut trees still living and producing nuts, which includes sprouting from diseased chestnut stumps that may survive long enough to produce nuts before dying back again (Johnson 1956). Occasionally, scattered pockets of native chestnut trees or plantings of blight resistant varieties are located in isolated areas in sufficient numbers to sustain active weevil populations (Brooks and Cotton 1929, Johnson 1956).

3. Current chestnut tree research

In the Midwestern United States, the survivable range of the American chestnut tree (*C. dentata*) and its close relatives Allegheny Chinquapin (*C. pulmila pumila*) and Ozark Chinquapin (*C. pumila ozarkensis*), has been scattered if not eliminated due to the persistent presence of a highly virulent chestnut blight, *Cryphonectria parasitica* (Gold and Hunt 2002a, Hunt et al. 2004a, Anagnostakis 2005). However, current research has established several cultivars of chestnut that are blight resistant and thus have revived the tree crop industry of growing chestnuts in the Midwest, especially in central Missouri (Hunt et al. 2006). Tree improvement specialists with the Center for Agroforestry at the University of Missouri, have established an extensive chestnut production orchard to examine nut yield, nut quality, and tree hardiness, as well as cultivar resistance to the devastating chestnut blight that has plagued the native chestnut varieties since the early 20th century. There are three world-recognized commercial chestnut production tree species, the Japanese, *Castanea crenata*, European, *Castanea sativa*, and Chinese chestnut, *Castanea mollissima* (Hunt et al. 2006). However, current research by the

Center for Agroforestry has identified Chinese chestnut, *C. mollissima*, as the best adapted chestnut for Missouri, as it displays good hardiness and adequate tolerance to chestnut blight. Although the Center for Agroforestry has over 50 Asian chestnut varieties under production to establish recommendations for commercial growers (Hunt et al. 2004a,b), current nut varieties (*C. mollissima*) suggested for the commercial establishment of chestnut production in Missouri include Eaton, Peach, Gideon, Sleeping Giant, Qing and Auburn Homestead (Hunt et al. 2006).

While relatively new to many consumers and producers in the Midwest, chestnuts and their associated products are currently under a growing demand both domestically and world-wide (Gold et al. 2005a,b, Hunt et al. 2006). The Center for Agroforestry at the University of Missouri has conducted extensive and continued research concerning the market value chain associated with the production and sale of chestnut (*Castanea* spp.); moreover, the Center has focused upon three key areas, national market research, production techniques/orchard management and increasing consumer demand and awareness (McEowen 2005). Market analysis and economics of chestnut as a viable horticultural crop for both commercial growers and small farmers across the United States is ongoing, and researchers at the Center for Agroforestry have produced several publications (see Gold and Hunt 2002a,b; Gold et al. 2005a,b, 2006). With the resurgence of both the demand for chestnuts and the viability of chestnut production, multiple aspects for commercial production in the Midwest need to be addressed including control of potential pests. Insects such as the yellow neck caterpillar (*Danata ministra*) and the potato leafhopper (*Empoasca fabae*) are pests of the foliage of chestnut, especially younger trees, though treatment practices of regular pesticide application

during peak outbreaks of these pests are well established and effective control measures (Hunt et al. 2006). The single greatest insect risk to the chestnut crop comes from two weevil species, and although there is a paucity of research in the United States on these insects, they are often cited as devastating pests (Brooks and Cotton 1929, Johnson 1956, Jaynes 1979, Horton and Ellis 1999, Hunt et al. 2006).

B. Biology and Taxonomy of Chestnut Weevil Pests

1. General descriptions of *Curculio*

Weevils from the insect genus *Curculio* (Coleoptera: Curculionidae: Curculioninae) select nuts for reproduction from the following tree species: *Carya* (hickory), *Castanea* (chestnut and chinquapin), *Castanopsis* (chinquapin), *Corylus* (hazelnut), *Lithocarpus* (tanoak), and *Quercus* (oak). There are 27 documented species of *Curculio* north of Mexico (Brooks and Cotton 1929). Johnson (1956) states that the genus *Curculio*, formerly known as *Balaninus*, comprises a well defined group of species which includes the nut weevils. Johnson (1956) further states that the distinguishing phenotypic characters of the genus *Curculio* are the extremely long and slender rostrum, or beak, and the vertical mandibles.

There are two primary species of nut weevils (Curculioninae: *Curculio*) that cause damage to chestnut fruit during harvest in the United States, and these insects bare the common name chestnut weevil. These weevils cause the majority of their damage during the larval stages where they consume and nearly hollow-out the inside of the mature nut (Brooks and Cotton 1929, Johnson 1956, Ihara et al. 2003). Males and females of the species are readily separated by the length of their proboscis. Male chestnut weevils tend

to have a rostrum or proboscis that is shorter than their body length, where the female's mouthparts are longer than her body (Johnson 1956).

The phenotypic uniformity of many of the nut weevil species has historically made this genus difficult to study and differentiate, and as many as eight to ten synonyms of names have been documented for the American chestnut weevil species (Johnson 1956). *Curculio proboscideus* was described in 1775 by Fabricius, though it is now referred to as *Curculio caryatrypes*, or the greater chestnut weevil. Casey (1910) described *Balaninus auriger*, but it is now referred to as *Curculio sayi* (Gyllenhal 1836) or the lesser chestnut weevil. Many synonyms of both species exist and this gives evidence for the difficulty in identification due to phenotypic variations within the species. Consequently, as these synonyms arise in the older literature, it is often difficult to determine what species the author was discussing unless the host plant is mentioned. Casey (1910) and Johnson (1956) list 4 synonyms for the *C. caryatrypes* (the greater chestnut weevil): *C. proboscideus*, *B. caryatrypes*, *B. hariolus*, and *B. cylindricollis*. Casey (1910) and Johnson (1956) list 8 synonyms for *C. sayi* (the lesser chestnut weevil): *C. auriger*, *B. auriger mollis*, *B. strigosus*, *B. algonquinus*, *B. acuminatus*, *B. setosicornis*, *B. macilentus*, and *B. perexillis*.

The greater chestnut weevil, *C. caryatrypes* has a slightly larger adult form than its counterpart, the lesser chestnut weevil (*C. sayi*). *C. sayi* can also be separated from *C. caryatrypes* by their smaller size, more slender form, and a tendency to be shorter in body length with a greater curvature of the beak or rostrum (Johnson 1956). The greater chestnut weevil is considered the largest of the *curculio* species in the United States and can be distinguished from all other nut weevil species by the antennae, which have the

second funicular segment longer than the first (Brooks and Cotton 1929, Johnson 1956). Adult female *C. caryatrypes* length varies from 8 to 11 mm, with a body width of 4 to 5 mm. Adult female *C. sayi* length varies from 5 to 9 mm, with a body width of 2.5 to 3.5 mm. Genitalia descriptions of nut weevils are not very common, as only one of the 39 *Curculio* species has been studied using genitalia as a taxonomic characteristic (Johnson 1956). Brooks and Cotton (1929) state that, “the [larger chestnut weevil] is distinguished from the lesser chestnut weevil by its larger size, more robust form, straighter and longer beak, and, in life, by its slower movements and habits of carrying the beak projecting more directly forward.”

During all stages or instars of development, the teeth on the mandibles of the larvae of the greater chestnut weevil are deeper than those of the lesser (Johnson 1956). Lesser weevil larvae have an additional and distinct adfrontal area near the fork of the epicranial suture. The pupae of lesser chestnut weevil are missing a pair of short hairs on the beak at the base of the antennae, which are present on the pupae of the greater chestnut weevil (Brooks and Cotton 1929, Johnson 1956).

Johnson (1956) also describes in detail variations in sizes of larval and pupal characteristics based on measurements to the head capsule, though again, the greater and lesser weevil are separated by size variations.

2. Life history of the lesser chestnut weevil (*Curculio sayi*)

Curculio sayi (Gyllenhal) is the only documented chestnut weevil to emerge in the spring. Upon emergence (typically during the month of May), the adults remain on the ground for 1-2 days and then fly to the tree tops to feed on the spring catkins. After the catkins wither, the beetles disappear and are not found on the trees again until the

middle of August (Johnson 1956). Mating occurs on or near the chestnut trees in August and September, with egg laying taking place soon thereafter. *C. sayi* is the only chestnut curculio that is not observed to mate soon after emergence, but rather seemingly remains celibate until fall, or roughly 3 months after its initial spring emergence. The female typically begins to lay eggs in September, after chewing through the nut and sometimes the bur. Larvae develop inside the nuts in about 21 days and soon after, the fully grown larvae emerge from the chestnut and burrow into the ground to a depth of about 15 to 20 cm (Brooks and Cotton 1929, Johnson 1956). The larvae cut a circular exit hole in the nut of about 2 mm in diameter and quickly enter the soil. Johnson (1956) reported the emerging larvae to crawl no more than 8 cm from the point where they first contacted the ground. They burrow straight downward with little or no lateral movement for several centimeters and construct a smooth-walled earthen cell. The depth at which these cells are formed varies from 5 to 25 centimeters, and one of the factors which seem to determine the depth of the cells is the nature of the soil. Van Leeuwen (1952) reported that 97% of the larvae are found in the first 20 centimeters of soil. Once underground, the larvae construct earthen cells where they remain as larvae until September of the following year, at which time about 90% pupate. Those that pupate remain in the soil for three to four weeks prior to changing into the adult form. These adults then remain in the soil over a second winter, and emerge the following May or June (or about 21 months after they entered the soil). A few larvae will remain in the soil over three winters (or about 33 months). Some of this variation in range of lifecycles may be caused by larvae size prior to entering the soil (Menu and Desouhant 2002), as well as the depth to which

the larvae burrow (Hall and Austin 2002). Typical weevil emergence begins early May through mid-June (Brooks and Cotton 1929, Johnson 1956, Menu and Desouhant 2002).

3. Life history of the greater chestnut weevil (*Curculio caryatrypes*)

Curculio caryatrypes (Boheman) is even more poorly documented than *C. sayi*, as it does not tend to exist in as economically damaging numbers as its smaller counterpart (Johnson 1956, Hunt et al. 2006). Though very similar to *C. sayi* in lifecycle, *C. caryatrypes* is commonly referred to as completing its life cycle in only one year, thus passing only one winter underground in the larval stage, though the literature states the occasional individual will periodically pass two winters underground (Brooks and Cotton 1929, Johnson 1956). The adults only emerge in the fall, between August and September, usually just as the chestnut burs are opening. While *C. caryatrypes* adults emerge in the fall, as opposed to the spring emergence of *C. sayi*, most other life history characteristics are similar between these two closely related species (Johnson 1956). Adult female *C. caryatrypes* deposit their eggs into the nut after chewing a small hole through the bur, and the eggs will hatch within a few days. Larvae begin to consume the interior of the fruit for three weeks after hatching and then emerge from the nut to burrow into the soil. Depth of larval burrowing is estimated to be between 5 and 25 centimeters underground (Johnson 1956).

4. Life history of the European chestnut weevil (*Curculio elephas*)

The chestnut weevil *Curculio elephas* (Gyllenhal) is an important pest of the European chestnut, *Castanea sativa*. According to Menu and Debouzie (1992) and Desouhant (1998), this species can attack chestnuts and acorns (*Quercus* spp.), a behavior

not reported for the two American chestnut weevil species. Desouhant (1998) also states that mating and egg-laying occurs soon after adult emergence, which only occurs once and in the fall, beginning in late August and continuing through September. Larvae leave the fruit and burrow into the ground to over-winter. This underground diapause can extend for two, three or four winters (Menu et al. 2000, Soula and Menu 2003, Venette et al. 2003, Soula and Menu 2005). Adult weevils live for only 28 days on average and females can lay up to 28 eggs (Desouhant 1998, Debouzie and Menu 1992). Dispersal is thought to be limited, as adults tend to remain in or near the chestnut trees they emerge under (Debouzie and Pallen 1987, Venette et al. 2003). Females do not select nuts for oviposition based on the size of the nut, nor does the presence of others eggs or larvae of the same species deter further oviposition by another female (Desouhant 1998, Debouzie et al. 2002).

5. Life history of the Italian chestnut weevil (*Curculio propinquus*)

Adult *Curculio propinquus* (Desbrochers), the Italian chestnut weevil, emerge in the fall between August and mid-September. The specific emergence time may relate to geographical position of the trees and on the amount of rainfall at the end of the summer months (Paparatti and Speranza 2005b, Chen and Scherm 2006). Females lay their eggs by piercing a hole in the husk of the nut with their rostrum and inserting an egg into the hole. There are no differences between the feeding hole and the egg laying hole on the nut. Larvae feed on the amylaceous substratum of the kernel, and generally each nut hosts no more than 2 to 3 larvae. At the end of the larval stage, the larvae cuts through the nut shell and drops to the ground. In Central Italy, the larva buries itself at a depth ranging from 5 to 15 cm. The pupae appear in the soil late June and through July of the

following summer. There is a strong synchronism between adults emerging from the soil in the fall, and the degree of chestnut fruit ripening (Paparatti and Speranza 2005a,b).

This insect completes one life cycle per year, although some larvae remain in the ground in the larval state for several years (prolonged diapause), as observed on *C. elephas* (the European chestnut weevil).

C. Tree and Nut Damage by *Curculio* spp.

The primary mode of injury by the lesser and greater chestnut weevils, *C. sayi* and *C. caryatrypes*, respectively, begins with the eggs being laid inside the kernel while the nut is still growing, or even after the nut has fallen to the ground (Brooks and Cotton 1929, Johnson 1956). These eggs hatch within a few days (usually less than one week) and the larvae that infest the kernel can devour the entire content of the chestnut. Any portion of the nut which is not consumed is of little or no commercial value, thus any level of infestation within a nut removes that nut from any possibility of sale for human consumption (zero tolerance) (Boethel et al. 1974, Neel 1985, Collins et al. 1997). Weevil injury varies greatly in different chestnut growing localities but the variation is mostly due to age of the planting and thoroughness of the insect control program (Johnson 1956, Bessin 2003). It is not unusual for 50-75% of the nuts to be wormy and often infestation can reach 90-100% if left unchecked (Johnson 1956, Johnson 1957, Debouzie and Pallen 1987, Horton 2005). The lesser chestnut weevil tends to do more damage to a crop due to the fact that it can reproduce in higher numbers than the greater chestnut weevil (Brooks and Cotton 1929, Johnson 1956, Payne et al. 1972, 1975).

Several *C. sayi* larvae have been noted to emerge from a single nut, and not all larvae make their own exit holes. Johnson (1956) noted a maximum of 26 larvae from a single nut in Maryland. Van Leeuwen (1952) reports 58 larvae from a single Japanese chestnut. Typically, two to four *C. elephas* larvae emerge per infested nut (Desouhant 1998, Menu and Debouzie 1995, Desouhant and Debouzie 2000).

Feeding damage done by adults may result in the point of entry for fungal and yeast organisms (Johnson 1956), these organisms may cause the kernel to decay and are seldom noticed until the nuts are placed in storage. Though feeding on the catkins in spring is noted by several sources (Brooks 1929, Menu 1993a,b, Soula and Menu 2003), it is doubtful that this results in any appreciable damage to the tree.

Several attempts by Johnson (1956) to produce larvae from acorns, including both laboratory and field trials, were all unsuccessful. Therefore it seems that the lesser chestnut weevil, *Curculio sayi*, cannot survive apart from their primary host, chestnut, or their secondary host, chinquapin.

CHAPTER II
The Seasonal Occurrence of the Adult
Lesser Chestnut Weevil, *Curculio sayi*, in Mid- Missouri

A. Introduction

Chestnut trees were once a dominant sight across the deciduous forest of the eastern and central United States, but following a devastating blight in the early 1900's, much of the native range for this tree species has been lost (Anagnostikas 1987, Davelos and Jarosz 2004, Anagnostikas 2005). As interest in the restoration of the American chestnut tree increases, and as commercial production of chestnut fruit is being developed using blight resistant cultivars from Asia, a large quantity of both native and hybridized trees are coming into maturity and nut production (Gold and Hunt 2002a, Hunt et al. 2004a,b, 2006).

The two weevil species in the United States, the greater chestnut weevil (*Curculio caryatrypes*, Boheman) and the lesser chestnut weevil (*Curculio sayi*, Gyllenhal) attack ripening chestnut fruit, and they can devastate a commercial chestnut operation. Of these two species, the lesser chestnut weevil (*C. sayi*) has long been reported as the most common and most damaging chestnut pest insect species (Johnson 1956, Bessin 2003, Hunt et al. 2006). There is a paucity of recent literature that examines the ecology and life history of this pest insect, information that is imperative to establish basic biological parameters in order to generate a long-term management practices. The objective of this study was to ascertain adult emergence patterns and periods of activity of *C. sayi* in central Missouri.

B. Materials and methods

1. Field site

This study was conducted near Glasgow (Saline County), Missouri, on a private farmstead. Several nut trees of varying ages had been planted in the area including black walnut (*Juglans nigra*), pecan (*Carya illinoensis*), heartnut (*Juglans ailantifolia*) and several chestnut varieties (*Castanea* spp.); however, it should be noted that the nut trees had not been under any type of management program (pruning, fertilization, pest control etc) for several decades.

The chestnut trees, numbering 14 in total, were spaced at fairly even intervals of about 7 to 10 meters and their canopies were overgrown and overlapping. It is estimated that the trees were 40 to 50 years of age. For this study, five trees were selected to be monitored for adult *C. sayi* emergence and activity. The trees were of a grafted variety, and a forestry expert had classified them as a cross between Asian and American chestnut species (Ken Hunt, University of Missouri). The trees were 15 to 18 meters tall, and they produced reproductive catkins in late April and May. Annual nut drop started by late-August and continued until early-October across the five trees used in this study.

2. Cone traps

Traps used to collect ground emerging weevil were designed after the model for pecan weevil emergence traps described by Mulder et al. (1997). These ‘cone’ traps were constructed with two major layers of material. The first layer was composed of galvanized screen (0.4 cm mesh) that was cut in a half circle of a 1 meter radius (Fig. 1). This layer was reinforced by a 76 cm wooden lath, and the straight edges were overlapped and stapled in place to form a cone. The center, which encompasses the top

portion of the formed cone, was cut to remove an 8-10 cm radius section that was replaced by adding a prefabricated, commercial boll weevil trap top. The second wall layer was cut in a similar 1 meter radius half circle from heavy duty steel fence material; this fencing is then bent into another cone that is placed over the top of the original mesh screen cone, thus completing the two layer design and adding increased strength and durability. A commercially available boll weevil trap top (Great Lakes IPM, Vestaburg, MI) (Fig. 2) was placed on top of the cone's opening. This top portion contains all insects that emerged from the ground covered by the 2 meter diameter of the cage.

The emergence cone trap covered a surface area of ground equal to roughly 4 square meters, and the sturdy outer fence layer was suited to hold up against falling branches as well as high winds (Fig. 3). All cages were fastened to the ground using bent wire and remained in place throughout the season. Each year, the cone emergence traps were removed for the winter and were placed the following year at a slightly different location under the canopy of the same five chestnut trees, since no chestnuts would have fallen under the previous years trap location and no larvae would have entered the soil in those previously covered locations.

As *Curculio sayi* (and any other insects) emerge from the soil, they crawl up the inside of the cage towards the narrow opening at the apex and into the container. The trap is designed so that insects cannot find their way back down once inside the top container section.

Each chestnut tree had a unique canopy cover that was assessed visually and graphed to account for ground cover and potential nut drop. Using these canopy estimates, emergence cone traps were evenly dispersed under the potential canopy zone

for nut drop from the pervious year (Fig. 4). Each tree was assigned either five or six cone traps depending on canopy size, thus a total of 26 cone traps were used each year at the Glasgow site. Collection cages were setup and monitored during May through October for three years (2005, 2006 and 2007).

3. Tree-mounted circle traps

In order to monitor for weevil presence in the trees, a second trap type known as the circle trap was employed to capture *Curculio sayi* as they climbed up the tree trunks. This trap type was orginally designed for both plum curculio (*Conotrachelus nenuphar*) and pecan weevil (*Curculio caryae*) (Mulder et al. 1997). It is composed of a large cone of galvanized 0.4 cm mesh screen. The circle traps are stapled to the bark of the chestnut tree trunk so as to catch insects moving up the tree trunk or branches (Fig. 5). Again, a boll weevil trap container top is used to hold the insects that are funneled to the apex of the trap. Because of some of the variation in lower trunk branching among the five chestnut trees used in this study, two to four circle traps were attached per tree. Circle traps can be useful in areas where extensive mowing or livestock make ground-based emergence cone traps less desirable. The traps were obtained from a commercial source (Great lakes IPM, Vestaburg, MI), and were placed at the field site in 2006 and 2007.

4. Pyramid traps

A third trap type, known as the pyramid trap or silhouette trap, was also used in 2006 and 2007. Pyramid traps, which were first developed in 1994 for pecan weevil and plum curculio monitoring, simulate the dark upright object a weevil would interpret as a tree trunk (Mulder et al. 1997) (Fig. 6). At the field site, the pyramid traps were placed at

least 10 feet from the actual chestnut tree, and spaced from each other to maximize ground coverage in conjunction with the emergence cone traps. One pyramid trap was associated with each of the five sampling trees (Fig. 4).

5. Data analysis

The data were analyzed using analysis of variance (ANOVA), Chi-square and T-tests procedures (SigmaStat 2004).

C. Results

1. Adult emergence data

The 2005 data revealed a spring ground emergence of adult *C. sayi*, beginning the week of May 17th (2 adults collected), peaking the week of May 23rd (19 adults), and falling back off to low levels of emergence for most of June (averaging 1-2 adults per week) (Fig. 7). The trap data also revealed a second ground emergence period occurring in the fall that began the weeks of August 25th (with 7 adults) and August 30th (with 5 adults) with emergence continuing into October (averaging 1-2 adults per week). The total number of weevils collected in 2005 was 45 adults.

In 2006, the first captured weevils in the ground emergence cone traps occurred during the week of May 12th (18 adults) (Fig. 8). The following two weeks had 129 and 49 adults emerging from the ground, respectively. The spring emergence period continued into June though with decreasing numbers of adult *C. sayi* emerging. A second emergence period started to occur during the week of August 31st (46 adults) and continued into October (with average emergences of 4-5 adults per week). A total of 286 adult weevil were collected in the cone emergence traps in 2006.

In 2007, the adult emergence began the week of May 6th (12 adults) and peaked the following week of May 13th (27 adults) with numbers of emerging adults trailing off into June (Fig. 9). The second emergence period of 2007 began the week of August 26th (4 adults), peaked the week of September 2nd (12 adults), and continued to decline into October. A total of 75 adults were collected in the ground emergence cone traps in 2007.

2. Adult activity data

Circle and pyramid traps (hereafter referred to as ‘activity’ traps) were open to the environment and captured beetles actively moving about the field site. In 2006, these two trap types collected 389 adults. Spring adult activity was relatively low, as only 36 adults were collected. Peak collection weeks in the two activity traps always followed weeks of peak ground emergence data (Fig. 8). While ground emergence numbers in the spring of 2006 peaked during the week of May 19th, adult activity numbers peaked the following week of May 26th. More adults were collected in the emergence traps than in the activity traps during the spring (Fig. 8).

Fall adult activity (as measured by the two activity trap types) was much higher than that recorded in the spring as 353 adults were collected, beginning in August and extending through October (Fig. 8). Activity traps began capturing adult *C. sayi* during the week of August 31st (12 adults) and peaked the week of September 21st (177 adults). Adult weevils continued to be captured in relatively high numbers through October. Fall activity trap data in 2006 were much greater than the fall emergence data (353 adults versus 83 adults, respectively) over the three month period from August through October (Fig. 8). Thus it appears that most of the adults at the field site had migrated into the area.

In 2007, collection in the spring activity traps peaked (with 7 adults the week of May 20th) following the spring ground emergence trap peak (of 27 adults the week of May 13th) (Fig. 9). Spring ground emergence traps produced more adults than the activity traps. Fall adult activity was higher than in the spring in terms of total adults collected; however, the activity levels were not a parabolic curve as was recorded in 2006. Activity traps recorded a peak activity in the chestnut trees the week of October 17th (17 adults), though adults were collected in the activity traps in moderate numbers from August through October (Fig. 9). More adults were captured in the activity traps in the fall than were recorded that fall in the ground emergence traps.

D. Discussion

The spring emergence ground trap data at the Glasgow site were very consistent over the three years of sampling, though amplitude of emergence numbers varied by year. During 2005, the spring emergence began the week of May 17th; in 2006 it began the week of May 12th, and during 2007, the week of May 6th. Also of similar timing was the peak of spring emergence each year, which occurred on May 23rd (2005), May 19th (2006) and May 13th (2007). This seems rather reasonable considering in the literature the reported obligate nature of *C. sayi* diapause, a hibernation that is controlled by a biological clock and not related to environmental cues (Menu et al. 2000, Menu and Desouhant 2002)). It may also be the case that because of the proposed two to three year diapause, overlapping generations of emergence may create an alternating pattern of large (2006) and smaller (2005, 2007) emergences every other year.

Perhaps the single most interesting result of the three year study was the second ground emergence phenomenon that occurred in the fall, an event that has not been

previously documented for *C. sayi*. This second emergence was typically observed to begin in late August, and often continued through September and sometimes into October. It is commonly reported in the literature that emerging adults in the spring feed on the catkins in May and June, and then disappear for the summer months only to return in the fall when the fruit of the chestnut tree are exposed for oviposition.

There are numerous conjectures as to what happens to the adults of the lesser chestnut weevil that emerge in the spring time. The two most commonly reported suppositions in the literature, though neither have any experimental evidence, are that (1) the adults that emerge in the spring leave the site of their emergence and disperse to neighboring areas, only to fly back in the fall to the chestnut trees (Brooks and Cotton 1929, Menu 1993a,b), or that (2) the adults bury themselves in the soil again, lie dormant for the summer months and reemerge in the fall when the burs are opening and the nuts are ready for feeding and egg laying (Brooks and Cotton 1929, Johnson 1956, Menu 1993a,b, Desouhant 1998).

Based on the data collected from the activity traps in 2006 and 2007, we can elaborate further on the two aforementioned possibilities for *C. sayi* disappearance during the summer months. First, note the relatively small amount of *C. sayi* captured by the activity traps (the tree mounted circle traps and ground-based pyramid traps) during the spring compared to the large ground emergence numbers recorded both in 2006 and 2007. This suggests that relatively few adults that emerge in the spring are actively seeking the nearby chestnut trees. However, note the relatively high amount of *C. sayi* captured by the activity trap types during the fall months, which is especially interesting given the smaller amplitude of fall emergence from the soil. This strongly suggests that

insect activity is high in and around the chestnut trees during the fall when compared to the activity in the spring, and moreover, that fall adult emergence alone cannot account for the large numbers of *C. sayi* captured during the fall. It seems apparent that *C. sayi* does in fact return in high numbers to the chestnut trees in the fall, though this evidence does not directly address the two possibilities of summer disappearance, namely whether or not the insects disperse by flight from the site, or rebury themselves over the summer. The circle and pyramid trap data does indicate that adult *C. sayi* activity at the site during the summer months, especially around the trunk of the trees, was relatively non-existent. One can assume that the spring emerged weevils had left the chestnut tree area. However, we cannot conclusively discount the possibility that the weevils spent the summer in the trees' canopy, since canopy samples were not taken. But the nature of the circle and pyramid traps do strongly suggest that the adult *C. sayi* were moving back into the area during the fall, being attracted to the silhouette of the trunks and being captured moving back up the chestnut trees.

The seasonal occurrences of the European chestnut weevil, *C. elephas*, and the Italian chestnut weevil, *C. propinquus*, are well documented and report only a single emergence period that occurs in the fall (Menu 1993a,b, Desouhant 1998, Desouhant and Debouzie 2000, Paparatti and Speranza 2005a,b). The seasonal occurrence of *C. sayi*, which was the only chestnut weevil to have been reported in the literature to emerge in the spring, appears to also have the unique characteristic of two annual emergence periods. Further study is required to ascertain the ecological importance of this spring emergence, as adults are active long before their host plant is generating nuts for female *C. sayi* oviposition.

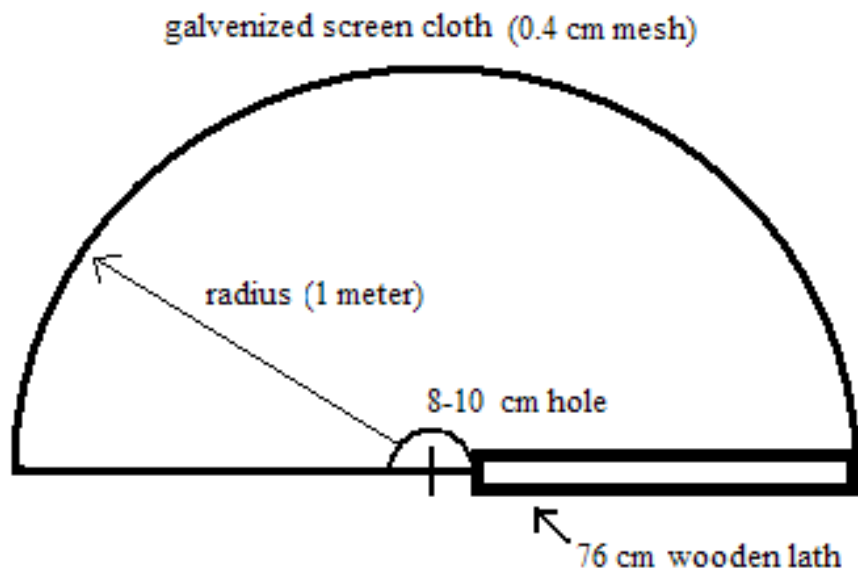


Figure 1. Schematic pattern and dimensions of the ground emergence cone traps (modified from traps described by Mulder et al. 1997). The flat edges are stapled together over a wooden lath to form a cone. The center of the semicircle, which becomes the top of the cone upon folding, is removed to allow the addition of a pre-fabricated boll weevil trap top to collect insects.



Figure 2. Commercial boll weevil trap top (disassembled) placed on top of the cone trap (see Fig. 1) (trap tops were obtained from Great Lakes IPM, Vestaburg, MI).



Figure 3. The emergence cone trap covered an area of ground roughly 2 square meters and stands at just over 1 meter tall. The inner galvanized mesh layer directs all emerging insects including *C. sayi* to the boll weevil trap top for collection. These traps were used in 2005 through 2007.

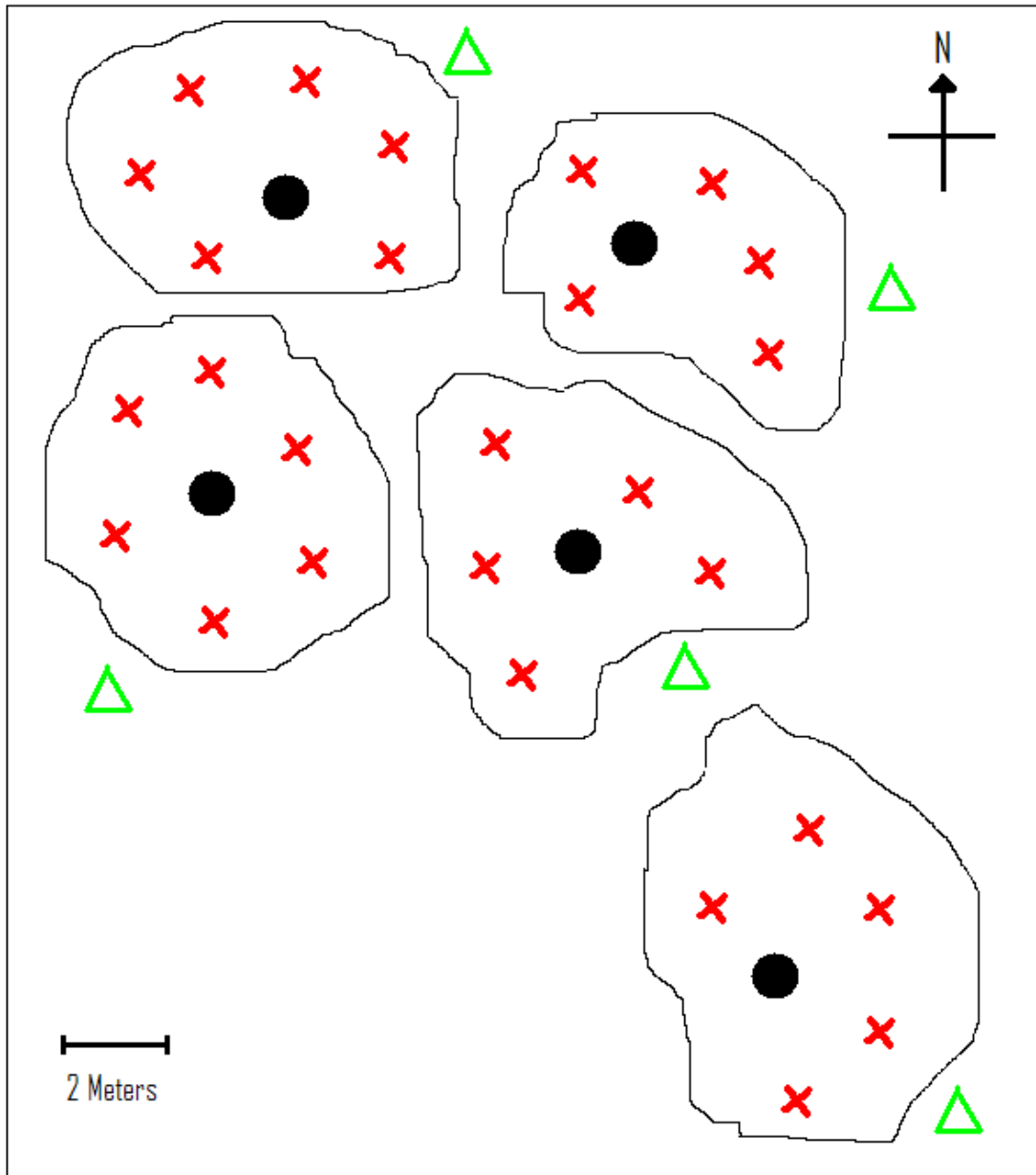


Figure 4. Schematic depiction of the location of the five selected chestnut trees at the Glasgow, MO, site. Tree trunks are shown as blackened circles. Trees were on average spaced 7.5 to 9 meters from one another. The emergence cones traps, shown as red crosses, were evenly dispersed under the tree's canopy and the approximate position of the pyramid traps are shown as green triangles.



Figure 5. Tree mounted circle traps that were positioned to collect adult weevils as they move up the chestnut trees.



Figure 6. Pyramid traps act as a false tree trunk and capture the weevils as they climb upward and become trapped in the boll weevil trap at the top (traps obtained from Great Lakes IPM, Vestaburg, MI).

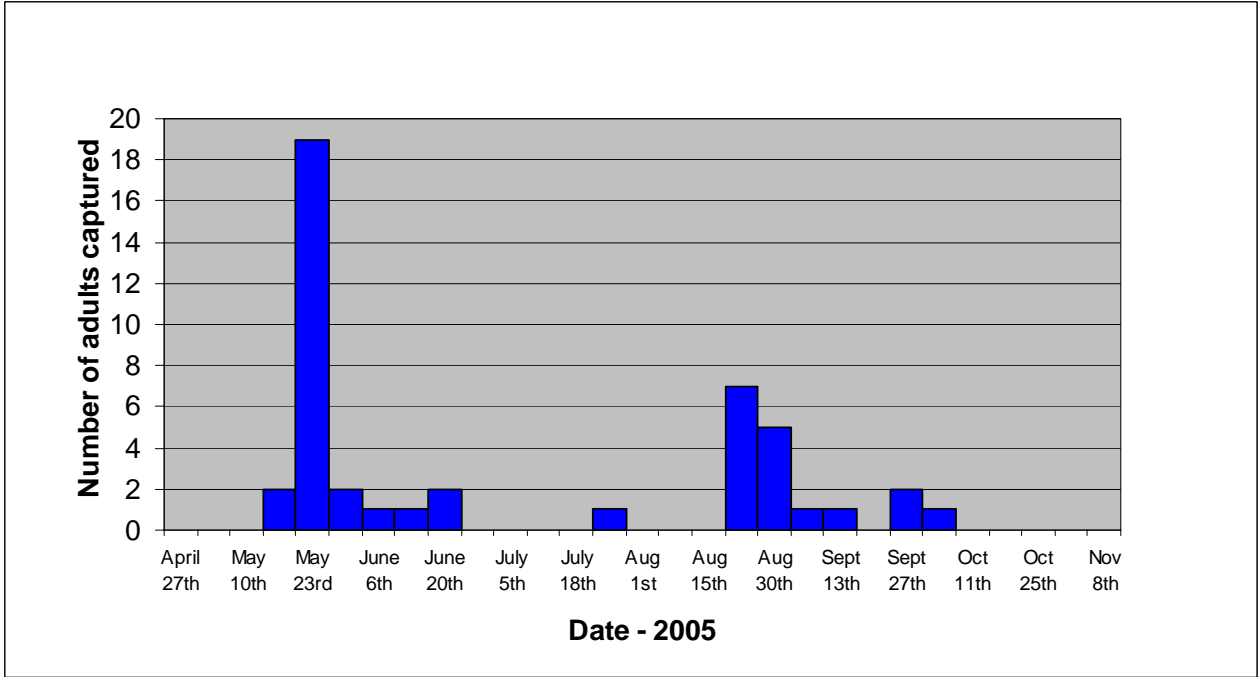


Figure 7. Numbers of adult *Curculio sayi* captured emerging from the ground in 2005.

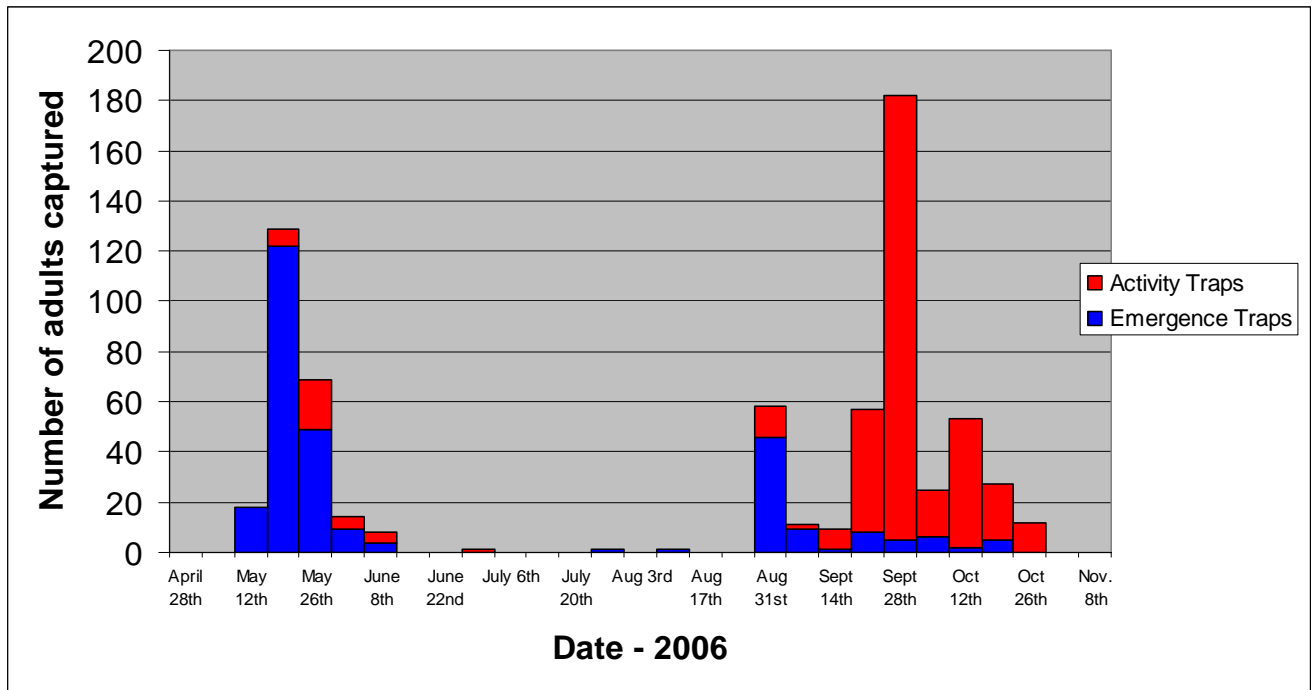


Figure 8. Numbers of adult *Curculio sayi* captured emerging from the ground (data in blue) and crawling up the tree trunks (data in red) in 2006.

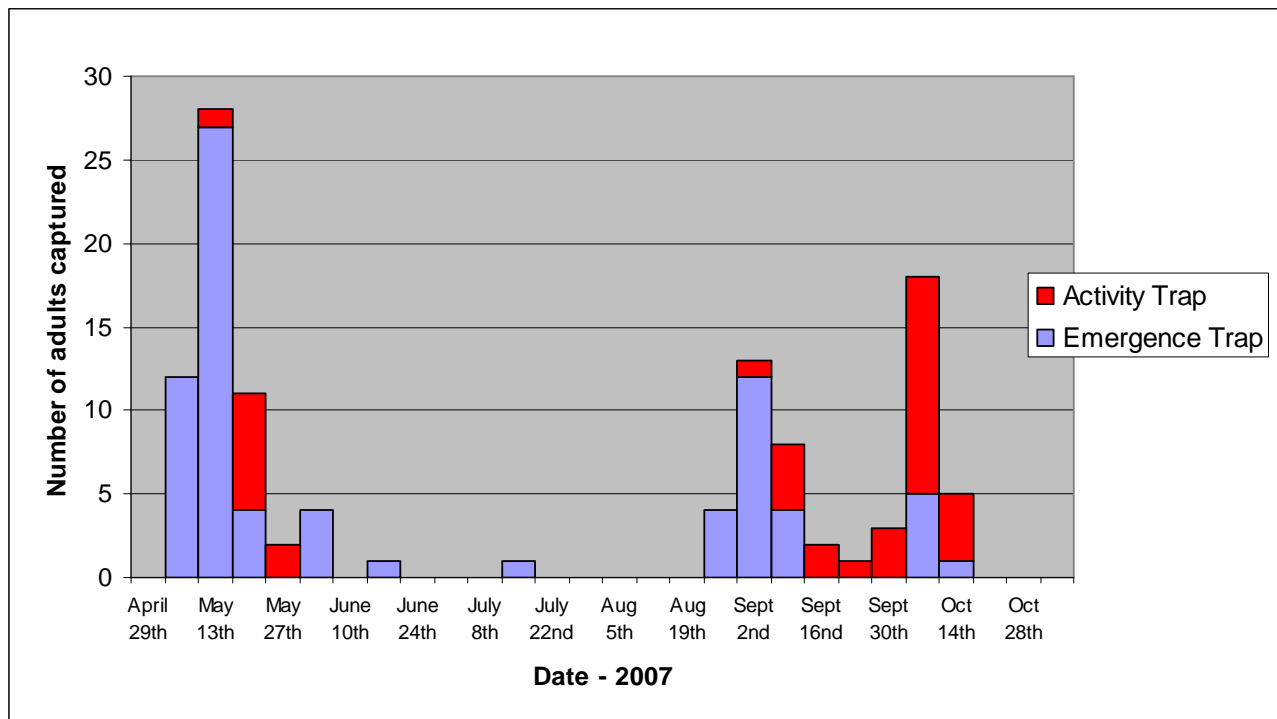


Figure 9. Numbers of adult *Curculio sayi* captured emerging from the ground (data in blue) and crawling up the tree trunks (data in red) in 2007.

CHAPTER III
Within-Soil Distribution and Development of the
Lesser Chestnut Weevil, *Curculio sayi*

A. Introduction

Chestnut trees were once a dominant sight across the deciduous forest of the eastern and central United States, but following a devastating blight in the early 1900's, much of the native range for this tree species has been lost. As interest in the restoration of the American chestnut tree increases, and as commercial production of chestnut fruit is being developed using blight resistant cultivars from Asia, a large quantity of both native and hybridized trees are coming into maturity and nut production.

The two weevil species in the United States, the greater chestnut weevil (*Curculio caryatrypes*, Boheman) and the lesser chestnut weevil (*Curculio sayi*, Gyllenhal) attack ripening chestnut fruit and they can devastate a chestnut operation. Of these two species, the lesser chestnut weevil (*C. sayi*) has long been reported as the most common and thus most damaging chestnut pest insect. There is a paucity of recent literature that examines the ecology and life history of this pest insect, information that is imperative to establish basic biological parameters in order to generate a long-term management practices. The objective of this study was to assess the soil distribution of mature larvae leaving the chestnut and their subsequent development through time.

B. Materials and Methods

1. Field site

This study was conducted at the University of Missouri, Horticulture and Agroforestry Research Center (HARC), New Franklin (Howard County), MO. Soil cages were placed in Block E of the research center, which is a 1.2 ha block of alternate plantings of “Red Delicious” and “Jonathan” apple cultivars. The tree planting distance was 5.5 m rows and 7 m between rows. The trees were 6-years old, approximately 3 m tall. With the exception of 2005, the apple trees in Block E had received irregular applications of insecticide spray.

2. Soil cage containers

Sixteen cage containers were constructed from 25 cm diameter PVC tubes that were cut into seven 7.5 cm tall sections. Seven ring sections, composing a total of 53 cm in length, were fastened together using wooden support slates and held in place by wood screws (Fig. 10). Each cage was buried so that only a small portion of the upper-most ring was still visible (Fig. 11). The cages were buried, and filled with soil and given one month to settle prior to introduction of the weevil larvae (Fig. 11).

3. Insects

Infested chestnuts were collected off the ground from a private farm near Glasgow (Saline County), Missouri, during September of 2005. The chestnuts were suspended over a laboratory table top in an open-ended box with a heavy gauge wire mesh floor. As the larvae emerged from the nuts they fell through the mesh and into a collection tray below. Emerged larvae were then collected and transported in plastic

containers stored in insulated boxes to the HARC field site. The larvae were then placed on top of the soil in each cage and they immediately began to burrow downward. The larvae that had emerged each day from the infested chestnuts were divided into 16 groups, and distributed evenly across each of the buried cages. This was done to avoid any possible differences in larvae fitness based on the time of their emergence from the nuts. By mid-November, a total of 85 larvae had been placed in each of the buried cages.

4. Sampling procedures and schedule

Forty-eight hours after the last of the larvae had been added to each cage soil surface (November 2005) a single cage container was removed from the ground to assess immediate larval activity. This initial tube extraction was used to ensure that larvae were in fact tunneling and burying themselves in the ground after being released. Five cages were removed in January of 2006, three months after they had last been stocked with larvae, and a second set of five cages was removed in May of 2006. A single cage was removed in April of 2007 (after seventeen months in the ground). The remaining four cages were removed in October of 2007, almost two years after their initial burial.

Once removed, the individual cage assembly was dismantled into the seven separate sections that correspond to the various soil depths. The soil contained in each section was carefully broken apart by hand to reveal the presence, location and number of *C. sayi*, as well as its life stage (i.e. larva, pupa or adult). Both live and dead weevils were recorded.

5. Data analysis

Statistical analyses of the data included analysis of variance (ANOVA), Chi-square and T-test procedures (SigmaStat 2004).

C. Results

In the first soil cage removed from the ground in November 2005, approximately 48 hours after the last of the 85 larvae had been placed on top of the soil, 62 larvae were recaptured for a 72.9% survivorship. The vast majority of the larvae were located in the first section of the tube ($n = 50$), within 7.5 cm from the soil surface (Fig. 12). From Section 2 (7.5 – 15 cm depth) and Section 3 (15 – 23 cm depth), 9 and 3 larvae were recovered, respectively (Fig. 12). The remaining four sections contained no larvae.

In January 2006, three months after initial burial, five soil cage tubes were removed from the ground. No larvae were recovered in the deepest three sections, five through seven (30 - 53 cm depth). From Section 1 (0 - 7.5 cm depth) an average of 21.6 larvae were recovered. From Section 2 (7.5 - 15 cm depth) an average of 33.6 larvae, from Section 3 (15 - 23 cm depth) a mean of 19.0 larvae, and from Section 4 (23 - 30 cm depth) an average of 1.4 larvae were recovered (Fig. 13). Mean survivorship of *C. sayi* from the five soil cages in January 2006 was 88.7%. An analysis of variance procedure (ANOVA) revealed no significant differences between sections 1-3 (where 99% of the larvae were recovered), but these three sections were significantly different from sections 4-7 ($P < 0.001$). All recovered *C. sayi* from January 2006 were in the larval stage.

In May 2006, six months after the larvae were released into the ground, another set of five soil cage containers were removed. No larvae were collected in sections 4-7 (23 – 53 cm depth). On average, from Section 1 (0 – 7.5 cm depth) 20.2 larvae were

recovered, Section 2 (7.5 – 15 cm depth) 38.2 larvae were recovered, and from Section 3 (15 – 23 cm depth) 10.6 larvae were recovered (Fig. 14). Mean larval survivorship average for these five containers was 81.18%. All individuals recovered were still in the larval stage.

In April 2007, seventeen months after the larvae were initially released into the soil, a single soil cage container was removed and analyzed (Fig. 15). No *C. sayi* were recovered in Sections 4-7 (23 – 53 cm depth). From Section 1 (0 - 7.5 cm depth) 12 larvae and 2 pupae were recovered. From Section 2 (7.5 - 15 cm depth) 22 larvae and 2 pupae were recovered. From Section 3 (15 - 23 cm depth) 4 larvae were recovered (Fig. 15).

During May 2007, several adults were captured by a cone trap apparatus covering the tops of each of the buried soil cages. Adult emergence from the four remaining soil cages began May 7th and continued through May 16th, for a total of 20 adults (Fig. 16).

In October 2007, nearly two years after the larvae were first released onto the soil surface of the cages, the final set of four soil cages were removed from the ground. No *C. sayi* were recovered in Sections 4-7 (23 – 53 cm depth). A total of 43 insects were recovered from Sections 1-3 (0 – 23 cm depth) (Fig. 17). The vast majority of the recovered *C. sayi* (n = 31) were in the adult stage, with a few in the pupa (n = 9) or larva stages (n = 3). From Section 1 (0 – 7.5 cm depth) one pupa and three adults were recovered (Fig. 17). From Section 2 (7.5 – 15 cm depth) three larvae, six pupae and 24 adults were recovered, and from Section 3 (15 – 23 cm depth) two pupae and four adults were recovered. It should be noted that from these four cages 20 adults had emerged

earlier in the year (May). If these 20 adults are included into the survivorship of these final four soil cage tubes, then survivorship of *C. sayi* after two years was 18.5%.

D. Discussion

The results of this study are consistent with that reported in the literature that *C. sayi* larvae burrow to a depth of 8 to 25 cm underground (Johnson 1956). There does not seem to be any cannibalism or direct competition for space underground, as it was observed that several larvae seem to follow the same tunnels underground and then branch off to form individual clumped earthen cells in close proximity to others in their cohort. Observations made during this study and noted in the literature concerning *Curculio*, illustrate that often during the exodus from the fruit or nut to the soil, several larvae may utilize the same exit hole generated by a single larvae (Stamps and Linit 2002). Thus it again seems reasonable that larvae may share initial pilot tunnels when burrowing underground. The lack of cannibalism or lack of competition among a cohort has also been reported by researchers examining chestnut weevil larvae feeding within the fruit of the chestnut tree, and that as many as eight larvae could be found inside a given nut and the larvae would still be devoid of direct physical damage to each other (Menu 1993a,b, Menu and Desouhant 2002).

There is a strong and consistent pattern of distribution across the first three sections of the soil cage tubes beginning in January of 2006, and continuing through October of 2007. The only distribution that does not match this pattern occurred in the first sample date in November 2005. The distribution noted at this time (48 hours after the larvae were released on top of the soil) suggests that final depth takes more than 2 days to achieve, as a skewed number of individuals were still located in the first section,

with very few spread out into Sections 2 and 3. All the subsequent sample evaluation periods revealed the same even spread of insects across the first three depth sections.

No change in the life stage of the seeded larvae was observed until the April 2007 sample date (or 17 months after the larvae entered the soil cage tubes). Thus it seems that there is at least a 17 month period that is the minimum duration *C. sayi* remains in diapause underground prior to completing its lifecycle. In fact, adult emergence from the soil cage tubes was noted a few weeks later in May 2007, when 20 adults were captured in the emergence traps that were placed over the top of the soil cage containers. Therefore, the first weevil to complete their lifecycle took roughly 18 months.

In the final sample of October 2007, only a handful of individual weevil were recovered, which supports that reported in the literature where a few *C. sayi* emerge the 3rd year after entering the soil as larvae. The vast majority of recovered insects in October 2007 were in the adult stage, though few recovered adults were still alive. It should be noted that at this time many of the soil cage tubes were quite dry and the clay-based soil was heavily solidified. There were two possible explanations for the number of dead adults recovered in October 2007. First, had the soil been under less drought strained conditions, the adults may have been free to emerge in the fall of 2007 through the softened soil (Manel and Debouzie 1997, Lapointe and Shapiro 1999). The second possibility is that these adults were going to stay underground for another winter, and were not emerging in the fall, but rather the following spring. Regardless, it appears that these insects were killed by soil entrapment and the ensuing desiccation.

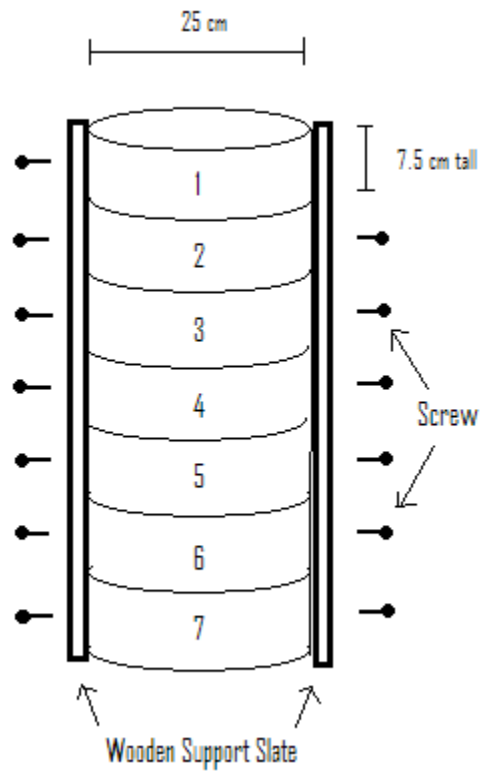


Figure 10. Schematic diagram of soil cage showing the seven ring sections of PVP pipe that were attached together using two wooden support beams.



Figure 11. A soil cage tube assembly above ground (left) next to a buried tube (right). Each soil cage tube was filled with soil and allowed to settle for one month prior to adding the weevil larvae.

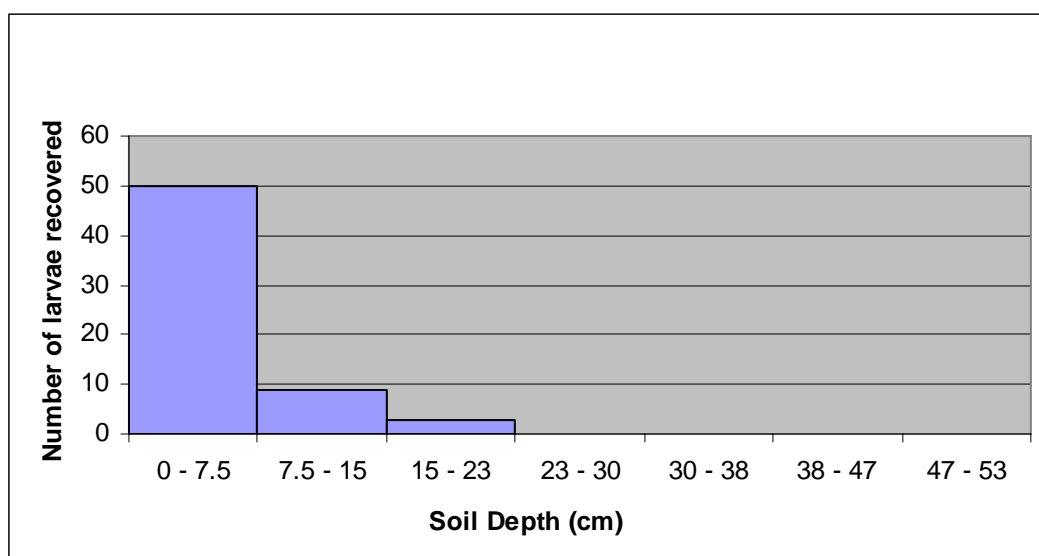


Figure 12. The number of *Curculio sayi* larvae recovered from a single soil cage container unearthed 48 h (November 2005) after the final larval introduction on the soil surface.

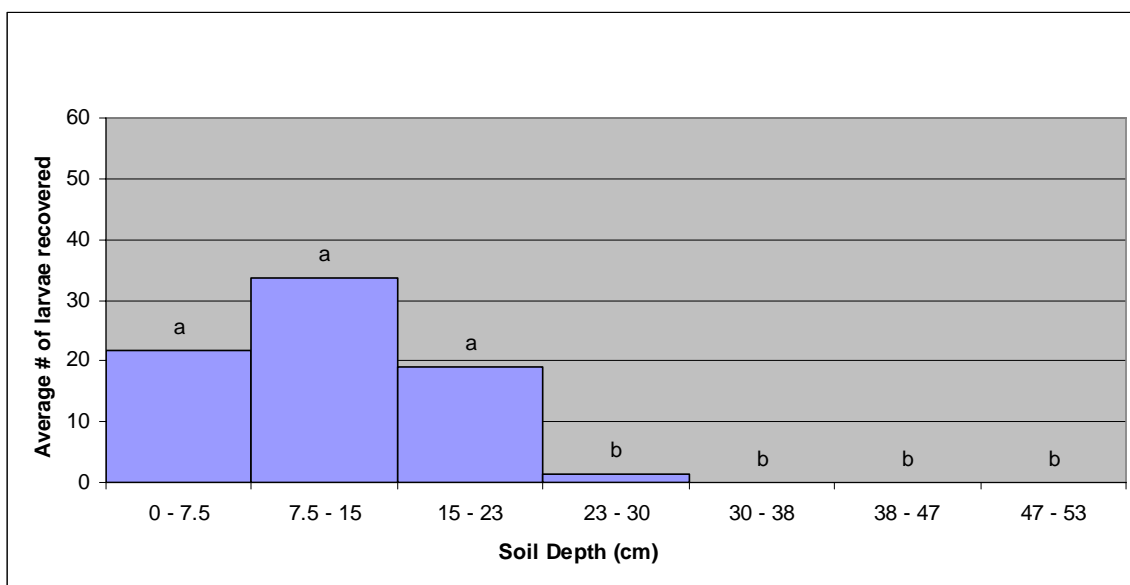


Figure 13. The mean number of *Curculio sayi* larvae recovered from five soil cage containers unearthed approximately 3 months (January 2006) after the final larval introduction on the soil surface. Mean numbers of larvae found at each soil depth followed by the same letter are not significantly different ($P < 0.05$).

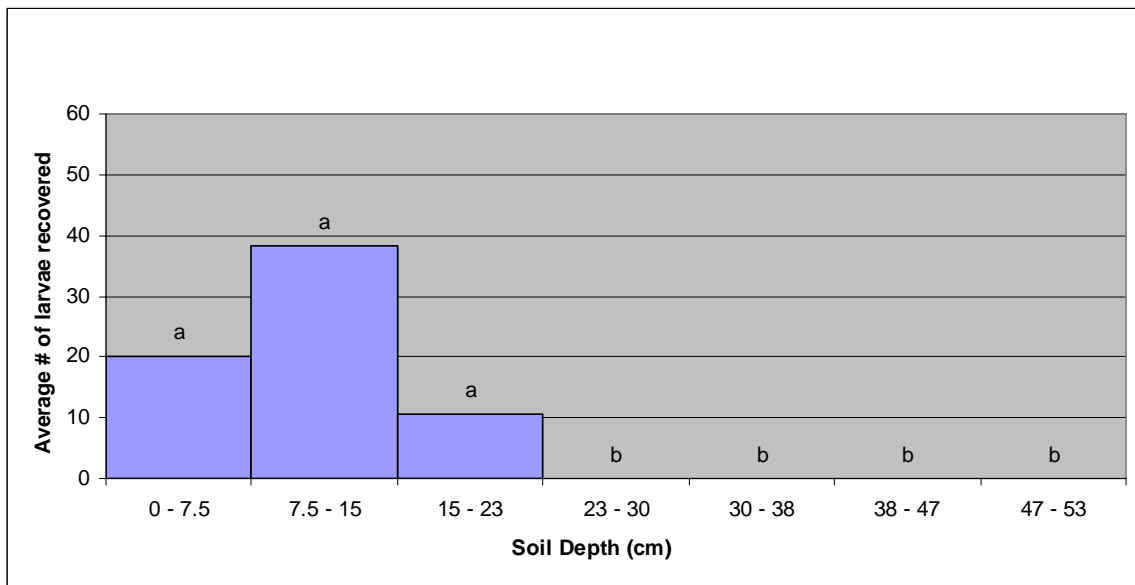


Figure 14. The mean number of *Curculio sayi* larvae recovered from five soil cage containers unearthed approximately 6 months (May 2006) after the final larval introduction on the soil surface. Mean numbers of larvae found at each soil depth followed by the same letter are not significantly different ($P < 0.05$).

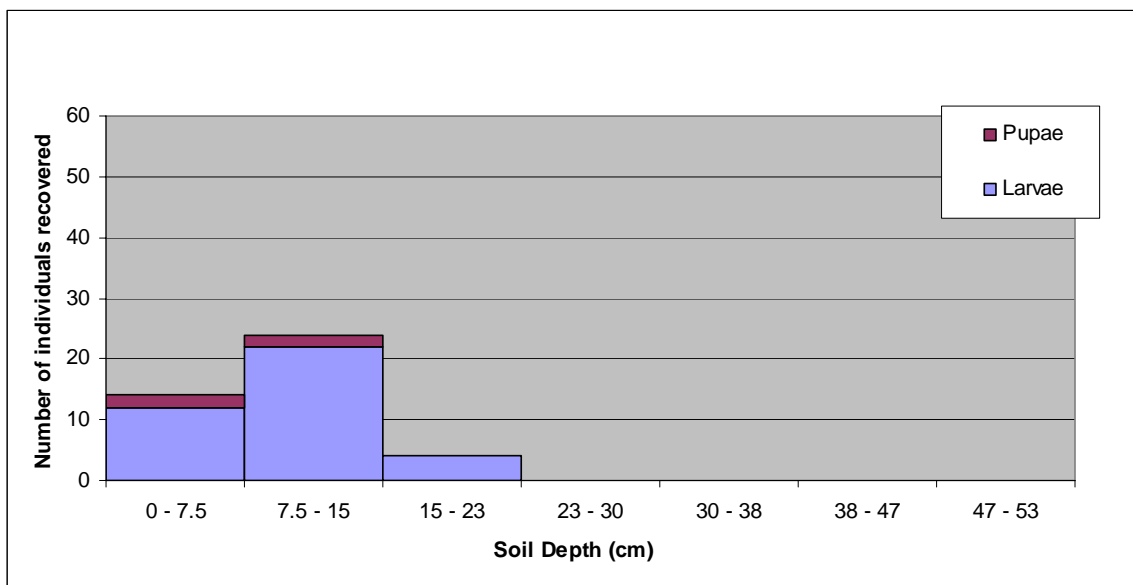


Figure 15. The number of *Curculio sayi* larvae (blue data) and pupae (red data) recovered from one soil cage container unearthed about 17 months (April 2007) after the final larval introduction.

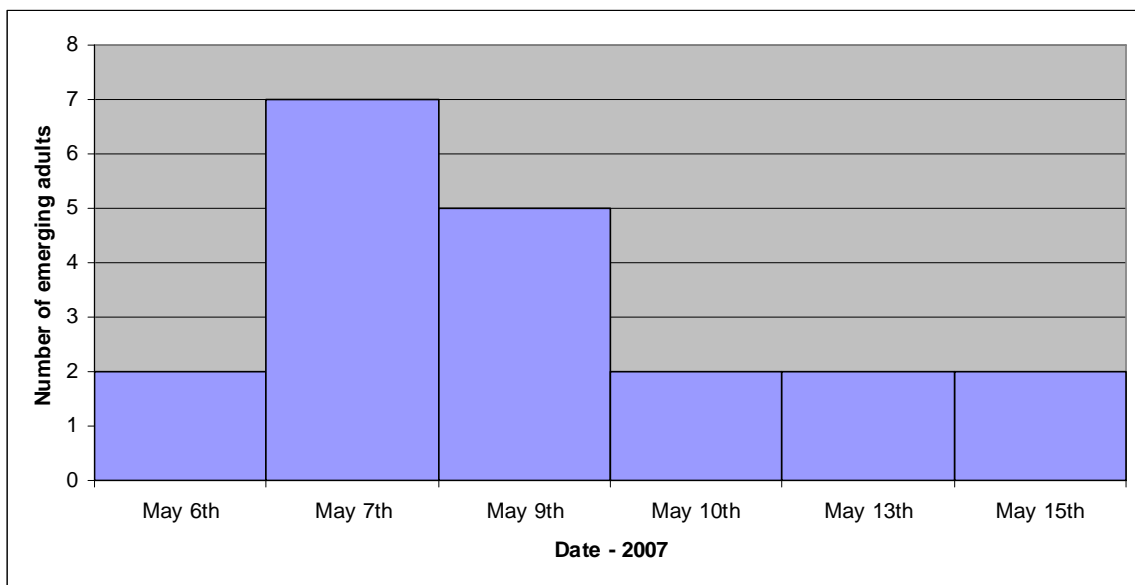


Figure 16. Numbers of adult *Curculio sayi* emerging in May 2007 from four soil cage containers.

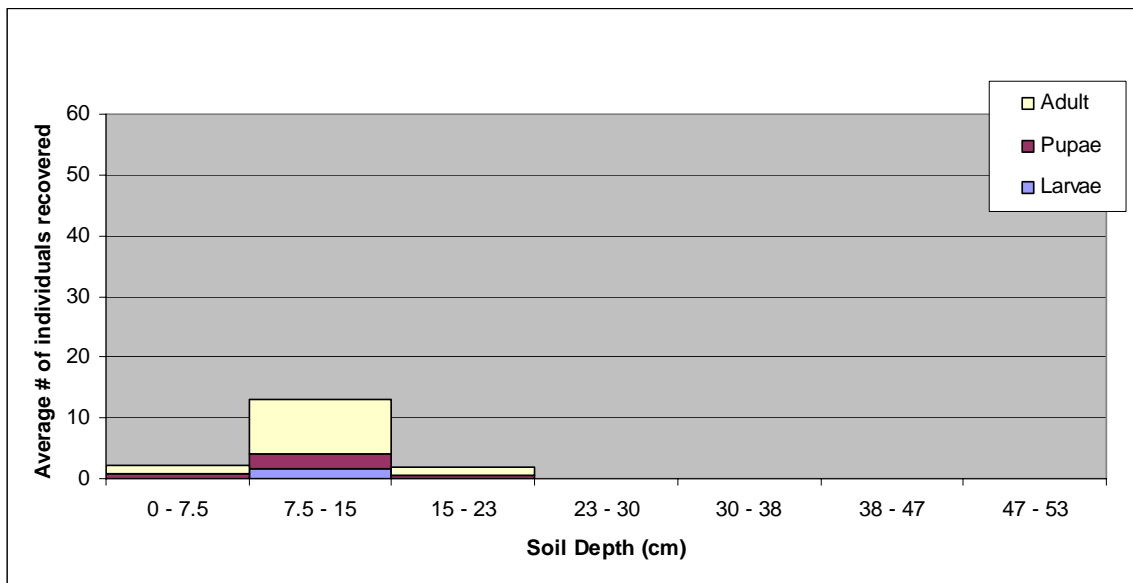


Figure 17. The mean number of *Curculio sayi* larvae, pupae and adults recovered from four soil cage containers unearthed approximately 2 years (October 2007) after the last of the larval introduction.

CHAPTER IV

Assessment of the Flight Characteristics of the Lesser Chestnut Weevil, *Curculio sayi*, from a Flight Mill

A. Introduction

Chestnut trees were once a dominant sight across the deciduous forest of the eastern and central United States, but following a devastating blight in the early 1900's much of the native range for this tree species has been lost. As interest in the restoration of the American chestnut tree increases, and as commercial production of chestnut fruit is being developed using blight resistant cultivars from Asia, a large quantity of both native and hybridized trees are coming into maturity and nut production.

The two weevil species in the United States, the greater chestnut weevil (*Curculio caryatrypes*, Boheman) and the lesser chestnut weevil (*Curculio sayi*, Gyllenhal) attack ripening chestnut fruit, and they can devastate a chestnut operation. Of these two species, the lesser chestnut weevil (*C. sayi*) has long been reported as the most common and most damaging chestnut pest insect species (Johnson 1956, Bessin 2003, Hunt et al. 2006). There is a paucity of recent literature that examines the ecology and life history of this pest insect, information that it is imperative to establish basic biological parameters in order to generate long-term management practices. For example, with the current degree of noncontiguous chestnut plantings across the Midwest and East coast, it is vital to understand the flight abilities of the primary nut pest, *C. sayi*, and establish an understanding of safe buffer zones between areas of infestation and clean areas of commercial nut production.

Studies have been conducted on other beetle species to establish flight parameters necessary to estimate dispersal ability, most of which concentrate on distance, duration and speed of the insect's flight (McKibben 1985). The use of flight mills typically allows the tethering of an insect that can be induced to fly given the appropriate conditions necessary for flight. Beetles that migrate or travel great distances are often the subject of such studies, especially those that are capable of tree disease pandemics through nematodes (Akbulut and Linit 1999) or fungal blights (Raffa and Berryman 1987). In order to assess the infestation capabilities of *C. sayi* in Mid-Missouri through flight dispersal, the objective of this study was to ascertain the flight characteristics of adult *C. sayi* through the use of a laboratory flight mill.

B. Materials and Methods

1. Insects

Adult *Curculio sayi* were collected from traps located in a chestnut tree planting on a private farmstead near Glasgow (Saline County), Missouri. Three trap types were used in obtaining the live weevils, including ground based cone traps, tree mounted circle traps, and free standing pyramid traps (for descriptions of these traps see chapter II). The adults used in this study were captured in the spring of 2007 between May and June. Collections from the traps occurred twice per week so the adult weevils were not left in the traps for more than 3 days at a time prior to being transported to the laboratory. Chestnut weevils were separated by gender upon collection in the field and placed in separate containers. Their mating status was assumed to be virgin.

Prior to their exposure to the flight mill, adults were placed in an environmental chamber (Percival, Model I30BLL) set 24° Celsius, a photoperiod of 12:12 h (light:dark),

and a 30-40% relative humidity. Adult weevils remained in this chamber with access to sugar water for 24 to 48 hours prior to being attached to the flight mill.

2. Flight mill and procedures

A flight mill that had previously been designed for the study of the flight characteristics of a large wood boring Cerambycidae (Coleoptera) (see Akbulut and Linit 1999), was modified slightly for the smaller and lighter weight *C. sayi*. The radius of the flight path, as measured along the flight mill arm, spanned 33cm; one revolution equating to about 208 cm travelled (Fig. 18).

The mill was placed on top of a 1.2 x 1.8 meter poster board with an alternating pattern of white and black stripes to aid in the retinal stimulation often required to induce normal insect flight (and possibly required by the insect for continued flight). Four incandescent lights (60 watt) were setup in a square around the flight mill, which helped control the temperature around the flight path (Fig. 19).

While many tethers were examined (including sewing thread, fine copper wire and insect mounting pins) it was ultimately noted that paper strips were the most effective in inducing spontaneous flight and for allowing the insects to be removed from the tether to rest before another flight assessment. A key component in inducing flight seemed to be the adult weevil orientating its body angle and body direction before uncasing the wings. Ultimately, a thin strip of paper that was cut into a point and affixed to an insect mounting pin was the most successful in meeting these pre- and post flight needs of the chestnut weevil. The fine, pointed tip of the paper was glued to the top of the insect head segment, just to the rear of the antennae and compound eyes (Fig. 20). The paper was

then angled 45 degrees from the line of symmetry through the insect, which allowed the paper to both be out of the way of the insect elytra once the wings were uncased, as well as to keep the paper out of the reach of the insect legs that tend to be splayed outward during flight (Fig. 20).

Prior to flight, tethered insects were given a small section of paper to hold onto and maneuver. Many insects orientated themselves towards one of the four corner lights and upon dropping the paper section began to fly. Some insects were induced to fly by removing the paper strip, thus removing the tarsi from tactile contact. The temperature that was maintained during each session was between 31 – 33° Celsius. Once flight began, as defined by the uncasing of the wings, flight duration and the number revolutions around the mill were recorded (Fig. 19).

The flight capabilities of 11 females and 10 males were assessed in this study, with each insect being examined 2 to 3 times (repetitions). Adults were given a 5 minute rest period between their flight attempts before the tether was removed and the insect was returned to the growth chamber. All insects were flown between one and three days after capture at the field site.

C. Results

The average flight duration for females was 578 seconds (9.6 minutes) (Table 1). The average flight duration for males was 688 seconds (11.4 minutes). The male mean flight duration was significantly greater than the female mean ($P < 0.05$). Maximum flight duration for females was 7205 seconds (~ 2 hours) and for males the maximum flight duration was 7035 seconds (~ 1.9 hours).

For females, the mean distance traveled was 226.6 meters, while males flew an average distance of 247.1 meters (Table 1). There was no significant difference between males and females for average distance traveled. The maximum distance traveled by a female was 3044.13 meters, and for a male, the maximum distance recorded was 2517.41 meters.

Male *C. sayi* generated a mean flight speed of 41.39 cm/sec (n = 10), while females had a mean speed of 41.97 cm/sec (n = 11). These two means were not significantly different (Table 1). Female flight speed ranged from 16.34 cm/sec to 75.87 cm/sec. Male flight speed ranged from 29.14 cm/sec to 60.15 cm/sec (Table 1). It was noted that as flights increased in duration the flight speeds decreased over time for both males and females (Fig. 21). Female flight speed decreased at 1.13 cm/sec² while males flight speed decreased by 1.91 cm/sec². Over the course of a female flight period that had lasted 2 hr, the insect decreased its flight speed from 47.8 cm/sec to 39.6 cm/sec, or roughly a total decrease in speed of 8.2 cm/sec. A male on the other hand, also flying for close to 2 hours, decreased its flight speed from 50.4 cm/sec to 35.8 cm/sec, or roughly a total decrease in speed of 14.6 cm/sec. The male was observed to decrease flight speed over time at a higher rate than that of the female.

D. Discussion

There was not a significant difference between male and female *C. sayi* in mean flight distance or speed; however, there was a significant difference between the sexes in flight duration. The maximum flight distances recorded for both male and female insects would allow both sexes to travel approximately between 2.5 and 3 kilometers respectively. Interestingly, this was similar to a distance predicted by Johnson (1956):

“That *Curculio auriger* (*sayi*) is capable of rather lengthy flight seems certain. At a Prospect Plantation Orchard, near Graysonville, Maryland the small chestnut weevil was found for the first time in 1949. These trees had been producing nuts for about four years. The orchard is bordered on the west by Chesapeake Bay and the south and southeast by a rather extensive marsh. The nearest wooded area is approximately one mile to the northeast. No chinquapin or chestnuts have ever been known in this area. The only access to this orchard would require the adults to fly at least two miles.”

Though average flight distances were much lower than the maximum distances observed for both male and female *C. sayi*, it may be possible to repeatedly induce such lengthy flights if preflight behavioral orientation requirements are more readily achieved in the laboratory.

Inducing *C. sayi* to fly proved to be the most difficult aspect of this study. Based on observations in the laboratory it appears that there is a cascade of necessary behaviors required before *C. sayi* is willing to take to flight. Body orientations, including posturing, angle to the light source and position of the tarsi on the preflight paper strip appeared to be the most influential predictors of willingness to uncase the wings and begin flight behavior. It should be noted that insects that performed flight durations of greater than 10 minutes were more apt to repeated lengthy flight durations and showed an increase in the ease of flight inducement. Thus there may be a physiological change associated with prolonged flight similar to that observed in migration states of some insects (Dingle 1972; Blackmer et al. 1994). Moreover, studies with female whiteflies (*Bemisia tabaci*) have shown an increase in the proteins required for egg production in long-distance flyers when compared to short-distance flyers or settled individuals. Thus, one could

hypothesize that a lengthy spring flight may be required for egg production in *C. sayi* adult females.

Another important factor observed during this study was that any tactile disturbance to the insect's tarsi, mouthparts, antennae or wings resulted in immediate halting of flight, and the beetle simply refolded its wings. Also, the insect must maintain a certain speed around the flight mill to continue flying. If the insect cannot achieve a moderate flight speed initially, the insect will halt flight (or the movement of the flight mill arm). Thus there may be a correlation between willingness to fly and the speed at which it perceives motion based on the alternating patterns of black and white stripes as it progresses around the flight mill. This study employed a black and white pattern below the insect to stimulate the perception of motion, though future flight studies of this insect may benefit from a vertical wall around the flight pathway that also bares the alternating color pattern since the field of vision of *C. sayi* points more towards its periphery and not in a downward fashion. The addition of this wall, if it is able to increase the motion perception of the insect, may both increase the ease of which the insect takes to flight as well as increase the duration of the flight behavior once the wings are extended.

During the longer recorded flights for each gender, it was observed that flight speed decreases linearly over time. It was also observed that the arc or distance that the insect wing traced was reduced slowly as the insect flew for prolonged periods, which was a good predictor of when the insect would halt flight. Moreover during prolonged flight, while the distance the wing covered per stroke slowly decreased over time, it was observed that occasionally *C. sayi* appeared to catch its second wind and regain both an increase in flight speed as well as an increase in wing stroke distance. Flight speed in *C.*

sayi appears to be dependent on the distance covered by the wing during a single stroke, thus again stressing the importance of tethering the insect to eliminate obstruction to the natural flight stance and elytra positioning.

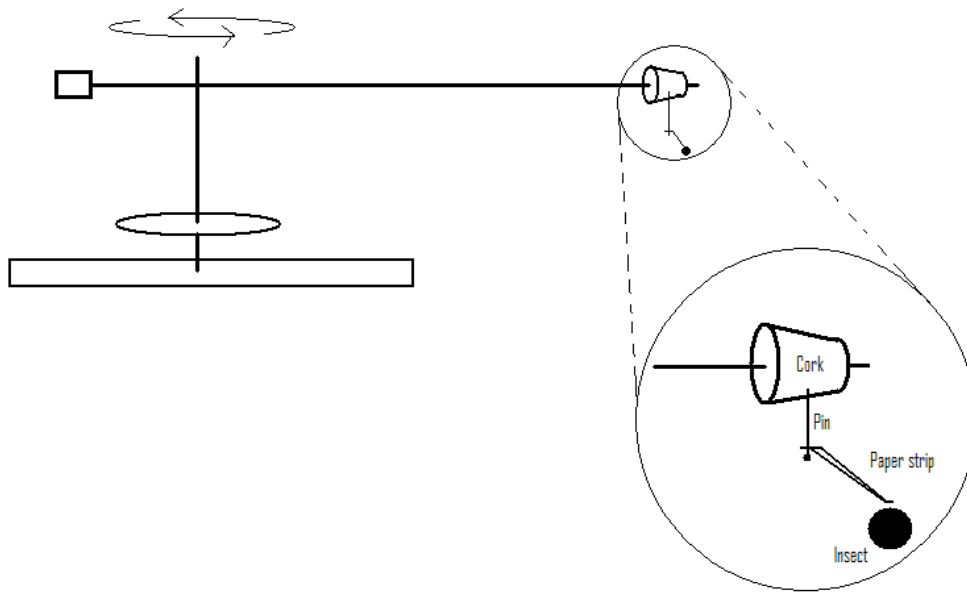


Figure 18. Flight mill apparatus. Enlargement window illustrates the orientation of the paper strip in relation to the tethered insect.

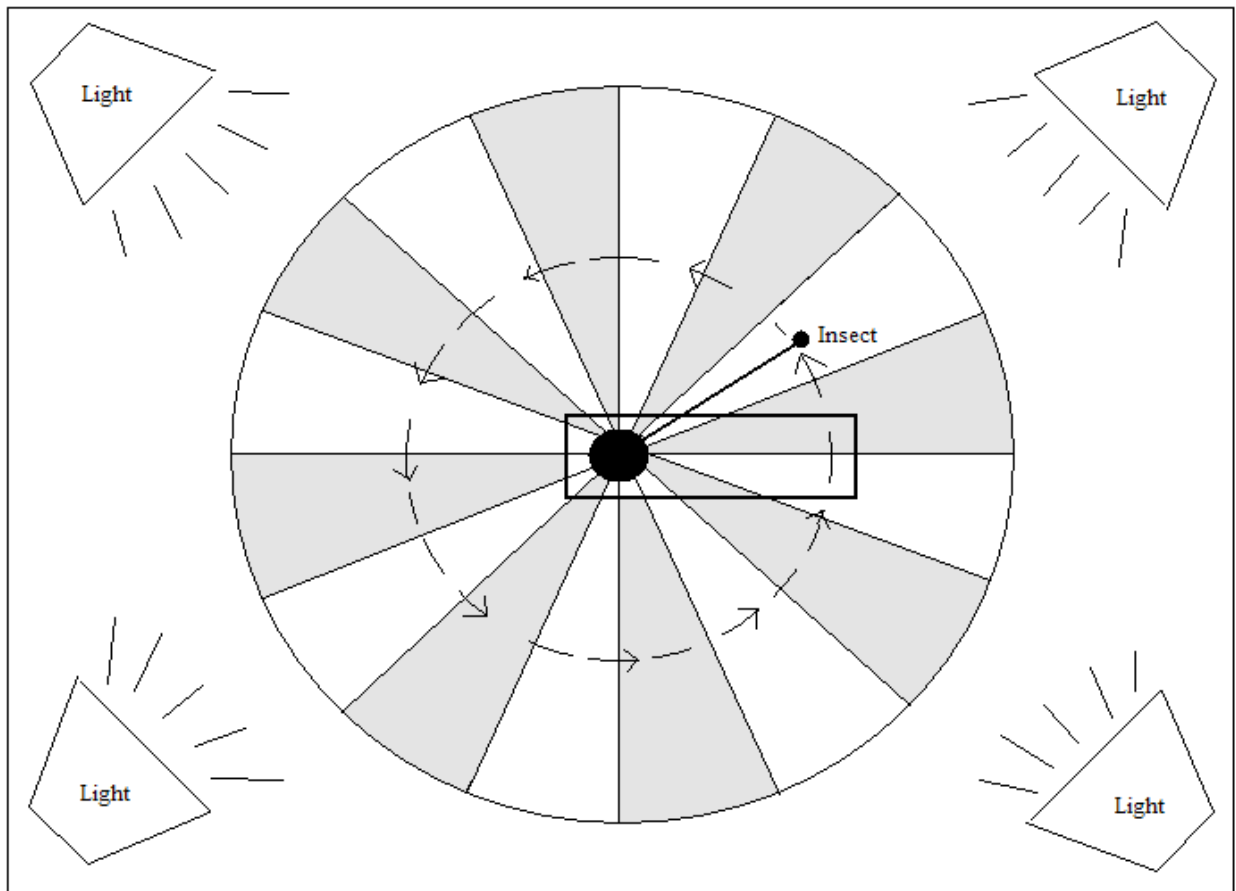


Figure 19. Top view schematic of the flight mill apparatus with the associated light/heat sources and alternating black and white stripes.

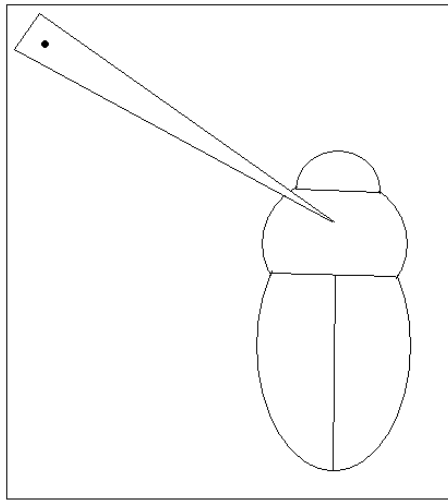


Figure 20. Schematic of tethering method used to attach adult weevils to the flight mill (paper strip is approximately 20 mm long).

Table 1. Mean flight parameters of male and female lesser chestnut weevil, *Curculio sayi*, based on a laboratory flight mill study.

Parameters	Female (n = 11)	Male (n = 10)
Mean Duration (in seconds)	577.6 a	687.8 b
Maximum	7,205	7,035
Minimum	13	31
Mean Distance (in meters)	226.6 a	247.1 a
Maximum	3,044	2,517
Minimum	8.3	10.4
Mean Speed (in cm/sec)	41.4 a	41.9 a
Maximum	75.9	60.1
Minimum	16.3	29.1

Mean per flight parameter row followed by the same letter are not significantly different (Fischer's PLSD, $P < 0.05$)

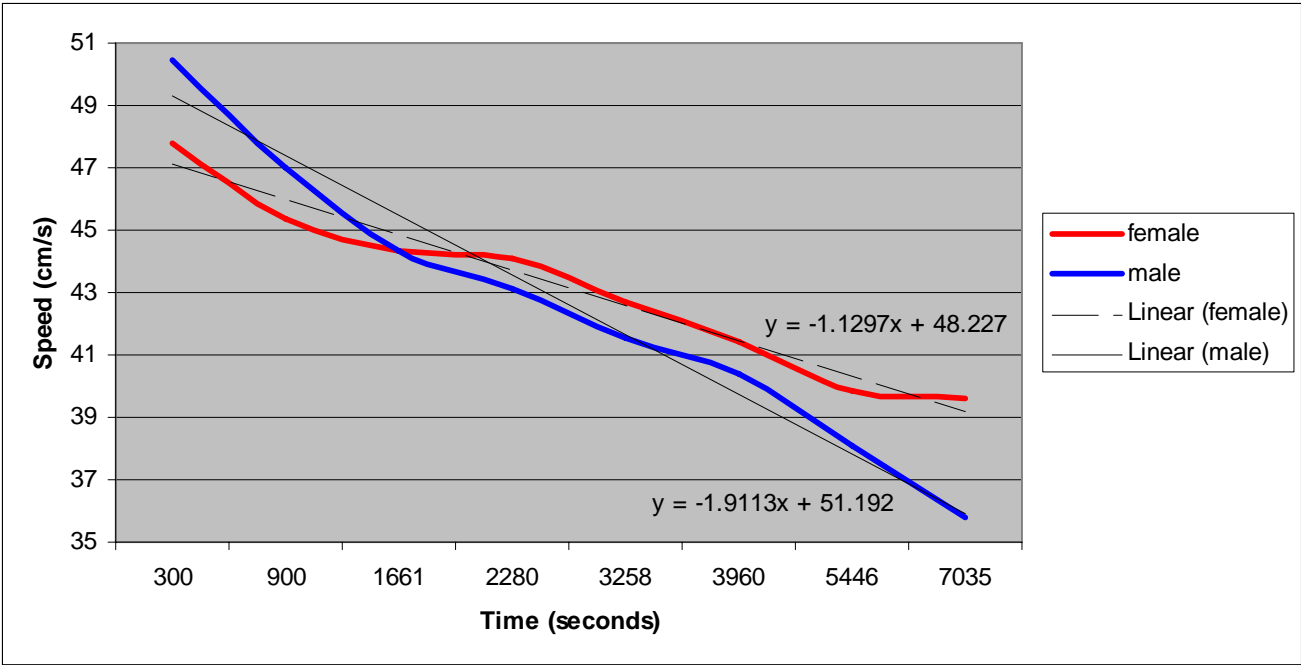


Figure 21. Flight speed over time of male and female lesser chestnut weevils, *Curculio sayi*, that had flown for approximately 2 hours.

CHAPTER V

Thesis Summary and Conclusions

The process of pest control often begins with a foundation of ecological research to establish baseline biological information about the target insect. Due the paucity of recent primary literature on the chestnut weevil species found in the United States, specifically the greater chestnut weevil (*Curculio caryatrypes*, Boheman) and the lesser chestnut weevil (*Curculio sayi*, Gyllenhal), it was necessary to examine their ecology thoroughly. More attention was given to *C. sayi* in this research as this species was reported to be the more economically important pest. Three major objectives of this research project were designed and conducted to address some of the basic ecological parameters necessary to prepare long-term control measures in commercial chestnut orchards.

First, it was established that *C. sayi* adults emerge twice per year, an occurrence that was not previously noted in the literature. Spring emerging adults were captured over the month of May, and occasionally into June. The fall emerging adults were captured during the month of August and into early-September. Peak activity in the trees, as measured by pyramid and circle traps, occurred in the fall. The heightened activity of the adults present in the fall, as compared to those in the spring, coincided with the tree's production of nuts for oviposition. Dates of emergence and activity were consistent over the tree years of this study (2004-2007).

Second, it was determined that *C. sayi* larvae burrow no more than 23 cm underground, a finding that was supported by the current literature on the European chestnut weevil and the older literature on the American chestnut weevil species. Based

on this two year study that examined the underground development of *C. sayi*, it seems apparent that the minimum duration of the larval diapause is 17 months under the natural environmental conditions of Mid-Missouri, with a few larvae requiring additional time before they entered the pupation and adult stages.

Third, the flight capabilities of *C. sayi* adults, both male and female, were estimated to be a maximum of 2 to 3 km in a single flight, though average flights were closer to 0.25 km for both genders. The adults flew on average for 9 – 10 minutes, though a few *C. sayi* flew for 2 hours. It seems apparent that this insect is perhaps better at flight than we initially estimated, and further study is required to ascertain the extent of its potential dispersal ability over multiple flights and a longer time frame. It should be noted that all adults used in the flight study were collected in the spring, and that it is possible there would be some variation in flight parameters when comparing adults collected in the fall.

Overall, a stronger grasp of the seasonal occurrence and ecology of *C. sayi* in Mid-Missouri has been achieved. Future research will progress towards establishing and testing control measures.

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VITA

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Ian plans to continue on to his PhD at the University of Missouri in 2008. Future research plans include continued work with *C. sayi*, with added emphasis on chemical ecology and establishing functional control measures in chestnut tree orchards through pheromone studies of chestnut volatiles.