Ecology and Management of Plum Curculio, *Conotrachelus nenuphar* (Coleoptera: Curculionidae) in Alabama Peaches

by

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Abstract

Peach production is a major industry in Alabama and many other southeastern states of the United States. Over 40,000 acres of fresh and processed peaches worth $65 million are produced annually in Alabama, Georgia, and South Carolina. In Alabama alone, approximately 22 million pounds of peaches were produced in 2001 with a market value of about $12 million. The plum curculio, *Conotrachelus nenuphar* Herbst (Coleoptera: Curculionidae) is the most serious economic pest of peaches in Alabama and other parts of the southeastern U.S. This study was conducted to develop and implement cost-effective and environmentally friendly pest management practices for plum curculio. Specific objectives are: (1) Field evaluation of traps and lures for monitoring plum curculio in Alabama peaches, (2) Laboratory evaluation of behavioral response of plum curculio to synthetic host plant volatiles and male-produced aggregation pheromone (grandisoic acid); (3) Seasonal occurrence and development of a degree-day model for forecasting spring emergence of plum curculio in Alabama peaches; (4) Field evaluation of targeted insecticide sprays against plum curculio in Alabama peaches and (5) Evaluation of the effects of soil and weed management practices on plum curculio pupal development and adult emergence in Alabama peaches.

In chapter II, the effectiveness of two widely used trap types (pyramid versus Circle traps) and commercially available synthetic lures for monitoring plum curculio was evaluated in two peach orchards in Alabama during 2008 and 2009. The lures evaluated alone or in combinations included benzaldehyde or BZ (a component of fruit odor), plum essence or PE (mixture of fruit odor extracted from food grade plum), and grandisoic acid or GA (male-produced aggregation pheromone of plum curculio). The results showed that pyramid traps captured more plum curculio adults than Circle traps, particularly, during the first generation. Trap performance was improved numerically by the addition of BZ, PE or GA alone (single lures), and was significantly enhanced by the addition of the combined BZ + PE lure. A follow-up study was conducted in the laboratory (Chapter III) to determine the influence of physiological factors (sex, age, diet and mating) on the response of plum curculio to the commercial lures (BZ, PE and GA) in four-choice olfactometer bioassays. The results showed that the physiological state of the weevils did not affect their response to the lures. In Chapter IV a degree-day model was developed for forecasting spring migration of plum curculio in peaches using historical temperature and trap capture data. The degree-day model predicted well the first and peak trap captures of plum curculio adults occurred in peach orchard. January 1 at a lower temperature threshold (LTT) of 10°C were found to be a better combination for accumulation of degree-days in peach orchards in Alabama. A six-order polynomial function fitted best to seasonal trap captures and cumulative degree-days, and revealed three overall seasonal peaks with the first (spring generation), second (summer generation), and third (summer generation) peaks occurring at cumulative degree-days of ca 220, 1122 and 1932 (base 10°C, biofix of January 1), respectively. The three-parameter Weibull model predicted the first trap and first peak (spring generation) trap captures to occur at mean cumulative degree-days of 108.02 ± 9 and 220.07 ± 16, respectively. In chapter V, studies were conducted in a peach orchard in Alabama during 2007 to 2009 to compare the conventional calendar-based insecticide spray program involving weekly applications of phosmet (Imidan®) to three different reduced spray programs using three targeted (well-timed) insecticide sprays (TIS) of phosmet, permethrin

(Arctic®), or thiamethoxam (Actara®) applied in an alternated fashion. All the three TIS programs significantly reduced plum curculio damage at harvest compared to the untreated control in two of the three years (2008 and 2009). Fruit damage due to stink bugs, which are emerging pests of peaches in the region, was also significantly reduced in the TIS programs in both years. In chapter VI the effects of soil and weed management practices on development and emergence of plum curculio was investigated in both field and greenhouse studies. Significantly fewer plum curculio adults emerged from centipede grass understory treatment than from other soil and weed management treatments. The results have identified promising tactics for the development of an IPM program for plum curculio in the southeastern United States.

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Dedication

This work is dedicated to my dear father, Mr. Ebenezer Kwesi Akotsen

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 History, Production and Uses of Peaches

Peaches (*Prunus persica* L.) belong to the family Rosaceae which has several species. The plant has been grown in the world since the past 4000 years (Malcolm 2006). Historically, the peach plant is believed to have originated from China and was spread through most parts of the world, particularly, Europe and England by the Greeks and Romans along the silk trading routes and most recent past the crop spread from its homeland to the western world through India and Persia (Iran) where it was cultivated before being introduced into Europe (Borris and Brunke 2006). Currently, China is the leading producer of peaches worldwide (Table 1). Peaches are now commercially grown in 71 countries around the world mainly for human consumption (http://www.uga.edu/fruit/peaches).

In North America, it is believed that peach trees were first introduced into the colonial settlements of the United States (U.S.) by the French explorers in 1562 at territories along the Gulf Coast region near Mobile, Alabama, then by the Spaniards who established Saint Augustine, Florida in 1565 on the Atlantic seaboard (Malcolm 2006). Since that time, peach orchards have spread throughout the northern, eastern and western U.S.

Peaches have a variety of uses among which include provisions of basic nutrients such as vitamins (vitamin A, vitamin B1, vitamin B2, and niacin), proteins, carbohydrate, minerals (calcium, phosphorus, iron, and potassium) and high fiber (Malcolm 2006). They also have medicinal uses (Seth 2004). For example, different parts of the plant have been used extensively to cure several diseases, such as relieve vomiting and morning sickness during pregnancy, constipation in the elderly, coughs and bronchitis, making it a good medicinal plant (Grieve 1984, Duke and Ayensu 1985, Brown 1995). Peaches can also be used to make dyes (Grae 1974).

Table 1. Peach production in leading countries and the world, 2001-2005 (1,000 short tons)

Country	2001	2002	2003	2004	2005
China	5,055	5,798	6,812	6,426	6,647
Italy	1,883	1,749	1,296	1,874	1,919
United States	1,479	1,568	1,533	1,576	1,509
Spain	1,193	1,406	1,401	1,010	1,246
Greece	1,040	815	275	872	751
Others	4,802	5,052	5,110	5,107	5,205
World	15,453	16,388	16,426	16,865	17,277

Source: Pollack, S. and A. Perez (2007) Fruit and Tree Nuts Situation and Outlook Yearbook/FTS-2007/October, ERS/USDA.

1.2 Economic Importance and Production Constraints

With the exception of grapes and apples, peaches are the most extensively grown temperate fruit in North America. In the U.S., peach related activities (planting, harvesting, distribution etc) contributes over $6 billion to national gross domestic product making it the 3rd largest non-citrus fruit crop in the country (USDA, NASS, 2006, 2008). Today's successful peach industry is concentrated primarily on the east coast from New Jersey to Florida and on the west coast in California. Large, sweet, white-fleshed clingstone peaches are preferred in most Asian and some European countries, while westerners prefer the yellow-fleshed freestone varieties. However, there is increasing western interest in the white-fleshed fruits.

The main problems associated with the peach industry in most countries are cold hardiness, number of chill hours (Byrne and Bacon 1992), low fruit quality, high production costs, international competition, overproduction (Fideghelli et al. 1998), and pests and diseases (Croft and Hoyt 1983, Prokopy and Croft 1994). Pests and diseases are the most important among these production constraints (Croft and Hoyt 1983, Prokopy and Croft 1994).

Several insects and mites can cause severe crop losses in peaches (Glass and Lienk 1971, Croft and Hoyt 1983, Prokopy and Croft 1994), particularly, in the southeastern U.S. (Horton and Ellis 1989). Notably among these pests and mites are plum curculio, *Conotrachelus nenuphar* Herbst, oriental fruit moth, *Grapholita molesta* (Busck), and several stink bug pest complex like the southern green stink bug, *Nezara viridula* L., the brown stink bug, *Euschistus servus* (Say), the green stink bug, *Acrosternum hilare* (Say), brown mamorated stink bug, *Halyomorpha halys* Stål, and mites pests like two-spotted spider mite, *Tetranychus urticae* Koch, and the European red mite, *Panonychus ulmi* (Koch) (Prokopy 1985, Prokopy et al. 1990, Horton and Ellis 1989, Horton and Johnson 2005). Among these pests, plum curculio is the most important key pest in the southeastern U.S. (Yonce et al. 1995, Johnson et al. 2002, Horton and Johnson 2005).

1.3 Plum Curculio, *Conotrachelus nenuphar* Herbst (Coleoptera: Curculionidae

1.3.1 Description, Biology and Life Cycle

Adult plum curculios are easily confused with other beetles that are captured in monitoring traps (Thomson 1932, Bloem et al. 2002, Fadamiro 2003). Some diagnostic features, however, differentiate them from other weevils. The adult is a small, rough snout beetle, about 4-6 mm long and mottled with black, gray and brown. There are 4 pairs of ridges that occur on the elytra, but the middle humps on each wing cover are larger, which makes it appear to have only two humps. The sharp, biting jaws are located on the tip of a long curved snout. Males are distinguished from females by the concavity of the metathoracic ventrites which is pronouncedly convex in the females (Thomson 1932). Also the two sexes can be differentiated by using the tibial spur which is large and modified in the males but absent in the female (Thomson 1932). Males produce aggregation pheromone which attracts both sexes. Adults begin mating shortly after emerging from soil.

The egg of plum curculio is about 0.4 mm wide, 0.6 mm long, pearly white and elliptical. Egg laying activity starts once the fruit begins to form, with egg hatch occurring after 3 to 12 days at mean daily temperatures of 25 to 18ºC (Whalon et al. 2000, Combs 2001 and reference therein). The female oviposits its egg singly by first constructing a cavity under the skin of the fruit using its beak and then deposits the egg near the mouth of the cavity (Chapman 1938, Armstrong 1958, Calkins et al. 1976). The female then forces the egg into the cavity with her ovipositor or beak (Mampe and Neunzig 1967) and left for the egg to hatch. A crescent-shaped, oblique slit is cut underneath the egg cavity to leave the egg in a flap of flesh (Chapman 1938, Butkewich et al. 1987). The reproductive activity of plum curculio is very high with each adult female capable of producing between 100-500 eggs in it entire life span of about 22 months under optimum laboratory and field conditions. Typically, one egg is laid by the female at a time in a single fruit. However, multiple eggs can be laid by a single female in one fruit, but it is not known whether the multiple eggs laid by a single female are made simultaneously or in two or more visits. The adult females does not host mark the fruit after oviposition so more than two female may lay their eggs in a single fruit (Butkewich et al. 1986). Egg survival rate is low, (about 15%) in apples (Stedman 1904, Whalon et al. 2000) but this can be high depending on the number of reproducing females in a season. No study has, however, been done to determine the oviposition rates as a function of weevil density.

Upon emergence the whitish and legless larva, immediately begins to feed and creates a tunnel into the fruit. The larva measures about 6-9 mm long when fully grown. It is slightly curved or bow-shaped and tapers slightly at each end. It has a brown head and a light brown shield behind the head. There are 4 larval instars (Snapp 1930, 1940). Each stage of the larva can be determined based on the head capsule width (Quaintance and Jenne 1912, Garman and Zappe 1929). A fully grown larva leaves the fruit 14-16 days after fruit drop and enters the soil at a depth of 3-8 cm where it forms a pupation chamber for an additional 10-12 days before transforming into pupae.

The pupa, which is found in the upper 3-8 cm below the soil surface (Horton and Johnson 2005) is whitish or cream-colored and measures 5-7 mm. All of the adult structures (such as eyes, mouthparts, etc.) are visible just before transformation into the adults.

The length of time between oviposition and larval entrance into the soil varies from 17-39 days depending on the type of fruit infested and also on environmental conditions such as temperature, precipitation (amount and time), humidity etc. Also the time taken for adult to emerge from the soil depends on soil conditions. However, it takes about 14-21 days for this to be completed in southern U.S (Lan et al. 2004). The complete cycle from egg to adult takes about 50-55 days (Garman and Zappe 1929, Lathrop 1949, Armstrong 1958, Mampe and Neunzig 1967, Mulder et al. 1997). After adults have emerged from the soil usually in summer, they feed on maturing fruiting and non-fruiting parts of host plants until cold weather forces them into hibernation.

Mating of newly emerged males and females begins shortly after emergence and the peak of mating is reached after 9 days of eclosion. The adult female after eclosion requires food to initiate and maintain oogenesis. Females oviposit 10-14 days after pupal eclosion. In the laboratory study Johnson and Hays (1969) found that plum curculio adults mated as young as 6 days old; a single female mated at the age of 5 days. Males were capable of inseminating matured sperm at this early age, but the females did not oviposit fertile eggs until 8 days old (Johnson and Hays 1969). Males can mate with as many as 16 different females by the time they are 30 days old (Johnson and Hays 1969). The average number of matings per male in a 30-day period is 10.4. Both virgin and non-virgin males were found to mate with more than one female in a 24-hr period. These females all produced large numbers of viable eggs (Johnson and Hays 1969). A single virgin male can mate with four females in one day, and a single non-virgin male can mate with two females in one day (Johnson and Hays 1969). The 1st as well as subsequent matings are responsible for large numbers of fertile eggs being produced. The average sperm content per female increases as the number of matings increases, although females that had mated only once may produce the average number of eggs per female reported by other workers (Johnson and Hays 1969). Females that mated 2 or 3 times produced more eggs during their lifespan than did those that mated only once (Johnson and Hays 1969).

The longevity of the adults varies depending on environmental and physiological conditions. Radiolabeled plum curculios were tracked for nine months after release (Rings and Layne 1953) indicating that the adult can live about 9 or more months. In the laboratory adults reared on thinning apples were in colony for 189 days. The adult can live without food for about 1 month (personal observation).

1.3.2 Distribution and Host Range

The plum curculio is native and endemic to the continental North America (Chapman 1938). Although some isolated populations have been found in Boxelder County in Idaho

(Kim and Alston 2008), plum curculio is generally distributed over the U.S. and Canada, east of the Rocky Mountains (Chapman 1938). The principal host plants include native and exotic rosaceous plant species, although it has been found feeding and developing on a number of other plant taxa (Jenkins et al. 2006a). The hosts of plum curculio include (in ranking order): apple, nectarine, plum, cherry, peach, cherry, apricot, pear, blueberry and quince spp (Beckwith 1943, 1944; Alm and Hall 1986b, Maier 1990, Polavarapu et al. 2004, Sridhar et al. 2004, Leskey and Wright 2007). Plum curculio can also survive on wild plum, as well as hawthorn (*Crataegus*), native crabapple and other tropical and nonhost fruits such as mangoes and passion fruit (Chapman 1938, Wylie 1966, Maier 1990, Hallman and Gould 2004, Jenkins et al. 2006a). Fruit damage from oviposition and larval feeding is economically important in apples, peaches, plums (Levine and Hall 1977), and cherries, where scarring and infested fruit render produce unmarketable. The importance of plum curculio as a pest varies from region to region. Stearn et al. (1935) reported that plum curculio damage peaches more than apples in Delaware. But in Connecticut plum curculio are more important in apples than peaches (Garman and Zappe 1929). Also in the Northeast U.S. plum curculio is important on apples than peaches. This is different in the southeastern U.S., where plum curculio is more important in peaches because peaches are widely grown in this region than apples.

1.3.3 Strains of Plum Curculio

Two strains of plum curculio occur in the United States (U.S.) (Chapman 1938, Bobb1952, Smith 1957, Hoffmann et al. 2004). The northern univoltine strain is usually found in the northeastern U.S., north of Virginia and Canada (Smith 1957, Hoffmann et al. 2004, Leskey 2008) and the southern multivoltine strain is found in the southeastern region (Hoffmann et al. 2004, Leskey 2008). The northern strain is characterized by a reproductive diapause that is broken during the course of overwintering. Diapause in the northern strain is obligatory whereas the southern strains show facultative diapause (Bobb1952). It has been shown that the northern strain does not mate or begin oogenesis until after spring emergence (Smith and Salkeld 1964) which in contrast to the southern strain does not require diapause to initiate reproduction development (Smith 1957, Smith and Flassel 1968).

The two strains are reproductively less compatible (Padula and Smith 1971, Zhang and Pfeiffer 2008). It has been reported that multivoltine females mated with univoltine males had reduced oviposition and egg hatch (Zhang and Pfeiffer 2008). Also laboratory-reared southern strain females have been shown to produce over twice as many eggs as their northern strain counterparts, possibly due to higher fecundity rates and longer period of oviposition.

1.3.4 Factors Affecting Plum Curculio Abundance and Activity

Comparatively little research has been conducted on the biology, host plant-relationships, and behavior of plum curculio. The reason is because of its cryptic coloration, nocturnal and thanatose behavior and lack of demonstrated strong response to visual and olfactory stimuli (Le Blanc et al. 1984, Racette et al. 1990), which makes it difficult to study the beetle in the field (Croft and Hoyt 1983). Field and laboratory data concerning daily and seasonal activity pattern are conflicting (Quaintance and Jenne 1912, Smith and Flessel 1968, Lafleur et al. 1987, Blanchet 1987, Racette et al. 1990, 1991). Although few studies have been done to describe the habitat preference, field observation has revealed that the adult prefer dark areas with relatively high humidity during feeding.

Temperature and moisture have been shown to be the most important environmental factors, which regulate plum curculio activity both in the spring and summer (McGiffen and Meyer 1986, Racette et al. 1990, Chouinard et al. 1992ab, Racette et al. 1998, 2002; Dixon et al. 1999, Mulder et al. 1997). For example, Racette et al. (1990) found in Quebec, Canada, that plum curculio’s activity pattern changes throughout the growing season. Plum curculios were found to drop from apple trees at night before apple fruit set when temperatures are cool. However, they remained active during day and night at fruit set and ‘June drop’ in the northeastern U.S. and ‘April drop’ in southeastern U.S. Also, Whitcomb (1929) found that plum curculio adults hide in protected sites on trees during cool weather. Moisture has been shown to be necessary early in spring to restore normal water relationships within the beetles. The exact role of water in the biology of plum curculio is, however, not clear but insects including plum curculio generally need water to meet their physiological needs. Also, plum curculios have been shown to be more active during warm, damp, cloudy days and in thick, heavy trees that provide abundant dampness in the centers. Other environmental conditions which have been observed to play a major role in plum curculio’s seasonal and diurnal activity include wind and photoperiod (Racette et al. 1990). For example, it has been shown that high winds and low humidity can affect beetles by forcing them to leave trees and burrow into the soil in search of moisture (Mulder et al. 1997).

1.3.5 Plum Curculio Behavior

1.3.5.1 Flight Behavior

Plum curculio is generally known as an infrequent flier (Racette et al. 1992 and references therein, Chen et al. 2006). Owens at al. (1982) and Chouinard et al. (1993) have shown that the most predominant behavior of plum curculio in nature is resting with flying accounting for <1% of the total time spent in trees or when in captivity. One report has shown that spring migration from overwintering sites is done by walking and flight (Prokopy et al. 1999). The extent to which flight is involved in the spring migration of plum curculio and its subsequent movement within and among trees is still unclear. Different results have been obtained on plum curculio flight behavior. For instance, while Owen et al. (1982) reported that plum curculio spent < 1% of the total time spent on apples flying. Prokopy et al. (1999) on the other hand found that flying accounted for 31% of total behavior of plum curculio under apples tree.

Both biotic and abiotic factors have been reported to affect plum curculio's flight behavior. Chen et al. (2006) have demonstrated in the laboratory that plum curculio can have a sustained flight of over 5 min on a flight mill. It was also shown that although females had significantly higher body mass than males, there was no significant difference in flight performed by the two sexes (Chen et al. 2006). It has also been shown that low temperatures significantly influenced flight of plum curculio in caged apple trees placed outdoor and that most flight was commonly observed at noon and midnight, whereas no flight was observed between midnight and 0600 h, a period of relatively low temperatures (Chouinard et al. 1993, 2002, Dixon et al. 1999, Lan et al. 2004). Also Owen et al. (1982) have shown that age-specific flight ability of plum curculio was greatest within a few days after adult emergence, nutritional status being equal, however, individual beetles that were provided with apples as a food source for 2 d after emergence showed considerably improved flight performance compared with those that had been given no food or only water during the same period (Chen et al. 2006). Food resource plays an important role in the development of insects. Plum curculios have been shown to have an outstanding adult longevity with the adult living for ~ 22 months under optimum field conditions (Armstrong 1958). The considerable long survival of plum curculio, particularly, in the absence of food may partly be because of their ability to feign death when disturbed thereby conserving expensive metabolic energy to be used only for survival. It has been shown, however, in the field that the potential longevity of plum curculio is greatly influenced by predation, environmental conditions and type of food (Armstrong 1958).

1.3.5.2 Sound Production

Sound is produced in many insect families. Unlike other organisms that produce sound using vocal cords, insect produce sound by rubbing body-parts together. Insects are small and therefore not easy to find each other. They find each by through a number of ways including sound production. Unlike other insects that produce sound when under some form of stress, sound production in plum curculio does not indicate any stress. The morphological structures involved in sound production were described by Carlysle et al. (1975). Sound is produced by muscular extension and contraction which move the sound structure (stridulitrum) that extend from the medial line of the posterior third of the left elytron that works in unison with a plectrum that is located on the 6th abdominal tergite (Carlysle et al. 1975, Combs 2001). Both males and females can produce sound (Mampe and Neunzig 1966). However, different sounds are produced by the males and females because each sex has a different configuration of the stridulitra (Carlysle et al. 1975).

1.3.5.3 Orientation Behavior

The mechanism involved with plum curculio adult host location and mate recognition is not well understood. Host and mate finding have been reported to depend on olfaction and possibly gustation. Gustatory sensilla have been described from the antennae of plum curculio and a number of other Curculionidae (Alm and Hall 1986a, Hatfield and Frazier 1976, Bland 1981). While most insects are known to use visual cues during foraging and other life processes for survival, there is no evidence which suggest that plum curculio uses visual cues when foraging within fruit canopy of host trees (Leskey and Prokopy 2002, Leskey 2006). Host plant volatiles cues have, however, been reported to be involved in plum curculio host finding (Butkewich and Prokopy 1993, 1997). Alm and Hall (1986a) investigated the antennal sensory structures on plum curculio and found similar structures which are similar to other curculionids used as pheromone receptors.

1.4 Integrated Management of Plum Curculio

Plum curculio pressure can be intense, but existing control measures are quite variable from region to region. Integrated pest management which is decision-based process involving coordinated use of multiple tactics for optimizing the control of all classes of pests (insects, pathogens, weeds, vertebrates) in an ecologically and economically sound manner (Prokopy 2003) is the only strategy that can effectively deal with many pest problems. The search for reliable monitoring systems for plum curculio has been carried out in many areas, particularly, the northeastern U.S. on apples (Prokopy and Wright 1997ab, 1998; Prokopy et al. 1999, 2003). The target stage of control has been theorized to be the adult rather than the larvae and pupae because the larvae are concealed in the fruit and soil. Also trap capture of adults are not correlated with damage and crop sanitation does not appear to be very effective. As a result several approaches to target the adults have been sought with limited success (Prokopy and Wright 1997, 1998; Prokopy et al. 1999, 2003, Piñero and Prokopy 2006, Leskey and Wright 2004ab, Leskey et al. 2005).

Although the control of most pests of pome and stone fruits including plum curculio have been extensively done by the use of insecticides, the situation has changed recently and that emphasis and philosophy toward research on control of fruit pests have rather been geared towards the use of integrated pest management rather than over-dependence on chemicals (Madsen et al. 1970, Hoyt and Burts 1974). The reason for this change was due largely to consumer perceptions about safety and also the fact that many insect pests developed resistance to these insecticides which required that insecticide volumes had to be increased resulting in many adverse effects such as health problems to man, non target organisms, contamination of water bodies etc. The emphasis of pest management through IPM was shifted to the studies on understanding of pest and host plant biology, monitoring, population dynamics, cultural control, biological control, host plant resistance with insecticides been the last resort when all control options fail.

1.4.1 Monitoring (sampling)

Pest monitoring is a critical component of integrated pest management (Bostanian and Coulombe 1986, Prokopy and Croft 1994, Vincent et al. 1999). Monitoring using traps and lure and degree-days assist in detecting the insect pests, timing of control measures, risk assessment and population density estimates (Reissig and Nyrop 1994, Reissig et al. 1998, Suckling 2000). Monitoring can also help in establishing a quantitative relationship between trap captures of a particular pest and the plant damage caused by it. The relationship is then used to define trap capture values that could be used to identify the economic threshold level of a pest for which control measures are necessary (Suckling 2000). Monitoring systems have enabled more effective targeting of major pest control tactics including pesticides and biopesticides (Suckling 2000).

Several traps and lures have been evaluated for their effectiveness in early detection of the arrival of dispersing and migrating of the spring migration of plum curculio in orchards (Tedder's and Wood 1994, Mulder et al. 1997, Prokopy and Wright 1997ab, 1998; Prokopy et al. 1999, 2000, 2001, 2002, 2003, 2004; Piñero et al. 2001, Leskey and Prokopy 2000, 2002, Leskey and Wright 2004ab, Leskey et al. 2001, 2005, 2009, Piñero and Prokopy 2005, 2006). Traps that have commonly been used include pyramid or Tedder's trap, Circle or screen trap, black cylinder, and sticky clear Plexiglas panel traps. The use of these traps has, however, achieved little and variable success (Prokopy et al. 1999, 2000 2003, Leskey and Wright 2004b). The pyramid trap was originally developed for pecan weevils (Tedder's and

Wood 1994) but has also been used to monitor plum curculios migrating from overwintering sites by walking or by flight (Tedder's and Wood 1994, Mulder et al. 1997, Leskey and Wright 2004a). Pyramid trap has performed better than other traps because its silhouette mimics a small tree which leads the adults to move towards it when crawling (Tedder's and Wood 1994, Mulder et al. 1997, Leskey and Wright 2004a). Although pyramid trap, in most cases, resulted in increased capture of plum curculio adult particularly when deployed in association with fruit based and pheromone lures in apple and peach orchards (Johnson et al. 2002, Piñero and Prokopy 2003). A recent study has also demonstrated that pyramid traps made of geotextile was a good alternative to wooden pyramidal traps (Lamothe et al. 2008).

Most of these traps have been used in association with host fruit-based attractants (Johnson et al. 2002, Piñero and Prokopy 2003, Leskey and Wright 2004ab) and a male aggregation pheromone which attracts both the males and females (Eller and Bartelt 1998). Synthetic host fruit volatiles evaluated to date include benzaldehyde, limonene, and ethyl isovalerate. However, results have been variable (Prokopy et al. 2000, Leskey and Prokopy, 2001). For example, studies in Massachusetts have reported that traps baited with grandisoic acid alone captured no more plum curculio's than unbaited traps (Prokopy et al. 2002, 2003). However, when grandisoic acid was combined with any one of the three different synthetic host fruit volatiles (benzaldehyde, ethyl isovalerate, or limonene), captures by baited traps resulted in about twice as high as capture recorded in unbaited traps (Prokopy et al. 2003). Addition of other synthetic fruit volatiles such as decanal, hexyl acetate, and trans-2- hexenal to grandisoic acid, however, did not enhance captures suggesting that benzaldehyde, ethyl isovalerate, or limonene are good attractants for plum curculio. Recent studies have shown that traps baited with compounds identified from volatiles released by foliar and woody tissues of European plum 'Stanley', in combination with benzaldehyde and grandisoic acid captured more plum curculio than traps baited with benzaldehyde or grandisoic acid alone, or a mixture of both (Leskey et al. 2005). However, it was found that the effectiveness of the lures and pheromones depended on field conditions, particularly, temperature, which has been observed to influence the release rate of lure treatments (Leskey and Zhang 2007).

1.4.2 Cultural Control

The purposeful manipulation of the environment to reduce pest infestation and damage is one of the oldest control methods used by early orchardists (Racette et al. 1992). It is currently considered the “cornerstone” of IPM because it involves some of the basic things that can be done without requiring the use of artificial inputs such as insecticides, herbicides fertilizers etc. For example selection of a good variety of a crop that is capable of reducing insect damage is one of the methods that can be used to reduce insect numbers. For plum curculio management the most commonly used cultural management practice is the removal and disposal of dropped fruits from the orchards. In many cases the disposable has involved burial of the fruits in big holes around the periphery of the orchard. Also spring burning of overwintering habitat have been recommended but it practices has been questioned because of the potential threat the burning can cause (Stearns et al. 1935). Another cultural management which has been used in plum curculio management is the removal of neglected or wild hosts (Maier 1990) which has been practiced in some orchards to reduce plum curculio development. Leskey et al. 2008 showed that planting of early-flowering hosts or wild plums could be kept in borders as refuge or a “trap crop,” could reduce migration into orchards. Plastic covers placed over damaged fruits have been evaluated but with little success.

1.4.3 Host Plant Resistance

Plant resistance is considered one of the safest and long term strategies for most insect pest control. Very little work has been done on the search for host plant resistance for pests of tree crops including the control of plum curculio (Myers et al. 2007). Some past studies have, however, been conducted on the breeding of apple cultivars resistant to other key apple pests (Goonewardene et al. 1975, 1979; Goonewardene and Kwolek 1985, Goonewardene 1987, Goonewardene and Howard 1989). Most of these works were conducted with disease-resistant *Malus* selections but not on *Prunus* spp. Field screening trials resulted in at least some apple germplasm accessions showing resistance or partial resistance to woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Knight et al. 1962); rosy apple aphid, *Dysaphis plantaginea* (Passerini) (Alston and Briggs 1970); and *Sappaphis devecta* (Walker) (Alston and Briggs 1968, 1977) but not to plum curculio. In terms of fruit-feeding pests, apple maggot, *Rhagoletis pomonella* (Walsh), has been shown to show differential levels of adult oviposition and larval mortality on certain species of crabapples (Neilson 1967, Pree 1970).

1.4.4 Biological Control

1.4.4.1 Arthropod and Other Natural Enemies

Tree fruits are long-term crops, offering continuity of overwintering sites and habitats for both pests and natural enemies, and hence show some degree of stability. The possibility of directly utilizing biological control agents is generally better in long term crops than short-term plantings. However, for plum curculio no such classical biological control involving the introduction of natural enemies from the host insect’s place of origin has been done because the species is native to North America. Racette et al. (1992) has provided a list of some of the important natural enemies reported by other researchers to attack adult and larvae of plum curculio. These include *Nealiolus collaris* (Brues), *N. rufus* (Riley), *Triaspis kurtogaster* Martin, *Bracon mellitor* Say, *B. politiventris* (Cushman), *B. variablilis* (Provancher)

(Hymenoptera: Braconidae), *Tersilochus conotracheli* (Riley) (Hymenoptera: Ichneumonidae), *Patasson conotracheli* (Girault) (Hymenoptera: Mymaridae), *Myiophasia aenea* Wiedemann, *Cholomyia inaequipes* Bigot (Diptera: Tachinidae), and *Pegomyia fusciceps* Zett. (Diptera: Anthomyiidae) (Riley 1871, Quaintance and Jenne 1912, Snapp 1930, Armstrong 1958, Jenkins et al. 2006b and references therein). All of these species, with the exception of *T. conotracheli* and *B. politiventris*, have been recorded in Georgia or Florida (Jenkins et al. 2006b) and are likely to be found in Alabama and other southern States. The vast majority of these parasitoids utilize a variety of other hosts, although many of their hosts are often found in fruit (Jenkins et al. 2006b). Also several ant species have been reported to attack plum curculios mostly at the stage that develop in the soil (Jenkins et al. 2006b). There is evidence too that vertebrates like toads can remove adult plum curculios or wormy fruit in orchards (Chouinard et al.1992). Recent study in Michigan is also showing renewed interest in the use of pigs to control plum curculio (Koan 2007). It has been reported that apple plots which had pigs feeding in an organic plot showed a significant reduction in plum curculio damage to apple fruit. Less than 3% damage was obtained from plum curculio in the plots with the young pigs and no other treatments used for plum curculio (Koan 2007) although many unmanaged orchards record more than 80% fruit damage.

1.4.4.2 Microbial Control

Several microorganisms have been reported to cause significant mortality to both field (Shapiro-Ilan et al. 2004ab) and laboratory populations of plum curculio (Garman and Zappe 1929, Shapiro-Ilan et al. 2002) and other related beetles (Shapiro-Ilan et al. 2000). Microbial agents that infect plum curculio are generally fungi, bacteria and nematodes. Fungi species that infect plum curculio include the green muscadine fungus *Metarhizium anisopliae* (Metchnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillimen (Garman and Zappe

1929, Pereault 2008). *Beauveria bassiana* was reported to have caused 90% adult mortality of field collected larvae of plum curculio from blueberry fields in North Carolina (McGiffen and Meyer 1986) although the source of contamination was not readily identified. Efficient and economically viable delivery systems still need to be developed. Field conditions are critical in the establishment of entomopathogens in orchards (Kim and Alston 2008). Without adequate establishment, entomopathogens will require repeated applications as a biopesticide. This as was reported by Pereault et al. 2009 will entail continued production, distribution and storage costs that will be passed on to the farmer.

In orchard production systems entomopathogenic nematodes have received wide attention as biological control agents because of their wide host range, ability to kill host rapidly with no reported adverse effects on environment. Strains of entomopathogenic nematodes Steinernematidae and Heterorhabditidae have shown great promise especially for the control of the adult (Shapiro-Ilan et al. 2002, Shapiro-Ilan et al. 2004ab, 2008; Pereault 2008, Pereault et al. 2009) and the immature stages (Shapiro-Ilan et al. 2002, Shapiro-Ilan et al. 2004ab, Pereault 2008, Kim and Alston 2008). The genera *Steinernema* and *Heterorhabditis* are known to have mutualistic association with the bacteria *Xenorhabdus* and *Photorhabdus* which are carried in the intestine of the nematodes to be microbivorous, not directly entomophagous.

1.4.5 Chemical Control

The discovery of DDT in the 1940s and other insecticides led to the focus of most control effort towards the use of insecticides against major pests of fruits (Hoyt and Burts 1974). Since then the control of plum curculio control has for the past 50 years depended largely on organophosphate insecticides, particularly, methyl parathion, azinphos-methyl (Guthion, Bayer CropScience, Research Triangle Park, NC) and to some extent on Phosmet (Imidan, Gowan Company, Yuma, AZ). The effectiveness of various insecticides against internally feeding larval fruit pests including plum curculio was noted in the 1950s (Snapp 1951, Cox 1951, Smith et al. 1956), when the chlorinated hydrocarbons and early organophosphates were being intensely evaluated as replacements for arsenic-based insecticides. Sprays of parathion, EPN, dieldrin, and benzene hexachloride (BHC, or hexachlorohexane-HCH) was observed to reduce larval plum curculio emergence after application to infested prunes (Cox 1949, 1951, Smith et al. 1956). Similar results were seen in peaches (*Prunus* spp.); BHC and parathion were effective curative agents for plum curculio (Driggers and Darley 1949, Bobb 1950, Driggers 1950).

Since the mid-1980s peach insecticide costs have risen significantly. The use of some of these insecticides is now being restricted by Environmental Protection Agency (EPA) in response to the Food Quality Protection Act (FQPA 1996). The restriction or loss of these insecticides will put the peach industry in jeopardy and likely force some peach growers, particularly, in Alabama to resort to the use of unsafe products to ensure the profitability of their investments. Alternative plum curculio control tactics based on effective monitoring and the bottom-up approach concept to pest management is therefore needed in Alabama.

The search for new reduced risks (RR) and organophosphate alternatives (OP-Alt) insecticides to replace Phosmet (Imidan®) and azinphosmethyl (Guthion®) is still on-going (Wise and Gut 2000, Wise et al. 2006, 2007). Recent laboratory and field studies have shown that neonicotinoid insecticides such as thiacloprid (Calypso®), thiamethoxam (Actara®) and imidacloprid (Provado®) can significantly prevent oviposition and feeding damage by plum curculio to apples (Wise and Gut 2000, Wise et al. 2006, Foshee et al 2006, 2008; Hoffmann et al. 2008, 2009). However, many of these materials have not been tested in peaches in Alabama. Other biorationals which have been reported to show some promise in the laboratory for the control of plum curculio is pyriproxifen (Esteem®) and novaluron

(Rimon®). However, these do not seem to have curative activity in the field at current application rates (Hoffmann et al. 2009). Exteem and Novaluron are insect growth regulators which are involved in either inhibiting chitin and subsequently break diapause. Thiamethoxam has also been shown to prevent egg laying of overwintering adult population. Foshee et al. (2006) reported that twice the recommended labeled rates of fipronil, bifenthrin, and malathion exhibited high mortality on adult plum curculios in the laboratory.

1.5 Justification of Study

Although literature on the biology, ecology and behavior of plum curculio seems to be many, several questions still remain unanswered concerning the ecology and management of plum curculio. For instance, the factors mediating movement of plum curculio from overwintering sites to orchards are not well known. There appears to be regional variation in some aspects of the biology of plum curculio including the host range (Jenkins et al. 2006b, Leskey and Wright 2007) and the timing of emergence of overwintering adults is poorly known.

Several lures and a male-produced aggregation pheromone (Eller and Bartelt 1998) have been evaluated in many trapping programs particularly, in the Northeastern U.S, in apples; however, results have been shown to differ from region to region and season to season. The effectiveness of these traps has not been evaluated extensively in Alabama peaches. In addition, no reliable degree-day model has been developed for forecasting activity of plum curculio in Alabama. The lack of accurate and convenient methods for estimating plum curculio population density, particularly, in the early crop season, and the associated inadequacy of information on plum curculio migration behavior have prevented the development of comprehensive integrated pest management programs for peaches and apples (Croft and Hoyt 1983, Whalon and Croft 1984, Lafleur and Hill 1987) in most part of the U.S. These and other problems provide the relevant justification for the development of effective control strategies for the management of plum curculio in Alabama peaches.

1.6 Dissertation Outline, Goals and Objectives

The goal of this dissertation is to develop, evaluate and implement ecologically-based pest management practices for managing plum curculio in Alabama peaches based on pest monitoring, pest modeling, and targeted/reduced use of pesticides and the effect of soil and weed management practices on plum curculio pressure and damage. The dissertation is arranged under three major sections with the basic aim of providing information on the performance of three commercially available lure types which are commonly used in association with some traps (mainly pyramid and circle) to monitor adult plum curculio population in orchards. Secondly, the study provides a testable degree-day model which can be applied for proper decision making on the timing of insecticides that will help in reducing the current calendar spray schedule for plum curculio. Thirdly, this research has resulted in the development of alternative management tactics for plum curculio including cultural and weed management practices. Fourthly, the study provides a better understanding of the ecology of plum curculio in Alabama and the southeastern U.S.

Section I (Chapters II, II and IV) focused on monitoring and pest modeling using degree-day. Section 2 (Chapter V) looked at targeted spray with the goal of reducing the number and cost of insecticide application. In section 3 (Chapter VI) the development of plum curculio larvae and pupae in difference soil and weed management practices maintained by growers in Alabama was studied.

In Chapter II, I evaluated the attractiveness of synthetic host plant volatiles (benzaldehyde and plum essence) alone and in combination with the synthetic male-produced aggregation pheromone, grandisoic acid (GA), using pyramid or Tedder’s and ‘‘Circle’’ or screen traps deployed in an unsprayed peach orchard in 2008 and 2009 peach crop seasons. The lures evaluated alone or in combinations included benzaldehyde or BZ (a component of fruit odor), plum essence or PE (mixture of fruit odor extracted from food grade plum), and grandisoic acid or GA (male-produced aggregation pheromone of plum curculio). The result showed that pyramid traps captured more plum curculio adults than Circle traps, particularly, during the first generation. Trap performance was improved numerically by the addition of BZ, PE or GA alone (single lures), and was significantly enhanced by the addition of the combined BZ + PE lure. The objective of Chapter III was to evaluate the behavioral response of plum curculio to synthetic host fruit volatiles and male-produce aggregation pheromone in the laboratory. The goal was to determine the influence of physiological factors (sex, age, diet and mating) on the response of plum curculio to the commercial lures (BZ, PE and GA) in four-choice olfactometer bioassays. Both BZ and GA lures were not attractive to plum curculio when tested in the laboratory as commercially formulated. PE was released at a much higher rate (1.51 mg/hr) than BZ (0.36 mg/hr) and GA (ca. 0.04 mg/hr), suggesting that the higher attractiveness of PE may be due to its relatively higher release rates and appropriate concentration of the active compound. The physiological conditions of the weevils had no significant effect on their response to the lures. While gas chromatography-mass spectrometry analyses of the lures showed benzaldehyde (BEN) was the main component in both BZ and PE lures, this compound was detected in BZ lure in amounts ~22-fold higher than in PE lure. Thus, the inhibitory effect of BZ lure may be due to the release of BEN at concentrations possibly too high for olfactometer tests, or to 1, 2, 4-trichlorobenzene (TCB), which was detected in BZ lure but not in PE lure.

In Chapter IV I used historical trap capture and temperature data from 2000 to 2008 to establish the seasonal occurrence and a degree-day model for predicting the early spring migration of plum curculios. Linear, polynomial and three-parameter Weibull functions were tested to describe the relationship between weekly trap capture and cumulative degree-day. A six-order polynomial function fitted best to seasonal trap captures and cumulative degree-days, and revealed three overall seasonal peaks with the first (spring generation), second, and third (summer generation) peaks occurring at cumulative degree-days of ca 220, 1122 and 1932 (base 10°C, biofix of January 1), respectively. The three-parameter Weibull model predicted the first trap and first peak (spring generation) trap captures to occur at mean cumulative degree-days of 108.02 ± 9 and 220.07 ± 16, respectively. Validation of the model in the unmanaged orchard in 2009 and 2010 and in a second unmanaged orchard (located 1.6 km from the first) in 2009 showed that the Weibull function was within seven days for its predictions for the first and peak trap captures of the spring generation in 2010 in the first unmanaged orchard.

In chapter V, I evaluated three targeted spray programs versus a conventional spray program evaluated showed that well targeted insecticide sprays of three applications to coincide with plum curculio development stages could be comparable with conventional spray of weekly insecticide application.

In chapter V, studies were conducted in a peach orchard in Alabama during 2007 to 2009 to compare the conventional calendar-based insecticide spray program involving weekly applications of phosmet (Imidan®) to three different reduced spray programs using three targeted (well-timed) insecticide sprays (TIS) of phosmet, permethrin (Arctic®), or thiamethoxam (Actara®) applied in an alternated fashion. All the three TIS programs significantly reduced plum curculio damage at harvest compared to the untreated control in two of the three years (2008 and 2009). Fruit damage due to stink bugs, which are emerging pests of peaches in the region, was also significantly reduced in the TIS programs in both years

In Chapter VI, I evaluated the effect of soil and weed management practices on plum curculio pupal development and adult emergence in Alabama peaches. The following orchard “understory” treatments were evaluated in plots (3 m × 3 m) located under tree canopies in peach blocks in Alabama: i) centipede grass understory, ii) weedy understory, iii) pine bark understory, and iv) no understory (bare soil). The emergence of plum curculio larvae placed in each plot covered with a cone trap was determined. Significantly fewer plum curculio adults emerged from the centipede grass understory treatment than from the other treatments. Additional tests conducted in the greenhouse showed a similar trend. The implementation of the results of this study has identified and provided IPM practices that is vital to the management of plum curculio and will help to maintain the survival of the peach industry in Alabama.

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CHAPTER 2

FIELD EVALUATION OF TRAPS AND LURES FOR MONITORING PLUM CURCULIO, *CONOTRACHELUS NENUPHAR* (COLEOPTERA: CURCULIONIDAE) IN ALABAMA PEACHES

2.1 Introduction

The plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae), is a key pest of tree fruit in eastern North America (Chapman 1938), and one of the most serious economic pests of peaches, *Prunus persica* (L.) Batsch, in the southeastern U.S. (Horton and Ellis 1989, Horton et al. 2008). Peach growers in Alabama and many other parts of the southeastern U.S. typically manage plum curculio by using a calendar-based insecticide program involving six to 12 sprays of broad-spectrum organophosphate and/or pyrethroid insecticides per growing season (Foshee et al. 2008). However, recent cancellations or restrictions of some common fruit insecticides by the Environmental Protection Agency have necessitated development of alternatives to the calendar-based insecticide program for plum curculio. Ongoing research by our program suggests that targeted insecticide spray programs in which insecticide sprays are timed to coincide with key phonological stages of plum curculio may provide a cost-effective and environmentally sound alternative to the calendar program by significantly reducing the number of plum curculio sprays per season. However, the success of a targeted spray approach is highly dependent on the ability to effectively detect and monitor plum curculio activity in the field.

In Alabama and other peach-growing regions, plum curculio adults are known to overwinter in wooded lots adjacent to orchards from where they immigrate into peach orchards in the spring beginning around bloom (Snapp 1930, 1940; Yonce et al. 1995, Johnson et al. 2002). This movement pattern underscores the need for effective monitoring systems, which can detect activity of plum curculio spring immigrants and track the development and activity of their progeny in orchards throughout the season. Studies in some fruit producing regions in the United States have identified candidate traps for monitoring plum curculio. The two most popular traps are black pyramid trap (also called Tedders trap) and “Circle” or screen trap (Tedders and Wood 1994, Mulder et al. 1997, Prokopy and Wright 1998, Prokopy et al. 1999ab, 2000, 2002; Leskey and Prokopy 2002). The pyramid trap mimics tree trunks and are usually deployed in the orchard border or in between tree rows where crawling or flying plum curculio adults are visually attracted to the traps (Tedders and Wood 1994, Prokopy and Wright 1998, Leskey and Wright 2004b). In contrast, the Circle trap, named after Edmund Circle, a Kansas pecan grower is a “passive trap,” that is encircled around the tree trunk to intercept crawling plum curculio adults (Mulder et al. 1997, Prokopy and Wright 1998, Prokopy et al. 1999b, Johnson et al. 2002).

The search for semiochemical attractants for plum curculio has resulted in the identification of various plant-based volatiles, the most attractive of which include benzaldehyde (BZ) and foliar and woody tissue of plum trees (Leskey and Prokopy 2000, 2001; Prokopy et al. 2001, Leskey et al. 2005). Grandisoic acid (GA), a male-produced aggregation pheromone of plum curculio, also was identified as attractive to both sexes of plum curculio (Eller and Bartelt 1996). To date, a synergistic lure composed of BZ and GA, developed in Massachusetts remains the most widely used attractant for monitoring plum curculio in apple (*Malus* spp.) orchards in the northeast (Piñero and Prokopy 2003, Piñero and Prokopy 2006), and in peach orchards in the mid-Atlantic (Piñero et al. 2001, Leskey and Wright 2004b, Leskey et al. 2005). Prokopy et al. (2003, 2004a,b) also developed the trap-tree approach, a simple and effective integrated pest management (IPM) tool that allows growers to determine need for and timing of insecticide applications based on occurrence of fresh oviposition injury by plum curculio to apple fruit that are monitored on a perimeter-row trap tree. The effectiveness of this approach to monitor oviposition activity of plum curculio has been demonstrated recently in seven northeastern states (Piñero et al. 2006), but not in the southern states. Plum essence (PE), a commercially available synthetic mixture of plant essence, has recently been shown to be effective in attracting plum curculio in apple orchards (Coombs 2001, Whalon et al. 2006). Despite the above-mentioned efforts, no truly effective and practical attractant-based monitoring systems are currently available for plum curculio. This is due to several factors including competition from natural odors from host plants and lack of adequate knowledge of the effect of environmental factors on plum curculio trap capture. The majority of the research on evaluation of monitoring traps and lures for plum curculio have been conducted in apple orchards in the northeastern United States (Piñero and Prokopy 2003; Leskey and Wright 2004ab; Leskey et al. 2005). Very little has been done to evaluate the performance of traps and lures for monitoring plum curculio in peach orchards in Alabama and other parts of the southeastern United States. An exception to this was the study by Johnson et al. (2002) that evaluated pyramid versus Circle traps baited with GA for monitoring plum curculio in peach orchards in Arkansas and Oklahoma. The authors concluded that captures of plum curculio in baited traps can be used in combination with fruit damage to time insecticide applications against plum curculio (Johnson et al. 2002). Because regional conditions differ considerably across the continental United States and because most volatile lures depend on temperature driven mechanisms of release of attractant molecules (Leskey and Zhang 2007), it is imperative that lures are evaluated on a regional and perhaps local basis before recommendation for grower use. Furthermore, it is possible that the two geographical strains of plum curculio in the United States, the northern univoltine strain and the southern multivoltine strain (Smith 1957, Hoffmann et al. 2004), may differ in their responses to traps and semiochemicals.

The objective of this study was to evaluate the effectiveness of two widely used trap types (pyramid versus Circle traps) and commercially available lures (synthetic fruit volatiles and aggregation pheromone) for monitoring populations of plum curculio in Alabama peaches. Data from this study, in addition to a degree-day model being developed (unpublished data) may aid the development of an effective IPM program for this pest in the region.

2.2 Materials and Methods

2.2.1 Study Sites. This 2-yr study was conducted during the 2008 and 2009 in two unsprayed peach orchards located at Clanton, Chilton County, AL. The predominant peach variety in each orchard was ‘David Sun’ (early season variety harvested in early to mid-June) and ‘Loring’ (midseason variety harvested in early to mid-July), respectively. The two orchards were 500m apart, each with row spacing of 6.7m and tree spacing of 4.9 m. The peach trees were ~12 yr old, with an average height of 4 m. The David Sun variety orchard (henceforth referred to as David Sun orchard) was bordered to the south by a stretch of woods across the breadth of the entire orchard ~ 30 m from the perimeter row on the west and east sides were two orchards of different cultivars. The west side was bordered by ‘Rich May’ variety that matured and was harvested earlier than David Sun, whereas the east side was bordered by ‘Fireprince’, which matured later than David Sun variety. The north side was an open field with no trees or shrubs. The Loring variety orchard (henceforth referred to as Loring orchard) was bordered to the north and south by open grassland with the closest peach orchard being ~100 m away, to the west by a wheat field, and to the east by a wood lot.

In the David Sun orchard, the first bloom was observed in mid-March, whereas bloom was recorded in late March in the Loring orchard. Except for the application of a fungicide

(Bravo 720 or Captan 50W) early in the season, no systemic or foliar pesticides were applied in both orchards during this study. However, the orchards were conventionally managed in the years preceding this study. Routine orchard floor maintenance was performed during this study by mowing the understory periodically to aid in trap placement and maintenance, and data collection.

2.2.2 Evaluation of Traps and Lures. Two trap types (black pyramid versus Circle) unbaited or baited with various types and combinations of commercially available lures were evaluated. All traps tested in this study were purchased from Great Lakes IPM Inc. (Vestaburg, MI). Trap placement followed that of Prokopy and Wright (1998) and Prokopy et al. (2003). In brief, pyramid traps were placed ~0.6 m from a tree trunk. Circle traps, with a string for attachment were wrapped around the main tree trunk of selected trees. The two trap types were alternated on every other tree along a peach row, which resulted in a ~10 m distance between two traps. Four replicated plots (blocks) were set up in each orchard and blocks were separated apart by at least 24 m.

The following synthetic lures were evaluated singly (alone) or in combinations: benzaldehyde (BZ), plum essence (PE), and grandisoic acid (GA). The following lure treatments were compared in David Sun orchard in both years: i) BZ only; ii) PE only; iii) GA only; iv) BZ + PE; v) BZ + GA; vi) BZ + PE + GA and vii) Control (no lure). However, only five treatments (treatments i, iv, v, vi, and vii) were evaluated in Loring orchard in both years because of its smaller size (i.e. the single PE and GA lure treatments were not tested). The BZ dispenser was a small polyethylene vial containing ~ 5 ml of lure consisting of BZ formulated with 1, 2, 4-trichlorobenzene (TCB) at a ratio of 9:1 (BZ: TCB). The TCB was used as a stabilizing agent to prevent the hydrolysis of BZ to *trans*-stilbene and benzoic acid under UV light and oxidation processes (Leskey et al. 2005). The PE lure was a blend of plant essences (Great Lakes IPM Inc.). The PE lure dispenser was a transparent polyethene sachet with a small cotton thread of about 6.5 cm long through which the lure is released. The GA lure dispenser consisted of a heat-sealed polymer membrane release device obtained from ChemTica International (San Jose, Costa Rica). The position of each lure treatment within a block was re-randomized bi-weekly (fortnightly) to minimize potential effect of treatment location on trap capture. All lures were replaced (with fresh ones) every 2-3 weeks depending on field conditions. For the pyramid traps, a single BZ dispenser was placed in the plastic, funnel shaped top attached to the tip of the trap. The PE and GA dispensers were each attached separately at random positions on the top corner of the pyramid trap using a small push pin or binder clip. In all cases plum curculio adults were captured in a boll weevil trap top attached to the top of the pyramid trap. Similar procedures were used for installing lures on the Circle traps, which also contained boll weevil trap tops for capturing beetles. Trap and lure treatments were deployed on 29 February (during bud swell) and checked weekly for plum curculio adult captures until 24 July (two-three weeks after during harvest) of each year. The date of first plum curculio capture was noted for each trap/lure treatment combination.

2.2.3 Estimation of Release Rates of Lures. The release rates of the BZ and PE lures were determined gravimetrically in the Loring orchard in 2008 and 2009 using the methods described by Leskey and Wright (2004b). Briefly, five fresh lures of each type (BZ or PE) were weighed on a balance (Acculab VI-6kg model, Precision Weighing Balances, Bradford, MA) to determine initial weight. Each lure was then attached to a pyramid trap and placed in the test orchard. The weight of each lure was determined weekly to estimate release rate per day under variable field conditions. The daily average temperatures were recorded to determine any relationship between temperatures and lure release rates. The release rate of the GA lure (25 mg) was not evaluated since it was determined by the manufacturer and in a previous study (Leskey and Wright 2004b) to be ~ 1 mg/day. The mean field release rate (mg/day) of BZ increased from 8.9 in early-mid April to 13.5 in late May-early June. Similarly, mean field release rate (mg/day) of PE increased from 244 in early-mid April to 648 in late May-early June. The mean daily average temperature within the period ranged from 12.7 ± 4.8 in early-mid April to 25.2 ± 3.5°C in late May–early June. In general, similar release rates were recorded in 2009.

2.2.4 Statistical Analyses. Data for each orchard and year were analyzed and presented separately. Trap capture data were not normally distributed and thus were transformed by using the $\sqrt{x} + 0.5$ transformation method. Because two distinct plum curculio generations were recorded in central Alabama in both years, the first (spring) generation from early March to late May and the second (summer) generation from early June to mid September, trap captures were compared by generation. Data were first analyzed by using standard least square analysis of variance (ANOVA) (JMPIN version 7.0.1, SAS Institute 2007) to test for effects of trap, lure, and interactions among both factors on plum curculio trap capture. Seasonal mean trap captures were then calculated for each lure treatment (data for each trap type analyzed separately by generation) and analyzed with ANOVA followed by Tukey-Kramer HSD to determine significant effects of lures and blocks (replicates). To measure the attractiveness of each lure, a response index or RI (Philips et al. 1993, Leskey and Prokopy 2000, Leskey et al. 2001) was calculated by subtracting the total number of plum curculio responding to an unbaited control trap (C) from the total number responding to its corresponding baited trap (BT) dividing by the total number of plum curculio captured by the C and BT traps, and multiplying by 100. Thus, $RI = [(BT-C)/BT+C)] \times 100$. RI was calculated for each replicate and this was used to calculate the mean RI for each lure. A lure was considered attractive only if it had a mean RI value of 25 or more (Leskey and Prokopy 2000). Ratios of interaction (ROIs) were calculated as described by Hammack (1996) and Piñero and Prokopy (2003) to determine the type of interactions (additive, inhibitory, or synergistic) among single and multiple component lure treatments in the David Sun orchard (single lure treatments were not evaluated in Loring orchard). The ROIs, calculated for each replicate, was based on the following relationship; ROI = [(A + GA) + control]/[(A) +GA)], where (A) represents plum curculio captures by traps baited with a particular fruit volatile or combinations of fruit volatiles, (GA) represents captures by traps baited with GA alone, (A + GA) represents captures in traps baited with either single or double fruit volatiles, and control represents the trap capture numbers in unbaited traps. We adopted the rule of thumb that ROI values significantly less than 1 indicate inhibitory effect, equal to 1 indicate additive or neutral effect, and significantly greater than 1 indicate synergistic interaction between lures (Pinero and Prokopy 2003). ROI values significantly less or greater than 1 were established by using Student's t-test (JMPIN version 7.0.1, SAS Institute, 2007). The specific interactions examined were between BZ and GA, BZ and PE, and BZ, PE and GA for the David Sun orchard in both years. Interactions between PE and GA could not be examined because there was no PE + GA treatment. For all data differences between/among treatments were considered significant at $P < 0.05$.

2.3 Results

In 2008, a total of 78 and 52 plum curculio adults were captured in the David Sun and Loring orchards, respectively. Higher trap captures were recorded in 2009 totaling 345 and 264 plum curculio adults in the David Sun and Loring orchards, respectively. Standard least square ANOVA revealed a significant effect of trap on adult captures in the David Sun orchard during the first ($F = 25.13$, df = 1, $P < 0.0001$) and second ($F = 10.37$, df = 1, $P =$

0.0025) generations in 2008, and during the second (F = 4.93, df = 1, P = 0.0319) generation in 2009. Standard least square ANOVA also showed a significant effect of lure on trap captures of plum curculio adults in the David Sun orchard during the first generation (F = 4.90, df = 6, P = 0.0007) in 2008 and during the first (F = 5.62, df = 6, P = 0.0002) and second (F = 4.46, df = 6, P = 0.0014) generations in 2009. In the Loring orchard, lure had a significant effect on adult trap captures during the second generation (F = 10.32, df = 4, P < 0.0001) in 2008 and during the first generation (F = 3.48, df = 4, P < 0.0189) in 2009. In general, the interaction between trap and lure (trap*lure) was not significant in six out of eight cases. A significant trap*lure interaction was recorded only during second generation in the Loring orchard in 2008 (F = 10.32, df = 4, P < 0.0001) and during second generation in the David Sun orchard in 2009 (F =2.97, df = 6, P = 0.0166). Since significant trap*lure interaction was not recorded in most cases, captures of adults in pyramid versus Circle traps (data pooled for all lures) were compared for each generation in each orchard and year using Student’s t-test analysis.

In 2008, significantly greater number of plum curculio were captured in pyramid traps than in Circle traps in the David Sun orchard during the first (t = 17.73, df = 1, P = 0.0001) and second (t = 11.16, df = 1, P = 0.0016) generations (Fig. 1A). Similar results were obtained in the Loring orchard in 2008 with significantly more plum curculios captured in pyramid traps than in Circle traps during the first (t = 6.50, df = 1, P = 0.0153) and second (t = 5.19, df = 1, P = 0.0289) generations (Fig. 1B). In 2009, no significant differences in trap captures were recorded between pyramid traps and Circle traps in the David Sun orchard during the first (t = 0.02, df = 1, P = 0.8923) and second (t = 3.18, df = 1, P = 0.0806) generations (Fig. 1A). In the Loring orchard in 2009, significantly more plum curculio adults were captured in pyramid traps than in Circle traps during the first generation (t = 6.16, df = 1, P = 0.0180) but not during the second (t = 0.14, df = 1, P = 0.7097) generation (Fig. 1B).

In general, ~ 2-5 times plum curculio adults were captured in pyramid traps than in Circle traps (Fig. 1). In 2008, no significant effects of block (replication) were recorded on plum curculio captures during the first (David Sun: $t = 1.15$, df = 3, $P = 0.3396$; Loring: $t = 0.80$, df = 3, $P = 0.5008$) and second (David Sun: $t = 1.38$, df = 3, $P = 0.2584$; Loring: $t = 0.50$, df = 3, $P = 0.6875$) generations. Similarly in 2009, no significant effects of block (replication) were recorded on plum curculio captures during the first (David Sun: $t = 0.33$, df = 3, $P = 0.8065$; Loring: $t = 0.17$, df = 3, $P = 0.9136$) and second (David Sun: $t = 1.62$, df = 3, $P = 0.1969$; Loring: $t = 2.41$, df = 3, $P = 0.0834$) generations.

Lure treatments had significant effect on plum curculio trap captures in both orchards and years of the study (Table 1 and 2). Significant differences among lure treatments were observed only in pyramid traps in both orchards and years (Tables 1 and 2). In general, more plum curculio adults were captured in pyramid traps baited with the combined BZ + PE lure or the three-component BZ + PE+ GA lure than traps baited with single lure or unbaited traps (Tables 2 and 3). Analyses of response index (RI) further confirmed that the combined BZ + PE lure was the most attractive odor treatment for plum curculio, particularly during the first generation (Tables 3 and 4). This was true for both trap types, although significant trap captures were recorded only in pyramid traps. Pyramid traps baited with BZ + PE had the highest RIs during the first generation in both orchards and years (Tables 3 and 4). In contrast, traps baited with BZ + PE + GA had the highest RIs during the second generation in both orchards in 2008, but not in 2009 (Tables 3 and 4).

The ROIs were calculated using the pooled data for the two generations per year, because the aim was to simply determine the type of interactions among single and multiple component lure treatments. In both years in the David Sun orchard, high ROI values were recorded for the combined BZ + PE (ROI = ~ 2) and BZ + GA (ROI = ~ 2.4) baits in both pyramid and Circle traps (Table 5). However, these values were not significantly different from 1 due to high sample errors. Thus, t-test showed only an additive effect of combining BZ and PE and BZ and GA. The ROI values for the three-component BZ + PE + GA lure ranged from 0.75 to 1.1 in both traps and years (Table 5), suggestive of a weak additive effect at best.

The seasonal captures of plum curculio in pyramid traps baited with BZ + PE is presented in Fig. 2 to illustrate the seasonal phenology of the pest in central Alabama. The first plum curculio captures were recorded around the same time (90% bloom) in the different treatments within each orchard. In 2009, plum curculio adults were recorded in the traps earlier in the David Sun orchard (March 13) than in the Loring orchard (March 20), possibly due to early blooming of the David Sun peach variety.

2.4 Discussion

The results showed that pyramid traps captured more plum curculio adults than Circle traps. Trap performance was improved at least numerically by the addition of host plant volatile lures (BZ or PE) and the male-produced aggregation pheromone (GA) of plum curculio. Among the lures, the combined BZ + PE lure increased plum curculio captures over unbaited traps by up to 21 fold. In both orchards and years, pyramid traps baited with the combined BZ + PE lure captured more plum curculio adults than traps baited with single component lures of BZ, PE or GA. The combined BZ + GA lure and the three-component BZ + PE + GA lure also captured numerically more plum curculio adults than unbaited traps or traps baited with the single components lures but the differences were rarely significant. The response index data also supported the above results, which generally hold true for both generations of plum curculio.

Our results agree with previous studies which reported the superiority of pyramid traps over Circle traps and other trap types for monitoring plum curculio adults in fruit orchards (Le Blanc 1982, Le Blanc et al. 1984, Yonce et al. 1995, Mulder et al. 1997, Johnson et al. 2002, Lafleur et al. 2007). In contrast, Johnson et al. (2002) reported similar plum curculio captures in pyramid versus Circle traps in most of their samples. However, Circle traps deployed on tree trunks with circumference < 38 cm had significantly lower plum curculio captures than pyramid traps. The length of the Circle trap bottom used in that study was 38 cm which overlapped on circumferences > 38 cm, thus reducing plum curculio captures (Johnson et al. 2002). Leskey and Wright (2004b) also reported that Circle traps captured significantly more plum curculio adults than pyramid traps in unsprayed orchards. Although not discussed by the authors, this result may also be due to larger tree circumference in the unsprayed orchards. The length of the Circle trap bottom used in the present study was ~ 33 cm, which is smaller than the circumference of most trees in the test orchards (the trees were > 12 years old). Thus, the lower plum curculio trap captures in Circle traps compared to pyramid traps recorded in the present study may not be explained by smaller tree circumferences.

Our data on lure performance are also consistent with previous reports which showed that the combined BZ + GA lure was more effective than single lures for monitoring plum curculio (Leskey and Wright 2004b, Piñero and Prokopy 2003, Leskey et al. 2005). However, it is difficult to completely compare our data with those reported by the above authors since PE was not evaluated in the studies. We recorded no significant differences in plum curculio trap captures among any of the single lures (BZ, PE or GA), or between traps baited with any of the single lures versus unbaited traps. These results are generally similar to those reported by Leskey and Wright (2004b). Among the combined lures, BZ + PE attracted numerically more plum curculio adults than did BZ + GA or BZ + PE + GA. The data which showed no significant effect of combining BZ with GA agree with those of Leskey (2006), who reported that the combined BZ + GA lure was more effective in apples than in peach orchards.

The results on lure performance may be related to the physico-chemical properties of the lures including release rates. We obtained an average field release rate of ~ 11 mg per day for BZ, which is similar to the 10 mg per day reported by Pińero et al. (2001). For PE, we obtained an average field release rate of 405 mg per day, which is ~ 36 times higher than for BZ. Although not determined in this study, the release rate of GA is ~ 1 mg/day (Prokopy et al. 2004a, 2004b; Leskey and Wright 2004b). It is not surprising that the PE lure had a higher release rate than the BZ lure since both lures had different components with different viscosities. The PE lure is composed of ethanol with viscosity of 1.07 cp at 25°C, while the BZ lure consisted of BZ and TCB with viscosities of 1.4 cp at 25°C and 1.89 cp at 25°C, respectively. Viscosity has a direct relationship with evaporation; compounds with high viscosity tend to be released more slowly than those with low viscosity. Given this, the higher viscosity of the lure might have contributed to its slower release compared with PE. Thus, the comparatively higher release rate of PE under orchard conditions may explain in part the enhancement of captures of plum curculio in traps baited with BZ + PE. Further studies are necessary to confirm this prediction.

The data from the analysis of ratios of interaction (ROI) suggest a trend for synergistic interactions between BZ and PE and between BZ and GA in both trap types but the data were not significant due to large standard errors, hence we concluded additive effects. Piñero and Prokopy 2003 reported a synergistic interaction between BZ and GA, a finding consistent with the general view that aggregation pheromones enhance the attraction of beetles to host volatiles (Landolt 1997, Landolt and Phillips 1997). The numerically lower plum curculio trap captures in the three-component BZ + PE + GA bait compared to the two-component BZ + PE bait, plus the < 1 ROI values obtained for the three-component lure in 2009, suggest the possibility of an inhibitory effect of combining BZ + PE + GA. Although an inhibitory interaction cannot be concluded due to lack of statistical significance, further studies are necessary to confirm this possibility. Nevertheless, our results provided no economic or scientific basis for using the three-component (BZ + PE + GA) lure to monitor plum curculio in Alabama peach orchards.

The low plum curculio trap captures recorded in this study are fairly typical of studies in commercial fruit orchards (Johnson et al. 2002, Leskey and Wright 2004b), and are not surprising given that the test orchards were conventionally managed (including routine applications of conventional insecticides) in the years preceding this study. Overall plum curculio trap captures appeared lower in 2008 than in 2009 but the difference was not statistically tested since this study was not designed to compare years. We recorded no significant block (replication) effects on plum curculio trap captures, contrary to previous a report by Leskey et al. (2001) in which the effect of replications was significant, which was suggestive of a border effect. The lack of a block effect in the present study may suggest that a significant proportion of plum curculio adults overwintered in the test orchards instead of in adjacent wood-lots, thus diluting potential border effect due to immigration of plum curculio adults. Other authors have also reported that plum curculio adults are capable of overwintering within fruit orchards (Lafleur et al. 1987, Leskey and Wright 2004b, Piñero and Prokopy 2006).

In summary, this study demonstrated the potential utility of pyramid traps baited with the combined benzaldehyde (BZ) and plum essence (PE) lure for monitoring plum curculio in peach orchards in Alabama and other parts of the region. The results which showed the efficacy of baited pyramid traps in detecting activity of plum curculio spring immigrants, suggest a role for this monitoring system in the development of a targeted insecticide spray and IPM program for plum curculio. Future studies are necessary to confirm the efficacy of PE established in this study, investigate factors affecting response of plum curculio adults to the lures, and test the ability of baited traps to predict fruit injury by plum curculio.

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Table 1. Mean (± SE) total number of plum curculio adults captured in pyramid and Circle traps baited with synthetic formulations of benzaldehyde (BZ), plum essence (PE), or grandisoic acid (GA) singly or in combinations, versus unbaited (control) traps during the first and second generations in David Sun peach orchard (Clanton, AL) in 2008 and 2009

	2008				2009			
Lure Treatment	First generation		Second generation		First generation		Second generation	
	Pyramid	Circle	Pyramid	Circle	Pyramid	Circle	Pyramid	Circle
BZ	1.00 ± 0.12b	0.00 ± 0.07	0.50 ± 0.34	0.00 ± 0.00	1.25 ± 0.36b	2.50 ± 0.74	1.75 ± 1.42b	1.50 ± 0.87
PE	1.50 ± 0.61ab	0.25 ± 0.19	0.00 ± 0.28	0.00 ± 0.00	2.75 ± 0.91b	1.75 ± 0.26	4.50 ± 0.84ab	3.50 ± 0.79
GA	1.00 ± 0.50b	0.00 ± 0.07	0.50 ± 0.30	0.00 ± 0.00	1.00 ± 0.45b	1.50 ± 0.78	1.00 ± 0.60b	1.75 ± 0.17
BZ+PE	3.75 ± 0.41a	1.25 ± 0.48	1.50 ± 1.16	0.00 ± 0.00	5.50 ± 1.65ab	5.00 ± 1.95	8.25 ± 1.39a	2.75 ± 0.38
BZ+GA	1.50 ± 0.48ab	0.50 ± 0.32	0.50 ± 0.37	0.00 ± 0.00	7.25 ± 1.30a	5.50 ± 1.17	2.00 ± 0.85b	3.00 ± 0.50
BZ+PE+GA	1.75 ± 0.67ab	0.50 ± 0.28	1.50 ± 0.13	0.00 ± 0.00	3.75 ± 1.01ab	4.75 ± 1.21	5.50 ± 1.29ab	2.50 ± 1.29
Control	0.75 ± 0.51b	0.25 ± 0.19	1.00 ± 0.81	0.00 ± 0.00	1.75 ± 1.22b	1.50 ± 0.54	1.50 ± 0.81b	1.50 ± 0.22
F	3.49	2.27	0.81	0.00	4.04	2.37	5.37	1.01
P	0.0182	0.0832	0.5756	0.000	0.0097	0.0733	0.0025	0.4513

Means within the same column having no letter in common are significantly different (ANOVA, Tukey-Kramer HSD, $P < 0.05$, df = 6, 18).

Table 2. Mean (± SE) total number of plum curculio adults captured in pyramid and Circle traps baited with synthetic formulations of benzaldehyde (BZ), plum essence (PE), or grandisoic acid (GA) singly or in combinations, versus unbaited (control) traps during the first and second generations in Loring peach orchard (Clanton, AL) in 2008 and 2009

	2008				2009			
Lure Treatment	First generation		Second generation		First generation		Second generation	
	Pyramid	Circle	Pyramid	Circle	Pyramid	Circle	Pyramid	Circle
BZ	0.50 ± 0.41	0.00 ± 0.13	0.75 ± 0.39b	0.00 ± 0.00	1.50 ± 0.68	2.50 ± 0.86	1.25 ± 0.85	2.00 ± 0.23
BZ+PE	1.75 ± 0.40	0.75 ± 0.37	5.25 ± 1.29a	0.00 ± 0.00	7.00 ± 1.19	3.25 ± 0.44	3.75 ± 0.42	2.75 ± 1.16
BZ+GA	1.50 ± 0.91	0.25 ± 0.29	0.00 ± 0.39b	0.00 ± 0.00	6.50 ± 1.99	3.00 ± 0.56	4.25 ± 2.00	4.00 ± 1.04
BZ+PE+GA	1.25 ± 0.73	0.25 ± 0.14	0.50 ± 0.22b	0.00 ± 0.00	6.75 ± 1.99	3.75 ± 0.82	2.50 ± 0.77	2.75 ± 0.19
Control	0.50 ± 0.32	0.00 ± 0.13	0.25 ± 0.29b	0.00 ± 0.00	3.25 ± 0.33	1.25 ± 0.79	2.50 ± 0.77	1.50 ± 0.41
F	0.74	1.36	11.90	0.00	2.49	1.42	0.92	1.04
P	0.5829	0.3036	0.0004	0.0000	0.0991	0.2871	0.4850	0.4272

Means within the same column having no letter in common are significantly different (ANOVA, Tukey-Kramer HSD, *P* < 0.05, df = 4, 12).

Table 3. Response indices of the various lure treatments evaluated in David Sun peach orchard (Clanton, AL) in 2008 and 2009

Lure treatment	2008				2009			
	Pyramid		Circle		Pyramid		Circle	
	First generation	Second generation	First generation	Second generation	First generation	Second generation	First generation	Second generation
BZ	0.00	-8.33	-25.00	0.00	-6.68	-16.68	27.50*	-33.35
PE	20.82	-25.00	0.00	0.00	29.18*	47.23*	25.83*	38.33*
GA	-25.00	10.00	-25.00	0.00	-20.00	30.00*	-5.83	5.00
BZ+P E	67.50*	25.00*	50.00*	0.00	57.48*	66.88*	45.00*	18.33
BZ+GA	12.50	10.00	25.00*	0.00	55.78*	20.00	65.90*	27.38*
BZ+PE+GA	8.33	66.68*	25.00*	0.00	37.78*	59.23*	63.90*	-10.48
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

*denotes response index greater than or equal to 25, which is significantly more attractive than control.

Table 4. Response indices of the various lure treatments evaluated in Loring peach orchard (Clanton, AL) in 2008 and 2009

Lure treatment	2008				2009			
	Pyramid		Circle		Pyramid		Circle	
	First generation	Second generation	First generation	Second generation	First generation	Second generation	First generation	Second generation
BZ	-16.68	0.00	0.00	0.00	-48.93	-36.68	6.67	-6.68
BZ+PE	45.83*	9.45	50.00*	0.00	33.58*	4.20	35.85*	23.23
BZ+GA	8.33	-25.00	25.00*	0.00	24.65	23.50	40.83*	13.23
BZ+PE+GA	15.00	25.00*	25.00*	0.00	26.78*	0.83	24.53	16.68
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

*denotes response index greater than or equal to 25, which is significantly more attractive than control.

Table 5. Type of interaction found between single and combined attractants (synthetic fruit odors and grandisoic acid) when evaluated as baits in pyramid and Circle traps in David Sun peach orchard (Clanton, AL) in 2008 and 2009

		2008		2009	
Trap type	Lure type	ROI (Mean ± SE)	Type of interaction	ROI (Mean ± SE)	Type of Interaction
Pyramid	BZ + GA	2.0 ± 0.8*	Additive	2.1 ± 0.7*	Additive
Circle	BZ + GA	N/A	N/A	2.4 ± 0.9*	Additive
Pyramid	BZ + PE + GA	1.1 ± 0.1	Additive	0.96 ± 0.1	Additive
Circle	BZ + PE + GA	0.9 ± 0.3	Additive	0.75 ± 0.3	Additive

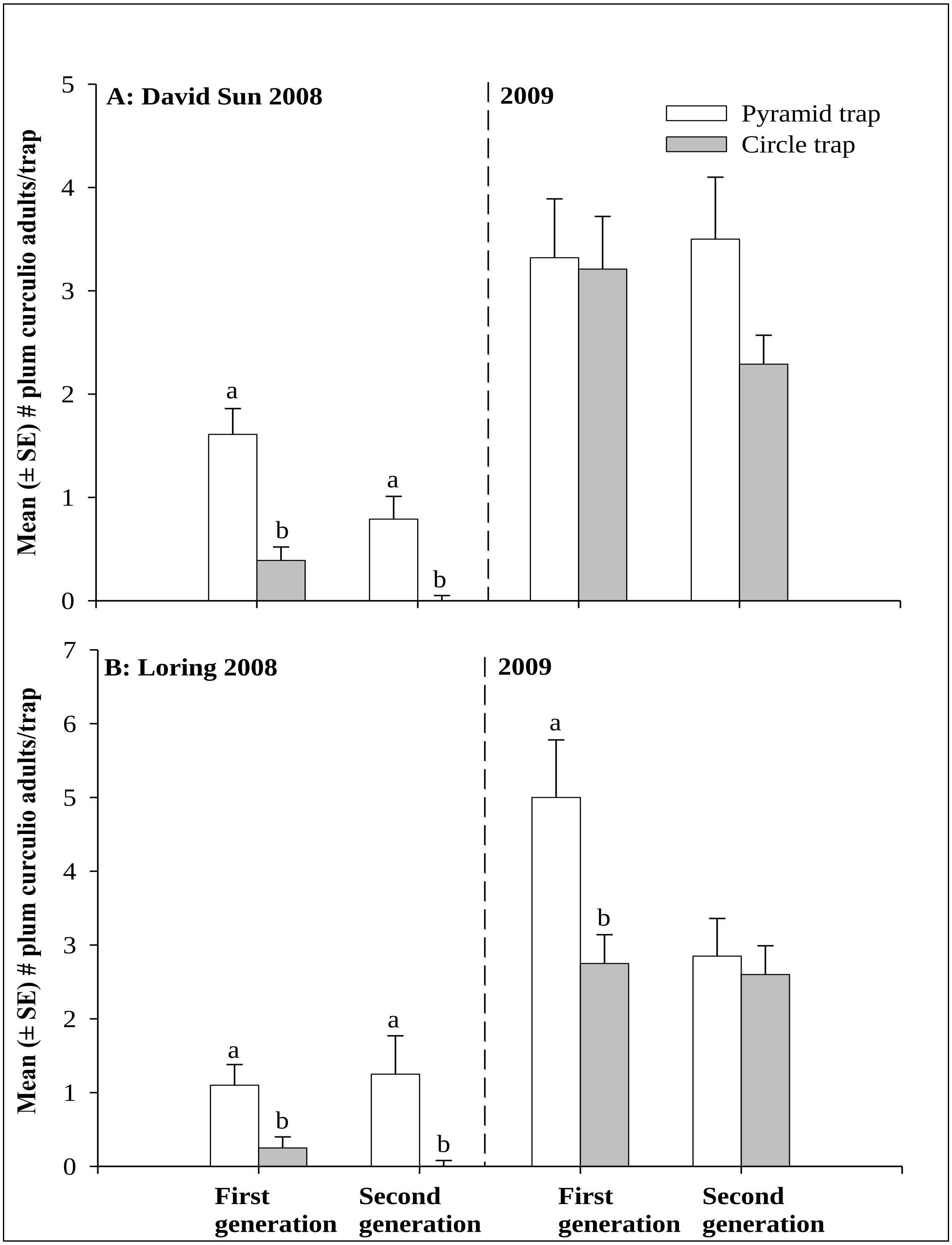
ROI = ratios of interaction; BZ = benzaldehyde; PE = plum essence; GA = grandisoic acid N/A = ROI was not calculated due to zero trap captures; *denotes high mean ROIs which could be indicative of synergistic interaction but not significant due to high sample errors.

Figure Legends

Figure 1. Mean (± SE) total captures of plum curculio adults in pyramid versus Circle traps during the first and second generations in (A) David Sun orchard (2008 and 2009), and (B) Loring orchard (2008 and 2009). Means for each generation having no letter in common are significantly different between trap types (ANOVA, t-test $P < 0.05$).

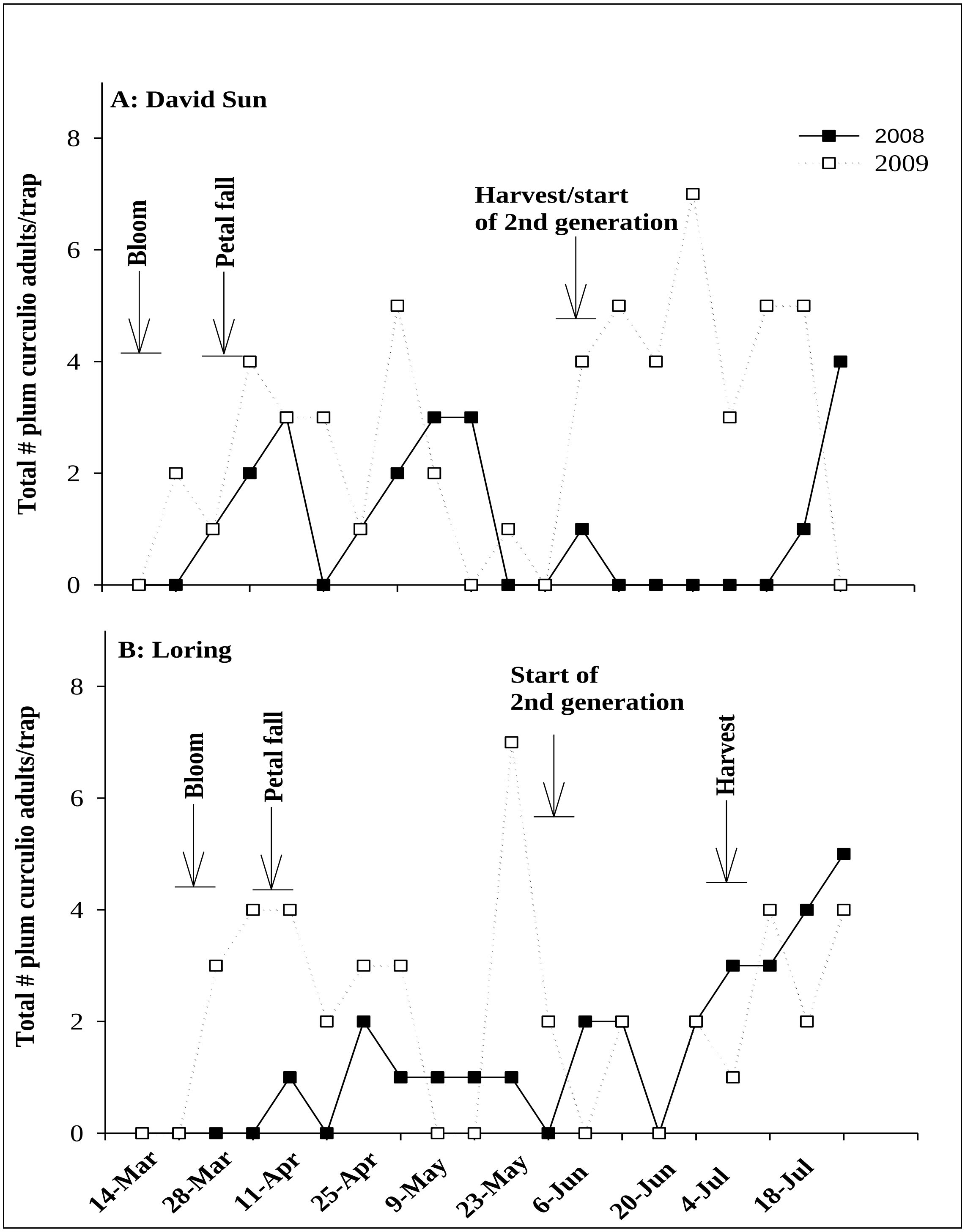
Figure 2. Captures of plum curculio adults in pyramid traps baited with BZ + PE in (A) David Sun, and (B) Loring orchards (Clanton, AL), in relation to peach phenology in 2008 and 2009.

Figure 1



A: David Sun 2008
2009
Pyramid trap
Circle trap
Mean (± SE) # plum curculio adults/trap
0
1
2
3
4
5
a
b
a
b
B: Loring 2008
2009
0
1
2
3
4
5
6
7
a
b
a
b
a
b
First generation
Second generation
First generation
Second generation

Figure 2


A: David Sun
Total # plum curculio adults/trap
0
2
4
6
8
Bloom
Petal fall
Harvest/start of 2nd generation
2008
2009
B: Loring
Total # plum curculio adults/trap
0
2
4
6
8
Bloom
Petal fall
Start of 2nd generation
Harvest
14-Mar
28-Mar
11-Apr
25-Apr
9-May
23-May
6-Jun
20-Jun
4-Jul
18-Jul

CHAPTER 3

BEHAVIORAL RESPONSE OF PLUM CURCULIO, *CONOTRACHELUS NENUPHAR* HERBST (COLEOPTERA: CURCULIONIDAE) TO SYNTHETICFRUIT VOLATILE LURES AND AGGREGATION PHEROMONE

3.1 Introduction

Plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae), is a major pest of many stone and pome fruit crops, which is widely distributed over the United States (U.S.) and Canada, east of the Rocky Mountains (Chapman 1938, Beckwith 1943, Mampe & Neunzig 1967, Racette et al. 1992). It is the most important direct pest of peaches (*Prunus persica* L.) in the southeastern U.S. (Horton and Ellis 1989, Horton 1998, Johnson et al. 2002, Lan et al. 2004, Akotsen-Mensah et al. 2010). Adult weevils typically overwinter in wooded lots adjacent to orchards or around fence rows from where they immigrate into peach orchards in the spring beginning around bloom (Snapp 1930, 1940; Lafleur et al. 1987, Johnson et al. 2002).

Like many insects, plum curculio uses olfactory cues; specifically host fruit derived volatiles, to locate host fruit (Butkewich and Prokopy 1993, 1997; Prokopy et al. 1995, Leskey and Prokopy 2000, Leskey et al. 2001, Prokopy et al. 2003, 2004). Earlier studies identified several active compounds from host plants (both fruiting and nonfruiting parts) which are attractive to plum curculio (Leskey and Prokopy 2000, 2001; Leskey et al. 2001, Prokopy et al. 2001). To date, the most attractive plant-based volatiles for plum curculio are benzaldehyde (BZ) and foliar and woody tissue of plum trees (Leskey and Prokopy 2000, Prokopy et al. 2001, Leskey et al. 2005). Benzaldehyde has since been formulated as an attractant for plum curculio and is commercially available as a lure. Plum essence (PE), a commercially available synthetic mixture of plant essence, is also a known attractant for plum curculio (Coombs 2001, Whalon et al. 2006). The male-produced aggregation pheromone of plum curculio, grandisoic acid (GA), which was identified by Eller and Bartelt (1996), was later shown to act synergistically with BZ in increasing adult trap captures in the field (Piñero and Prokopy 2003, 2005).

Consequently, a combined lure consisting of BZ and GA was developed in Massachusetts (Piñero and Prokopy 2003), and remains to date the most widely used attractant for monitoring plum curculio in orchards (Piñero et al. 2001, Piñero and Prokopy 2003, Leskey and Wright 2004b, Leskey et al. 2005, Piñero and Prokopy 2006). However, research has shown that captures of weevils in traps baited with this combined lure or other synthetic attractants can decline rapidly after fruit set due to intense competition from volatiles released by rapidly developing fruit (Prokopy et al. 2003, Leskey and Wright 2004b).

A recent study by our group in Alabama peach orchards showed that captures of plum curculio in pyramid (also called Tedders trap) and 'Circle' or screen traps (the two most popular traps for plum curculio), were improved numerically by the addition of BZ, PE or GA alone (single lures), and were significantly enhanced only by the addition of the combined BZ + PE lure (Akotsen-Mensah et al. 2010). The popular combined BZ + GA lure was significantly less attractive than the combined BZ + PE lure. The results suggested an additive interaction between BZ and GA, in contrast to the synergistic interaction reported in Massachusetts apples (Piñero and Prokopy 2003, 2006). These varying results on lure performance may be related to several factors including the physico-chemical properties of the lures, differential attractiveness of tree fruit species/varieties, prevailing orchard conditions, strain differences, and physiological state of weevils.

The physiological state of insects is known to play a major role in their response to odor cues (Walgenbach et al. 1983, Vet and Dicke 1992, Landolt and Phillips 1997, Fraser et al. 2003) however, we are not aware of any published studies on the response of plum curculio of different physiological states to currently available lures. This research was therefore designed to evaluate effects of physiological factors on the response of plum curculio to commercial lures. Specifically, we evaluated the response of weevils of different physiological status (sex, age, diet, and mating) to single and combined lures of BZ, PE and GA in laboratory olfactometer bioassays. Based on the knowledge of the field ecology and spring migration of plum curculio from overwintering sites, we tested the following key hypotheses: i) females will show greater response to the lures than males, ii) younger weevils will show greater response to the lures than older weevils, iii) starved weevils will show greater response to the fruit-based lures (BZ and PE) than fed weevils, and iv) mating will have no significant effect on response of weevils to the lures. Next, the release rates of the lures were determined gravimetrically and the chemical profiles of BZ and PE lures were characterized by coupled gas chromatography-mass spectrometry (GC-MS) to detect possible quantitative or qualitative differences that may provide an explanation for the results recorded in the olfactometer bioassays. Finally, follow-up olfactometer bioassays were conducted to determine the biological activity of the chemical components in each lure, as identified in the GC-MS analyses. It is hoped that the results of this laboratory study will assist with the interpretation of field data on captures of plum curculio in traps baited with these lures.

3.2 Materials and methods

3.2.1 Test Insects. The colony of plum curculio adults used for this study was maintained on pesticide-free green thinning apples in a growth chamber at 25 ± 1°C,65-70% RH, and 12:12 h (L:D) photoperiod. The weevils used to start the laboratory colony were collected from peach orchards in central Alabama and had been reared for more than 10 generations before the tests. The colony was periodically supplemented with weevils collected from the same field locations in Alabama. The rearing procedures followed that of Smith (1957) and Amis and Snow (1985). Females and males were separated using the methods of Thomson (1932) and then tested separately.

3.2.2 Lure Treatments. The lures evaluated were commercial formulations of benzaldehyde (BZ), plum essence (PE), and grandisoic acid (GA). The BZ and PE lures were obtained from Great Lakes IPM (Vestaburg, MI), while the GA lure was obtained from ChemTica International (San Jose, Costa Rica). The lures were used without any major modifications through either dilution or mixing with solvents.

3.2.3 Four-choice Olfactometer Bioassays. A four-choice olfactometer (Analytical Research Systems, Gainesville, FL) was used to determine the behavioral response of adult plum curculio of different physiological states to the above lures. The four-choice olfactometer system was similar to that described by Pettersson (1970) and Kalule and Wright (2004). Briefly, the apparatus consisted of a central chamber (30 x 30 x 5 cm) with orifices (arms) at the four corners through which purified and humidified air can be drawn in, creating four potential odor fields, and a central orifice where mixing of the airflow from the arms occurred. In the two experiments described below, only two (adjacent to each other) of the four orifices were designated for lure treatments while the other two adjacent orifices were designated for control (i.e. blank dispenser). In other words, only two lure treatments (binary tests) were compared at a given time. Although the device is designed to use push air we did not use push air in this study because preliminary experiments indicated that plum curculio responded better to the lures under minimal airflow (near still air) conditions, as has been previously reported (Leskey and Prokopy 2001). This observation may be related to the tendency of plum curculio to feign dead when disturbed.

The BZ and PE lures were dispensed by transferring aliquots into a 0.8 ml micro-centrifuge vials (USA Scientific Inc., Ocala, FL) in which a cotton string (~2.5 cm long) was threaded through a hole drilled through the lid of the cap to aid in the release of the lures. The GA lure dispenser consisted of a heat-sealed polymer membrane to protect the pheromone. The membrane was removed before placing the dispenser in the olfactometer arm. The lure treatments were placed in their designated olfactometer arms for 20 min prior to release of test weevils to ensure the stabilization of the diffusion of the lures.

3.2.4 Olfactometer Response of Plum Curculio to Commercial Lures. Two separate experiments were conducted to evaluate response of plum curculio to the three tested lures. In the first experiment, single lures were evaluated as binary or paired treatments (i.e. BZ vs. PE, BZ vs. GA, and PE vs. GA). In the second experiment, the most attractive single lure determined in the first experiment (i.e. PE) was selected and compared against combined (two component) lure treatments in binary tests (i.e. PE vs. BZ + PE, PE vs. BZ + GA, and PE vs. PE + GA) to determine the type of interaction (i.e. additive, synergistic, neutral, or negative/inhibitory) between any two lures. Weevils of different physiological states such as food deprivation (starved for 24 h vs. fed *ad libitum*), age (young or 10-14 days old vs. old or 20-24 days old), and mating status (unmated vs. mated), were tested in both experiments to determine the effect of the above physiological factors on the response of plum curculio to the lures. These resulted in a total of eight physiological treatments per sex. Groups of five females or males of each physiological treatment were released in the olfactometer and replicated six times per sex. Released weevils were given 30 min to respond by walking into one of the four olfactometer arms. Those that did not make a choice within this period were considered as "non-responders" and excluded from the test. A weevil was used only once. At the end of the test with each physiological treatment, the olfactometer set-up was rinsed with soap, water, and acetone. The glass wares were then heated in an oven to about 200°C for ~ 6 h before re-use. The position of each lure in the olfactometer was alternated after each replicate (i.e. lures were re-assigned to different olfactometer arms) to minimize position effect. All bioassays were carried out under red light in a dark room at 25 ± 1 ºC, 55 ± 10% RH between 1600 to 2400 h local time from June 2008 to December 2009. The time of day chosen was based on previous report which showed plum curculio adults to be most active in the field during scotophase (Racette et al. 1990, Lamothe et al. 2008).

Data from each experiment were first analyzed by using standard least square analysis of variance (ANOVA), a model capable of determining the effects of multiple factors, to test for effects of sex (male vs. female), age (older vs. younger), diet (fed vs. starved), mating status (mated vs. unmated), and lure treatments on the response of plum curculio. The model also allowed testing for effects of two-way, three-way, four-way, and five-way interactions (a total of 26 possible interactions) among the five factors. Because the model showed no significant effect of age, diet and mating or interactions among these factors on response of plum curculio to the lure treatments (see Results), data for these physiological treatments were pooled for each sex and analyzed using one-way ANOVA followed by the Tukey-Kramer honestly significant difference (HSD) test to determine significant differences in the response of each sex to the different lure treatments ($P < 0.05$, JMP 7.0, SAS Institute 2007).

3.2.5 GC-MS Analyses of Commercial Lures to Determine Chemical Components. The identities of the compounds released by the BZ and PE lures were confirmed by collecting headspace volatiles from each lure. The collection devise consisted of a small (150 ml) cylindrical glass jar. Four of the devices were used in order to simultaneously collect volatiles from each of the three lures and a control consisting of empty micro-centrifuge vial. An aliquot of each lure (BZ or PE) was transferred into a 0.8 ml-capacity micro-centrifuge vial (same type used for the olfactometer bioassays described above). The micro-centrifuge vials were each placed in one of the four chambers of the volatile collection system (Analytical Research Systems, Gainesville, FL) with each chamber connected to an air delivery system (ADS) that delivered air at the rate of 50 ml per min at room temperature. The other two chambers had only air and empty micro-centrifuge vial to serve as control. The air passing through the ADS was first purified by passing it through a three-step filtration system (Analytical Research Systems, Gainesville, FL) to remove water and any contaminants and then through two separate activated charcoal traps. Volatiles were collected from the lures at 1 (fresh lures), 24 and 48 hours after exposure in the laboratory, continuously for 30 min. Headspace volatiles from each lure (or control) were collected by using Super Q (Alltech Associates, Deerfield, IL, USA). Volatiles trapped in the Super Q were eluted with 2 ml of HPLC grade dichloromethane and then concentrated to ~ 1 ml. A 1-µL sample of the concentrate was injected into a GC (Shimadzu GC 17A, Shimadzu Corporation, Kyoto, Japan) equipped with flame ionization detector (FID) set at 280 ºC. The GC column used was a non-polar Restek® Rtx-IMS capillary column (30 m × 0.25 mm ID; 0.25-µm film thickness). The GC program used was as follows: initial temperature of 90°C, increase at rate of 15°C/min up to 270°C and held for 10 min. The injector temperature was set at 270 ºC and operated in splitless mode throughout the analyses. The carrier gas was helium (purity 99.96%) with a flow rate of 1.5 ml/min and at a constant pressure of 100 KPa.

The active peaks from each lure were identified by GC-MS using an Agilent 7890A GC coupled to a 5975C Mass Selective Detector, with an HP-5 ms capillary column (30 m×0.25 mm I.D., 0.25µm film thickness). One µl of each headspace extract was injected into the GC in a splitless mode using the GC conditions described above. Mass spectra were obtained by using electron impact (EI, 70 eV). Identification of the compounds was done by using NIST 98 library (National Institute of Standards and Technology, Gaithersburg, Maryland). The structures of the identified compounds were confirmed by using commercially available synthetic standards with purity >99% (as indicated on the labels) obtained from Sigma® Chemical Co. (St. Louis, MO, USA).

3.2.6 Olfactometer Response of Plum Curculio to Chemical Components in Commercial Lures. Follow-up olfactometer bioassays were conducted to evaluate the response of plum curculio to the key chemical components of the BZ and PE lures as identified above by GC-MS. The aim was to determine the biologically active chemical components in each lure. The synthetic pure compounds identified in each lure were obtained from Sigma® Chemical Co. (St. Louis, MO, USA) and tested against the commercial lures in four-choice olfactometer bioassays using the procedures described above for the lures. One difference was that three odor treatments were compared simultaneously (i.e. multiple treatment comparisons). For this, three of the four arms of the olfactomer were designated for odor treatments, while the remaining arm was designated as hexane control. Two experiments were conducted each comparing three odor treatments versus control consisting of empty micro-centrifuge vials.

In experiment 1, pure benzaldehyde (BEN) and pure 1, 2, 4-trichlorobenzene (TCB), the two key chemical components identified in the commercial BZ lure, were compared against BZ lure. The dose of BEN and TCB tested in the olfactometer was similar to the amount of each compound detected in BZ lure by GC-MS. Based on the results of the above first experiment we hypothesized that TCB, which is commercially formulated with BZ lure as a stabilizing agent (but not formulated with PE lure), is likely the compound responsible for the relatively lower attractiveness of BZ lure compared to PE lure. To test this hypothesis regarding “repellent” or “dampening” effect of TCB when formulated with the BZ or PE lure, a second experiment was conducted which compared PE lure (found to be highly attractive in the previous lure tests), pure TCB, and mixture of PE and TCB (PE + TCB). If TCB truly has a repellent or inhibitory effect, we would expect the combined PE + TCB treatment to be less attractive than PE lure. TCB was tested singly with PE at a dose similar to that detected in the BZ lure. In the mixed PE + TCB treatment both compounds were released from separate vials placed in the assigned olfactometer arm. For each experiment, groups of five female or male weevils (> 20 days old, mated, and starved for 24 h) were released in the olfactometer. The experiment was replicated 12 times per sex.

Data for each sex were analyzed using one-way ANOVA followed by the Tukey-Kramer HSD test to determine significant differences in the response of female or male weevils to the different odor treatments ($P < 0.05$, JMP 7.0, SAS Institute 2007).

3.2.7 Estimation of Release Rates of Commercial Lures and Synthetic Components. The release rates of the commercial lures (BZ and PE) and pure synthetic components identified as released by the lures (BEN/TCB for BZ lure and BEN for PE lure) were determined gravimetrically in the laboratory using the methods described by Leskey and Wright (2004b). Briefly, ~ 0.8 ml of each treatment (i.e. BZ lure, PE lure, BEN, or TCB) was transferred into a micro-centrifuge vial, which was then weighed on a microbalance (Ohaus® Adventurer® Analytical balance; model AR2140, Central Carolina Scale, Sanford, NC). Each vial was then placed in one of the chambers of the four-choice olfactometer. The experiment was conducted at 25 ± 1 ºC, 55 ± 10% RH between 1600 to 2400 h local time. The weight of each vial was determined again 24 h later. The experiment was repeated seven times using fresh vials and compounds each time. The release rate of GA lure was also determined in a parallel study using the same procedures. The mean release rates (mg/day) were calculated for each lure and component and analyzed using one way ANOVA followed by the Tukey-Kramer HSD test to determine significant differences in the release rates of the different compounds ($P < 0.05$, JMP 7.0, SAS Institute 2007).

3.3 Results

3.3.1 Olfactometer Response of Plum Curculio to Commercial Lures. Standard least squares analyses of the data from the first experiment (BZ vs. PE binary test) revealed no significant effect of sex (*F* = 0.11, d.f. = 1, *P* = 0.7413), age (*F* = 0.35, d.f. = 1, *P* = 0.5524), diet (*F* = 0.11, d.f. = 1, *P* = 0.7413), or mating status (*F* = 0.04, d.f. = 1, *P* = 0.8430) on plum curculio response. However, the effect of lure was highly significant (*F* = 259.93, d.f. = 3, *P* < 0.0001). The only two significant interactions recorded were sex x lure (F = 3.94, d.f. = 3, *P* = 0.0088) and diet x lure (*F* = 4.76, d.f. = 3, *P* < 0.0029). Similar results were obtained for the other binary treatment comparisons of single lures (i.e. BZ vs. GA, and PE vs. GA) or mixed lures (i.e. PE vs. BZ + PE, PE vs. BZ + GA, and PE vs. PE + GA). Based on these results which indicated that the physiological conditions of adult plum curculio have very little effect on their response to the lures, data obtained for weevils of different physiological states (i.e. age, diet and mating) were pooled by sex and analyzed using one-way ANOVA to compare response of each sex to different lure treatments.

Analysis of the pooled data showed that when BZ and PE were compared as binary odor treatments, plum curculio females (*F* = 92.29, d.f. = 3, *P* < 0.0001) and males (*F* = 175.6, d.f. = 3, *P* < 0.0001,) were significantly more attracted to PE than to BZ or the controls (Fig. 1A). Similarly, the results of the binary comparison of GA vs. PE showed significantly greater attraction of females (*F* = 69.06, d.f. = 3, *P* < 0.0001) and males (*F* = 104.4, d.f. = 3, *P* < 0.0001) to PE than to GA or the controls (Fig. 1B). The results of the binary comparison of BZ vs. GA showed greater response of females to one of the controls than to BZ or GA (*F* = 9.8, d.f. = 3, *P* < 0.0001), and no differences were recorded between BZ and GA (Fig. 1C). Similarly, significantly fewer males responded to BZ compared to GA or the controls (*F* = 16.4, d.f. = 3, *P* < 0.0001) (Fig. 1C). Together, these results indicate the non-attractiveness of BZ and GA lures in our olfactometer bioassays.

The results of the second binary experiments in which PE was compared against a combined lure treatment (i.e. BZ + PE, BZ + GA, or PE + GA) confirmed the superior attractiveness of the PE lure (Fig. 2). In the binary comparison of PE vs. BZ + PE, both the females ($F = 89.5$, d.f. = 3, $P < 0.0001$) and males ($F = 42.7$, d.f. = 3, $P < 0.0001$) showed greater response to PE than to BZ + PE or the controls (Fig. 2A). Similarly, females ($F = 65.9$, d.f. = 3, $P < 0.0001$) and males ($F = 76.4$, d.f. = 3, $P < 0.0001$) were more attracted to PE than to BZ + GA or the controls (Fig. 2B). Females ($F = 34.27$, d.f. = 3, $P < 0.0001$) and males ($F = 45.56$, d.f. = 3, $P < 0.0001$) also showed greater attraction to PE than to PE + GA or the controls (Fig. 2C). Similar results were also obtained when PE was compared against a treatment consisting of the three lures (i.e. BZ + PE + GA; data not presented). In addition to confirming the superior attractiveness of PE, these results also showed the non-attractiveness of BZ not only as a single lure but also when combined with PE.

3.3.2 GC-MS Analyses of Commercial Lures to Determine Chemical Components.

The results of the GC-MS analyses showed that the major compound released from both BZ (Fig. 3) and PE (Fig. 4) lures was benzaldehyde (BEN). In addition to BEN, 1, 2, 4-trichlorobenzene (TCB) was also released in high amounts (2.9×10^6) from BZ lure (Fig. 3). TCB was not detected in PE lure (Fig. 4). The abundance of BEN in BZ lure (9.0×10^6) was ~ 22.5 times greater than from the abundance in PE lure (4.0×10^5) (Figs. 3 & 4).

3.3.3 Olfactometer Response of Plum Curculio to Chemical Components in Commercial Lures.

Four-choice olfactometer tests were conducted to evaluate response of plum curculio to multiple treatments comprising of the lures and their key chemical components (i.e. 3

odor treatments and 1 control). In experiment 1, the following four treatments were compared: BZ lure, pure synthetic BEN, pure synthetic TCB, and control. The results showed that females (F = 9.64, d.f. = 3, P < 0.0001) and males (F = 6.87, d.f. = 3, P < 0.0007) were significantly more responsive to the control than to BZ lure or TCB (Table 1). Both sexes also showed slightly lower response to BEN than to the control. The results of experiment 2 in which PE was compared against TCB and PE + TCB showed that females were significantly more attracted to PE than to TCB or the control (F = 17.27, d.f. = 3, P < 0.0001). Also, females were numerically more attracted to PE than to PE + TCB (Table 2). Similarly, males were significantly more attracted to PE than to the remaining treatments (F = 12.50, d.f. = 3, P < 0.0001) (Table 1). These results again confirmed the attractiveness of the PE lure and the inhibitory effect of TCB when mixed with PE.

3.3.4 Estimation of Release Rates of Commercial Lures and Synthetic Components. Significant differences in release rates were recorded among the lures and compounds (i.e. BEN, BZ, PE, TCB) (F = 60.66, d.f. = 3, P < 0.0001). The gravimetric release rate of PE was much higher than the release rates of the other treatments (Table 2).

3.4 Discussion

The results of this laboratory study confirmed the attractiveness of the commercial plum essence (PE) lure (a synthetic mixture of plant essence) to plum curculio. Of the tested lures and synthetic components, PE was by far the most attractive to both sexes. This finding is in agreement with previous reports of PE as an attractant for plum curculio in the field (Coombs 2001, Whalon et al. 2006, Akotsen-Mensah et al. 2010). The relatively greater attractiveness of

PE lure may be due to its higher release rates, as determined in the release rate experiment. Our results, however, showed that the commercial benzaldehyde (BZ) lure was not attractive to plum curculio in olfactometer bioassays. This somewhat surprising finding is in contrast to the results of field studies which demonstrated attraction of plum curculio to traps baited with benzaldehyde (Leskey et al. 2001). In fact, BZ lure is commonly regarded as the most attractive lure for plum curculio when combined with GA and is widely used for monitoring the pest in the field (Leskey et al. 2001, Piñero et al. 2001, Piñero and Prokopy 2003, Leskey et al. 2008). The third commercial lure tested, grandisoic acid (GA), which is the male-produced aggregation pheromone of plum curculio (Eller and Bartelt 1996) was also not attractive to both sexes either when tested as single lure or in combination with other lures.

The results of the experiments in which combined lures were tested further confirmed the superior attractiveness of PE lure, which was more attractive than any combinations of the three lures. Combining BZ or GA lure with PE lure resulted in reduced attractiveness of PE lure. The data actually suggest a repellent or inhibitory effect of BZ when combined with PE, and a neutral effect of combining GA with PE. These results are again contrary to some field studies which reported either a synergistic effect of combining BZ and GA lures (Piñero and Prokopy 2003) or an additive interaction between BZ and PE and between BZ and GA (Akotsen-Mensah et al. 2010). The combined BZ + GA lure is presently the most widely used attractant for monitoring plum curculio in orchards (Piñero et al. 2001, Piñero and Prokopy 2003, Leskey and Wright 2004b, Leskey et al. 2005, Piñero and Prokopy 2006). Also, a recent field study by our group showed that the combined BZ + PE lure was the most effective lure for monitoring plum curculio in Alabama peaches (Akotsen-Mensah et al. 2010). However, both combined lures (BZ + GA and BZ + PE lures) were not as attractive as the single PE lure in the present study. The difference between the results of this laboratory study and the above field reports may be related to differences between experimental conditions and other factors such as the release rates of the lures. Chemical compounds such as the lures tested in this study, which depend on prevailing environmental conditions to be released in the right concentration are likely to vary in their performance in fluctuating field conditions compared to the more stable laboratory environment.

Intriguingly, the results of the GC-MS analyses of the lures showed that benzaldehyde (BEN) was the major component released from both BZ and PE lures. Why then is PE lure more attractive than BZ lure? The answer to this question is possibly related to the presence of 1, 2, 4-trichlorobenzene (TCB) in BZ lure. TCB is formulated with BZ lure as a stabilizing agent and was the only additional component released from BZ lure. TCB, however, was not detected in PE lure. This led us to hypothesize that the reduced attractiveness (or inhibitory effect) of BZ lure was due to TCB. We tested this hypothesis by comparing attraction of plum curculio to PE, TCB, and PE + TCB. The results indicated reduced attraction of plum curculio to PE + TCB compared to PE alone. However, it was unclear if TCB actually played a role in the observed non-attractiveness of BZ lure since the weevils were also not attracted to pure synthetic BEN. There is currently no evidence which suggests that TCB is a component of the general volatiles complex released by any of the host plants of plum curculio. Hence, we can only speculate that plum curculio is not likely to have evolved the ability to respond to TCB. However, the data which showed a three-fold reduction in the response of both sexes to TCB compared to the control may suggest the possibility of an inhibitory effect of TCB. The reduced attractiveness of the combined PE + TCB treatment relative to PE further supports this possibility. Although BEN has been shown to degrade rapidly to benzoic acid and trans-stilbene in the laboratory (Leskey and Wright 2004a, Leskey et al. 2005), we did not detect these compounds in our analyses when

BZ was exposed in the laboratory for up to 48 hours. Therefore the non-attractiveness of BZ lure and BEN could not be attributed to degradation to benzoic acid and other compounds. The results of the GC-MS analyses which showed that BEN was detected in greater amounts in BZ lure than in PE lure are not as relevant as the release rate results which showed that PE lure was released at a higher rate (~ 4-fold) than BZ lure.

The data showed no significant effect of physiological factors (i.e. age, diet, and mating) or sexual differences on the response of plum curculio to the tested lures. These results led us to reject most of our hypotheses and were somewhat surprising, in particular the null effect of diet on response. We had expected that plum curculio, which uses the same resources (fruit) for food and oviposition, will show greater response to fruit-based odor when starved than when fed. The results are in contrast to those reported by Prokopy et al. (1995), which showed that starved weevils responded more than fed weevils to hexane extract of wild plum. However, it should be noted that the study by Prokopy et al. (1995) used overwintering adults whose physiological conditions were largely unknown (with the exception of diet), whereas the weevils used in the present study were from a laboratory source and with known physiological conditions. Previous studies on the effect of physiological factors on response of other weevil species to odor have produced different results. While some studies have reported significant effect of some physiological factors on beetle response to host odor (e.g., Walgenbach et al. 1983, Vet and Dicke 1992, Landolt and Phillips 1997, Fraser et al. 2003, Addesso and McAuslane 2009), others have reported no effect (Walgenbach and Burkholder 1986).

In conclusion the results of this laboratory study demonstrated significant attraction of plum curculio to PE lure. Contrary to field reports, GA lure was not attractive, while BZ lure was inhibitory. The difference between our results and previous field reports may be due to several factors. The inhibitory effect of BZ lure may be due to the presence of TCB. Aside from this, it is plausible that the weevils reared in the laboratory are different from those that occur naturally in the field in their response to odor. In addition, the northern strain of plum curculio tested in most of the field studies and the southern multivoltine strain tested in the present study may differ in their behavioral response to odor. Future studies are necessary to further determine the basis for the inhibitory effect of BZ lure recorded in this study.

3.5 Acknowledgements

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Table 1. Response of plum curculio to the tested commercial lures and synthetic pure compounds in four-choice olfactometer bioassays

Experiment	Compounds/ lure treatments	Mean (± SE) no. responders	
		Female	Male
1	BZ	0.25 ± 0.10^{c}	0.50 ± 0.15^{b}
	BEN	1.17 ± 0.24^{ab}	0.92 ± 0.19^{ab}
	TCB	0.58 ± 0.15^{bc}	0.58 ± 0.15^{b}
	Control	1.83 ± 0.32^{a}	1.50 ± 0.19^{a}
	F	9.64	6.87
	P	< 0.0001	< 0.0007
2	PE	2.08 ± 0.26^{a}	2.42 ± 0.38^{a}
	TCB	0.17 ± 0.11^{b}	0.33 ± 0.19^{b}
	PE + TCB	1.42 ± 0.19^{a}	0.92 ± 0.19^{a}
	Control	0.58 ± 0.19^{b}	0.92 ± 0.19^{b}
	F	17.27	12.50
	P	< 0.0001	< 0.0001

BZ = commercial benzaldehyde lure; BEN = pure synthetic benzaldehyde; TCB = pure synthetic 1, 2, 4-trichlorobenzene; PE = commercial plum essence lure. For each experiment, groups of 5 weevils of either sex were released per test in the olfactometer and replicated 12 times. For each experiment and sex, means having no letter in common are significantly different (ANOVA, Tukey-Kramer HSD, $P < 0.05$; $df = 3, 44$)

Table 2. Release rates of the tested commercial lures and synthetic pure compounds under laboratory conditions

	Mean (± SE) release rates mg per hour
BZ	0.36 ± 0.05^{b}
PE	1.51 ± 0.13^{a}
BEN	0.29 ± 0.09^{b}
TCB	0.08 ± 0.01^{b}
GA*	ca 0.041

BZ = commercial benzaldehyde lure; PE = commercial plum essence lure; BEN = pure synthetic benzaldehyde; TCB = pure synthetic 1, 2, 4-trichlorobenzene; GA = grandisoic acid. Means having no letter in common are significantly different (ANOVA, Tukey-Kramer HSD, $P < 0.05$; n = 5). * Release rate was calculated based on manufacturer's recommendation.

Figure Legends

Figure 1. Response of plum curculio to commercial lures of benzaldehyde (BZ), plum essence (PE) and grandisoic acid (GA) in four-choice olfactometer bioassays. In each test two lure treatments (binary test) and two controls were compared (A) BZ vs. PE; (B) PE vs. GA, and (C) BZ vs. GA. In this and figure 2, Control 1 = air, Control 2 = empty micro-centrifuge vial. Groups of five weevils of either sex were released per test in the olfactometer and replicated six times. Means for each sex having no letter in common are significantly different (ANOVA, Tukey-Kramer HSD, $P < 0.05$).

Figure 2. Response of plum curculio to combinations of commercial lures of benzaldehyde (BZ), plum essence (PE) and grandisoic acid (GA) in four-choice olfactometer bioassays. In each binary test PE was compared against any two combinations of the three lures. (A) PE vs. BZ + PE, (B) PE vs. BZ + GA, and (C) PE vs. PE + GA. Groups of five weevils of either sex were released per test in the olfactometer and replicated six times. Means for each sex having no letter in common are significantly different (ANOVA, Tukey-Kramer HSD, $P < 0.05$).

Figure 3. Chemical analyses of commercial benzaldehyde (BZ) lure to identify key components (A) GC profile of BZ lure showing two major peaks: peaks 1 and 2 were identified as benzaldehyde (BEN) and 1, 2, 4-trichlorobenzene (TCB), respectively, (B) Mass spectrum of BEN, and (C) Mass spectrum of TCB.

Figure 4. Chemical analyses of commercial plum essence (PE) lure to identify key components (A) GC profile of PE lure showing one major peak identified as benzaldehyde (BEN), and (B) Mass spectrum of BEN.

Figure 1

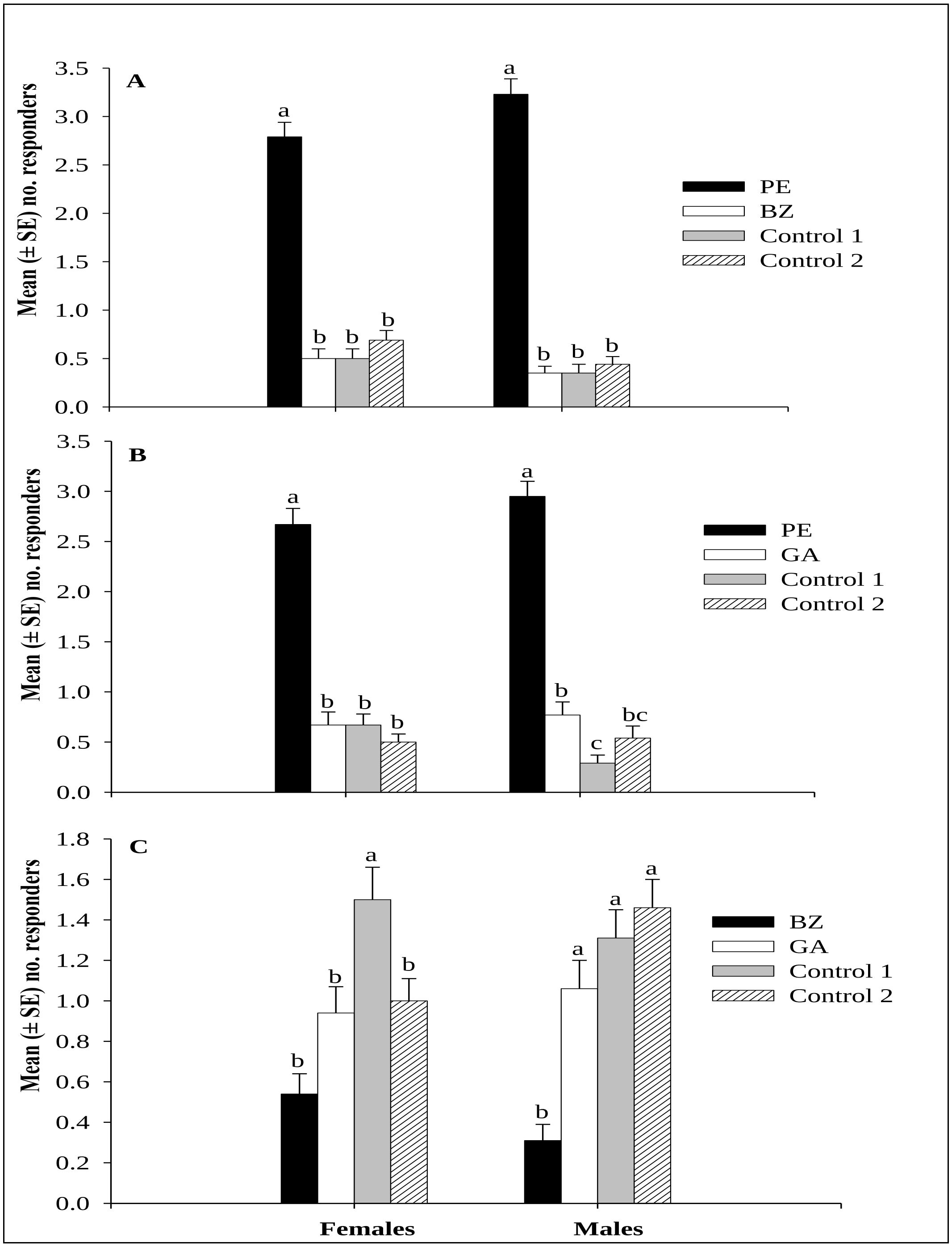

A
B
C
Mean (± SE) no. responders
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1.8
1.6
1.4
1.2
0.8
0.6
0.4
0.2
a
b
bc
c
PE
BZ
GA
Control 1
Control 2
Females
Males

Figure 2

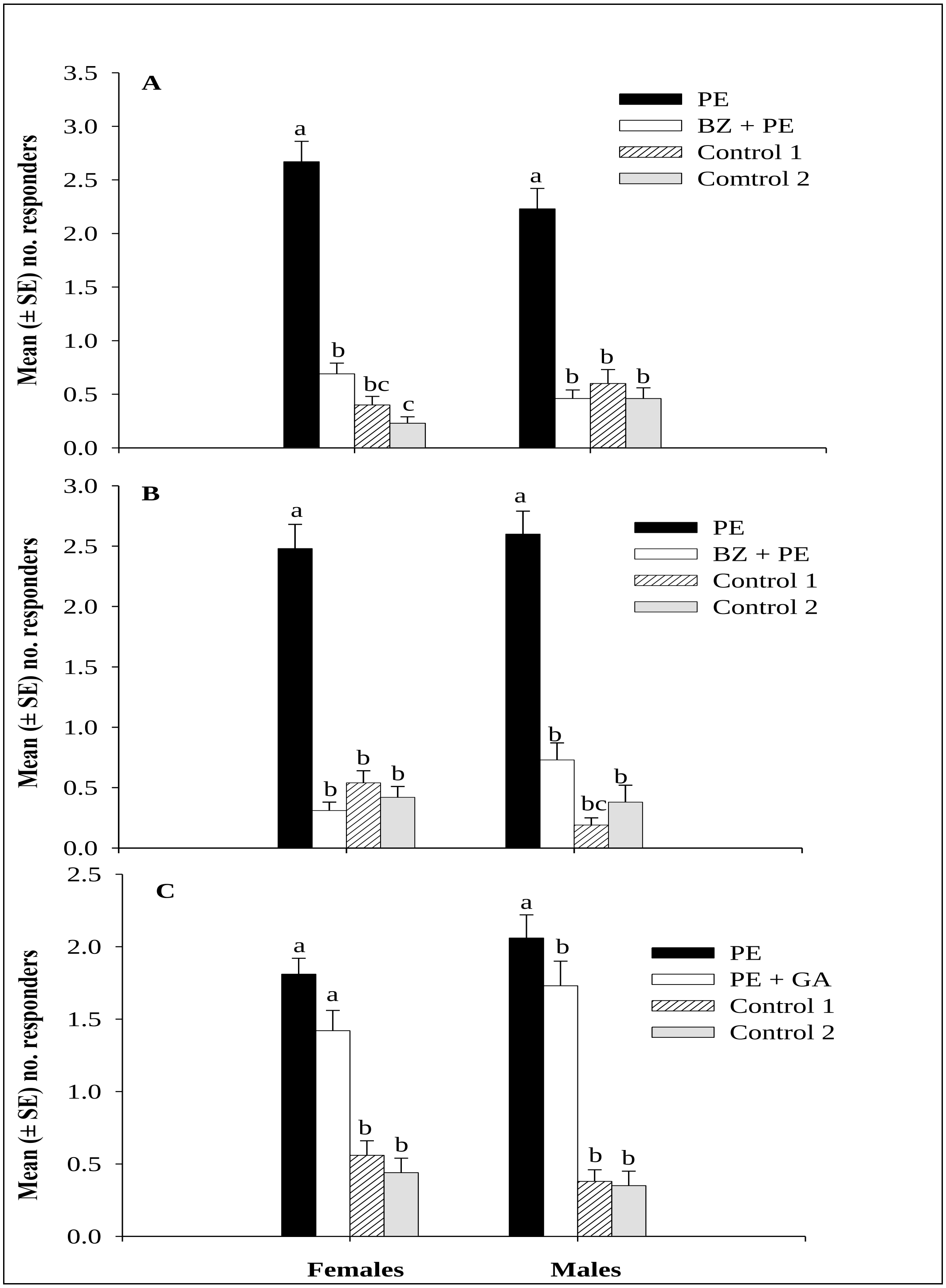
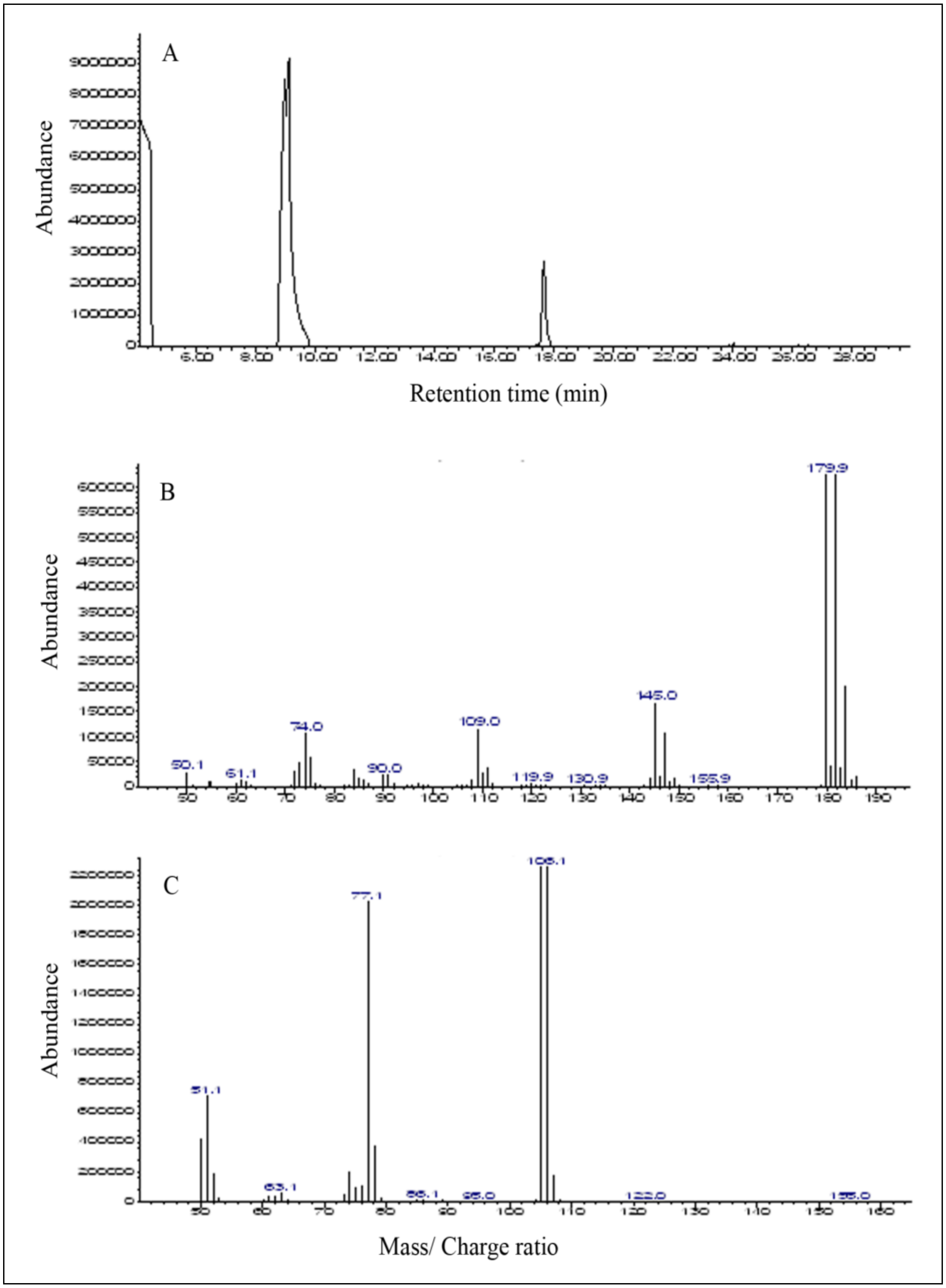

A
Mean (± SE) no. responders
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
PE
BZ + PE
Control 1
Comtrol 2
a
b
bc
c
a
b
b
b
B
Mean (± SE) no. responders
3.0
2.5
2.0
1.5
1.0
0.5
0.0
PE
BZ + PE
Control 1
Control 2
a
b
b
b
a
b
bc
b
C
Mean (± SE) no. responders
2.5
2.0
1.5
1.0
0.5
0.0
PE
PE + GA
Control 1
Control 2
a
a
b
b
a
b
b
b
Females
Males

Figure 3


A
Abundance
9000000
8000000
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
Retention time (min)
B
Abundance
600000
550000
500000
450000
400000
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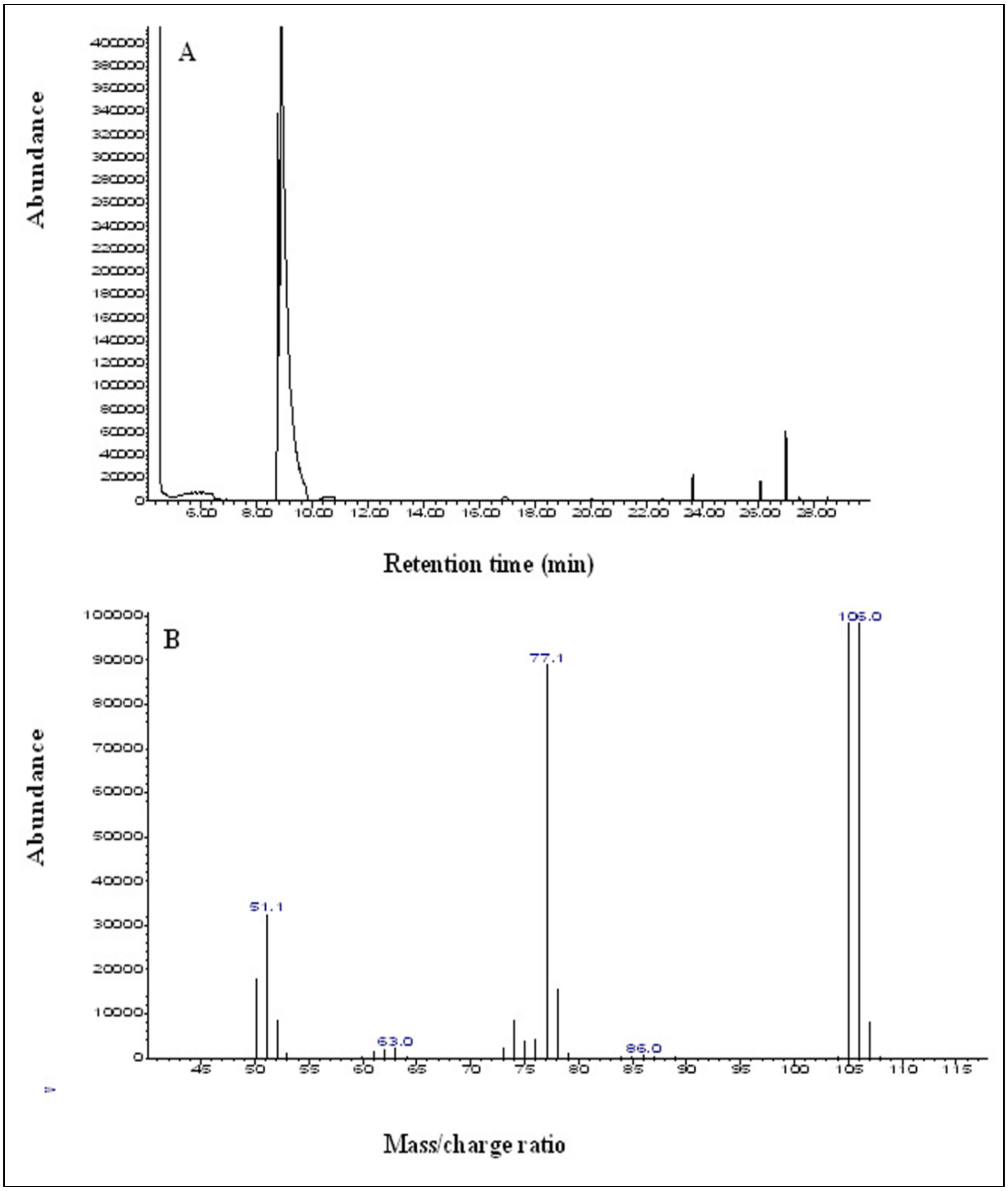
Mass/ Charge ratio

Figure 4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CHAPTER 4

SEASONAL OCCURRENCE AND DEGREE-DAY MODEL FOR FORECASTING SPRING EMERGENCE OF PLUM CURCULIO *CONOTRACHELUS NENUPHAR* HERBST (COLEOPTERA: CURCULIONIDAE) IN ALABAMA PEACHES

4.1 Introduction

Plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae), is a major pest of many stone and pome fruit crops, and is widely distributed across the United States (U.S.) and Canada, east of the Rocky Mountains (Chapman 1938, Armstrong 1958). In the southern U.S., plum curculio typically overwinter as adults in wooded lots adjacent to peach orchards from where they immigrate into the orchards in the spring beginning around bloom (Snapp 1940, Yonce et al. 1995, Johnson et al. 2002a). Development and activity of plum curculio, including spring migration, is greatly influenced by field conditions such as temperature, rainfall (amount and timing), humidity, and wind speed (Whitcomb 1932, Dixon et al. 1999). In the southern U.S. and most parts of North America, management of plum curculio is achieved mainly by the use of insecticides since no alternative control is currently effective. For instance, Alabama peach growers typically apply 6-12 calendar-based sprays of broadspectrum organophosphate and/or pyrethroid insecticides per growing season to control plum curculio (Foshee et al. 2008). A recent study by our program suggests that 3-4 targeted insecticide sprays may provide a cost-effective and environmentally sound alternative to the calendar-based spray program for plum curculio (unpublished). However, the success of a targeted spray management strategy is highly dependent on the ability to effectively detect and predict the activity of plum curculio in the orchards, so that insecticide sprays can be properly timed to coincide with the period of peak abundance and activity of the pest.

Models based on linear and nonlinear functions have been used to predict key insect events such as time of egg hatch, larval and pupal development times in the laboratory, and the first and peak trap captures and time of diapause in the field (Welch et al. 1981, Higley et al. 1986, Doerr et al. 2002, Blanco and Hernandez 2006, Broatch et al. 2006). Several predictive models such as degree-day models have been used to make important orchard pest management decisions with varying degrees of success (Dent 2000, Blanco and Hernandez 2006, Broatch et al. 2006). The concept of degree-days (DD) is that heat units accumulated over a 24-h period above a temperature development threshold could be used to predict insect development and activity patterns over time because temperature is the most important abiotic factor that affects the development of insects (Potter 1981, Higley et al. 1986).

Although degree-day models can provide proper timing of specific insect events, these temperature dependent models are not currently available for many species. The few degree-day models available for plum curculio have been used mainly to predict and forecast plum curculio activity in apple orchards (Reissig et al. 1998) with the assumption that they can be applied to other fruit crops such as peaches. However, Hoffmann et al. (2004) noted that this is usually not the case because the utility of degree-day models may vary from crop to crop and by region due to differences in latitude and strains of plum curculio found in the continental U.S. To date, the only degree-day model developed and applied at grower level is the egg hatch and oviposition model (Reissig et al. 1998). This oviposition model relates temperature (cumulative heat units) to cumulative fruit injury for scheduling insecticide applications against plum curculio in New

York. This model has had limited use in many of the peach growing regions in the southeastern regions including Alabama. Hence, a degree-day model to predict the emergence of adult plum curculio in peaches will be necessary to improve the timing of insecticides against plum curculio in Alabama peaches.

Developing a degree-day model for an insect requires an appropriate biofix: defined as the date to begin accumulation of degree-days (Flint and Gouveia 2001) and temperature developmental thresholds which consist of a lower and upper threshold (Flint and Gouveia 2001, Blanco and Hernandez 2006, Diaz et al. 2007). For most insects including plum curculio the lower and upper developmental thresholds are usually around 10°C and 35°C, respectively (Johnson et al. 2002a, Lan et al. 2004), and a biofix of January 1 is typically used in Northern Hemisphere (Hoffmann et al. 2004, Piñero and Prokopy 2006). However, since field conditions are variable and the development of insects can be influenced by microclimatic factors, population genetics, and host quality (Pitcairn et al. 1992), biofixes and temperature development thresholds used to determine degree-day requirements for insects could vary among different ecosystems. Temperature development thresholds are typically developed in the laboratory but their application in the field is usually limited because the degree-day requirements of insects may vary between laboratory and field (Hagstrum and Hagstrum 1970, Taylor and Shields 1990).

In this study, captures of plum curculio adults in unbaited pyramid traps recorded annually from 2000 to 2009 in an unmanaged peach orchard in central Alabama were used to determine the seasonal occurrence of the pest and to develop predictive degree-day models for critical decision making on the timing of insecticide applications against plum curculio in Alabama peach orchards.

4.2 Materials and Methods

4.2.1 Study Location. Trap captures data used in this study were collected at an unmanaged mixed variety peach block (referred herein as orchard) at the Chilton Research and Extension Center (CREC), Clanton (32°50′23″N 86°37′41″W), AL from 2000 to 2008 (nine peach growing seasons). The orchard had previously been used to evaluate different peach rootstocks and contained remnants of trees of several peach varieties such as ‘Nemaguard’, ‘Hagler’, ‘Rutgers Redleaf’, ‘Lovell’, and ‘Elberta’. The orchard had not received any insecticide or fungicide application since its establishment in 1985 and thus, high plum curculio populations had historically been recorded at the orchard. Routine maintenance was done by removal of dead tree branches and weeding with a mower mounted on a tractor. In all years, fruits were not harvested but allowed to remain on the trees until they dropped.

4.2.2 Seasonal Occurrence of Plum Curculio. Captures of plum curculio adults in four unbaited pyramid traps installed at random locations along the periphery of the orchard from 2000 to 2008 were used to determine seasonal occurrence. Trap placement procedures were as described by Prokopy and Wright (1997) and Akotsen-Mensah et al. (2010). All traps were installed in the orchard by 28 February of each year and were removed at the end of the seasonal activity of plum curculio, usually around August 5.

Traps were checked 2-3 times per week for plum curculio adults. Trap count data were pooled to obtain weekly trap captures with Fridays as the end of the week. This means that all trap captures from Saturday through Friday of each week were recorded as “Friday” trap counts. Degree-day data were handled the same way. The data obtained were then used to determine the seasonal occurrence. The following events were recorded yearly: first and peak trap captures of plum curculio, total number of plum curculios captured during spring generation, and phenology of peach trees at first trap capture.

4.2.3 Determination of Biofix and Lower Temperature Threshold for Accumulation of Degree-Days. To determine the best biofix and lower temperature threshold (LTT) for accumulating degree-days in peach orchards, seven sets of biofixes and four potential LTTs with no upper temperature threshold (UTT) were used to calculate cumulative degree-days using historic weather data obtained from the Alabama Mesonet database for Thorsby, AL (http://www.awis.com/cgi-bin/uncgi/awondasta.uncgi) and the National Weather Service, Raleigh, NC (http://www.weather.gov/climate). The biofixes evaluated were January 1, January 15, February 1, February 15, March 1, 1st plum curculio trap capture, and average temperature of >12°C occurring for 3 consecutive days. Cumulative degree-days were calculated for each of these biofixes at LTTs of 7.2°C (45ºF), 10ºC (50ºF), 11.1ºC (52ºF) and 12.8ºC (55ºF). These biofixes and LTTs were selected because they have been used in other studies to accumulate degree-days (Mulder et al. 1997, Mulder and Stafne 1998, Johnson et al. 2002b, Hoffmann et al. 2004, Piñero and Prokopy 2006). No UTT was used because examination of the historic maximum temperatures available to us showed that maximum temperatures within the period of the spring migration did not exceed the UTT of 35°C reported for plum curculio (Lan et al. 2004). In all cases cumulative degree-days (DD) were calculated using the simple average method given by $DD = \Sigma(T_i - LTT)$, where DD is degree-day; T_i is the mean daily temperature on day i and LTT is the base temperature. The few instances where temperatures were negative during the calculation of the degree days were assigned zero. To establish the best biofix and

LTT combination, a coefficient of variation (CV), which is a measure of variability within random sampling, was calculated for each of the observed cumulative degree-day at which a plum curculio event such as first and peak trap capture occurred during the spring of each year. The CV is the most commonly used method for measuring variability and was calculated by dividing the mean cumulative degree-day at each biofix and LTT over the entire 10-yr period by the standard deviation. The two most promising biofixes and LTTs, which produced the lowest CV were selected and used to calculate the degree-days for the models to predict the first and peak trap captures of plum curculio.

4.2.4 Models to Predict the First and Peak Trap Captures of Plum Curculio.

4.2.4.1 Models to Predict Seasonal Peaks. Several models were evaluated to predict the seasonal peak trap captures of plum curculio. This was done by fitting the weekly trap captures (dependent variable) and cumulative degree-days (independent variable) calculated by using the appropriate biofixes and LTTs obtained as described above for the 2000-2008 data, while the 2009 and 2010 data were used to validate the model. Due to year to year variability in population numbers, the weekly trap capture data was normalized within each year by calculating the proportion of weekly trap capture of the total trap capture within the year. Linear, quadratic, cubic and 4th – 6th order polynomial functions were then fitted to the normalized data and the cumulative degree-day to generate parameter estimates for both biofixes (January 1 and 15) and base temperatures of 7.2 and 10°C using the R package (R Development Core Team 2009). For each function an Akaike Information Criterion (AIC) (Akaike 1974, Bozdogan 1987), which is a criterion used to assess and evaluate statistical models, was generated and subsequently used to select the best model that fitted well to the weekly trap captures and cumulative degree-day data. Also a fitted curve was generated for the best model using SAS software (JMPIN version 7.0.1). The first and second order derivatives of the equations relating the dependent and independent variables function of the selected model was used to determine the maximum peaks that represented the function that best described the overall seasonal peaks (both spring and summer generation) of plum curculio.

4.2.4.2 Three-parameter Weibull Function to Predict First and Peak Trap Captures of Spring Population. To confirm the peak of the spring population (first peak) which is very crucial for the first insecticide application, a three-parameter Weibull function was fitted to the proportion of plum curculio captured during spring during each year as a function of cumulative degree-day proportion to predict the first and peak trap captures using Systat Inc. software 2002 (SigmaPlot version 8.0). The three-parameter Weibull function was selected because its parameters gamma (γ) and beta (β), represent the expected normalized time at onset of first migration and the estimate of the first peak trap capture, respectively. Also, the three-parameter Weibull function has been used to determine the relation between degree-days and several events in insects (Collier and Finch. 1985, Broatch et al. 2006) and some plants (Martinson et al. 2007, Royo-Esnal et al. 2010). The three-parameter Weibull function is represented by the equation 1 below:

$$Y = 1 - \exp(-(DD-\gamma)/\beta)^{\mu})\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

where Y represents the cumulative proportion over the entire spring generation period as related to the accumulated degree-days (DD). In this equation gamma (γ) represents the expected normalized time at onset of first migration (number of plum curculio per week) of the spring generation; beta (β) is a constant for rate of migration of the spring generation which is the estimate of the first trap capture; and mu (μ) is the estimate of the peak trap capture.

The coefficient of determination (R^2), and coefficient of variation (CV) were used to judge the goodness of fit of each parameter estimate. The model for each trap capture event such as first and peak trap captures was evaluated for each year by comparing the observed and predicted degree-days. Where the difference between the observed and predicted degree-days did not exceed ± 54 DD the model was considered to be accurate within ± 7-d. We used 54 DD because the average degree-day per day during the period of the spring migration in the study area was about 8 (base 7.2 and 10°C), so since the trap capture and degree-day was done every week, the cumulative degree-day for each predicted in theory occurred within a week. A negative degree-day difference between observed and predicted values indicated that the observed event occurred before predicted date and a positive difference indicated the event occurred after the predicted date.

4.2.5 Validation of the Degree-Day Model using Yearly Predictions and Historic Weather Data. The degree-day model was validated in 2009 and 2010 in the same unmanaged orchard where the seasonal occurrence data were collected. Validation was also conducted in a second unmanaged peach orchard in 2009. This orchard is located ~ 1.6 km from the first orchard and consisted primarily of the Loring peach variety. Use of a separate orchard for the validation allowed us to generate a dataset that is independent of the data used to generate the degree-day model. Methods used to obtain data from the validation orchard were similar to those described previously. The observed cumulative degree-days at which first and peak trap captures occurred during the validation period were recorded using the simple average method described previously. The observed cumulative degree-days for the first and peak trap captures were compared with the model prediction of these events using the Weibull function (2000-2008). The difference between the observed and predicted was used to judge whether the model was accurate in predicting plum curculio trap capture.

4.3 Results

4.3.1 Seasonal Occurrence of Plum Curculio. The first captures of plum curculio were recorded as early as the week of 3-10 March (2000) and as late as the week of 14-20 March (2001, 2005, and 2008). Total capture of plum curculio adults for all traps pooled from 2000-2008 was 5162 with a mean (± SE) of 516.2 ± 135.8 adults per four trap per observation year. Overall, plum curculio's migration began when peach trees were at varying development stages depending on the variety (i.e. at bloom stage for early maturing varieties, pink stage for mid-season and late maturing varieties). No plum curculio was captured prior to bloom. Plum curculio trap captures varied by year, but the seasonal occurrence observed throughout the 9-yr period (2000-2008) followed a similar pattern (Fig. 1).

4.3.2 Determination of Biofix and Lower Temperature Threshold for Accumulation of Degree-Days. The CV of the accumulated degree-days at the various biofixes increased as the LTTs increased (Fig. 2). The CV determined for the biofixes and LTTs showed that January 1 at LTT of 10°C and January 15 at LTT of 7.2°C produced the lowest CV values for accumulation of degree-days at which both the first (Fig. 2A) and peak trap (Fig. 2B) captures of the spring population occurred. These biofixes and LTT were subsequently selected and used to calculate all degree-days and to determine the models for predicting plum curculio seasonal activities. For comparison, the biofix of January 1 (Piñero and Prokopy 2006) and LTT of 10°C (Reissig et al. 1998, Hoffmann et al. 2004) have also been used to calculate degree-day.

4.3.3 Polynomial Models to Predict Seasonal Peaks. Among the several polynomial functions evaluated, a six-order polynomial (equation 3) produced the best fit with the least AIC (1244.30) (Table 1) using a biofix of January 1 and 15 and LTT of 7.2 and 10°C and highest R-square of 11.6. The parameter estimates for each term in the polynomial function that described the relationship between seasonal trap captures (2000-2008; number of sampling weeks = 171) and cumulative degree-day using a biofix of January 1 and LTT of 10°C were significant and the equation of the six-order polynomial function (Table 2). The polynomial function predicted that plum curculio has three major peaks (Fig. 3). Derivative analyses of the function showed three major peaks for plum curculio instead of two. The first (spring generation), second and third peak trap captures occur at accumulated degree-days of 220, 1122, and 1932 (Biofix at January 1 and LTT of 10°C), respectively (Fig. 3). There was a significant effect of intercept (P = 0.0178) indicating that the cumulative degree-day at which plum curculio started migrating into the orchard was significantly different than zero.

$$Y = -106.4 + 1.66\mathrm{DD}_{10} - 6.98x10^{-03}\mathrm{DD}_{10}^{2} + 1.21x10^{-05}\mathrm{DD}_{10}^{3} - 1.01x10^{-08}\mathrm{DD}_{10}^{4} + 4.03x10^{-12}\mathrm{DD}_{10}^{5} - 6.18x10^{-16}\mathrm{DD}_{10}^{6} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..(3)$$

4.3.4 Three-parameter Weibull function to Predict First and Peak Trap Captures of Spring Population. The predicted cumulative distributions of plum curculio trap captures versus cumulative degree-days using the three-parameter Weibull function is shown in Fig. 4. The summary of the best fit and mean (± SE) parameter estimates showed that the first trap capture (α) is predicted to occur at accumulated degree-days of 210.3 ± 14 (Biofix of January 1) and 222.1 ± 12 (Biofix of January 15) at LTT of 7.2°C ($P < 0.0001$) (Table 3). Also the best fit and parameter estimates for the first trap capture is predicted to occur at a mean accumulated degree-days of 108.0 ± 10 (Biofix of January 1) and 78.0 ± 9 (Biofix of January 15) at LTT of 10°C ($P < 0.0001$) (Table 3).

The three-parameter Weibull model also predicted the first peak trap capture (β) to occur at accumulated degree-day at the different biofixes and LTTs are as follows: 339.6 ± 23 (Biofix of January 1 and LTT 7.2°C; $P < 0.0001$); 244.9 ± 20 (Biofix of January 15 and LTT 7.2°C; $P < 0.0001$); 220.1 ± 16 (Biofix of January 1 and LTT 10°C; $P < 0.0001$); and 171.0 ± 14 (Biofix of January 15 and LTT 10°C; $P < 0.0001$) (Table 3). The coefficient of determination for each of the predictions was > 80% indicating the model explained > 80% of the variability within the data used to generate the model.

Comparing the observed and predicted degree-days on year-by-year basis for the first trap capture using the three-parameter Weibull function, January 1 and 15 at both LTT of 7.2 and 10°C showed 77.7% in the ability of the model to accurately predict the first trap capture within 7-d (54 DD) window (Table 4). This trend was not the same for the peak trap capture because the biofixes of January 1 and 15 at LTT of 7.2°C was only able to accurately predict the peak trap capture well by 50.0% within the 7-d (54 DD) window (Table 4). Based on the combined model-generated first and peak trap captures, January 1 (62.5% success) and January 15 (75.0% success) at LTT of 10°C were judged to be better at simultaneously predicting the first and peak trap capture within 7-d (54 DD) window (Table 4).

4.3.5 Validation of the Degree-Day Model. The results of the validation tests for the three-parameter Weibull model using dataset from the same unmanaged orchard in 2009 and 2010 and a second unmanaged orchard (Loring) in 2009 showed that only the first peak trap capture was predicted well in both orchards in 2009 using a biofix of January 1 at LTT of 10°C (Table 5). In 2010, however, both the first and peak trap captures of the spring generation were predicted well in the first unmanaged orchard (Table 5).

4.4 Discussion

The results of this study showed that migration (immigration and emigration) of plum curculio into peach orchards during spring is predictable using degree-days. Migration of overwintered plum curculio adults into peach orchards began in early-to mid-March of every year when peaches were at varying development stages depending on the variety (i.e., at bloom stage for early maturing varieties, pink stage for mid-season, and late maturing varieties). The population of the adults was sustained but gradually declined until the beginning of the emergence of the summer generation (late May to early June). The early March to early June period in central Alabama is usually associated with steady but fluctuated increases in air and soil temperatures, which are conducive for adults to begin their migration either by flying or walking to the trees (Prokopy and Wright 1998, Prokopy et al. 1999). Our results on the seasonal occurrence and onset of spring migration of plum curculio into peach orchards are similar to those reported in peaches in nearby Georgia (Yonce et al. 1995). Similar results were also obtained in apples and blueberries (Chapman 1938, Lafleur and Hill 1987, Polavarapu et al. 2004).

Based on the estimation of coefficient of variations, January 1 at LTT of 10°C and January 15 at LTT of 7.2 were the most accurate biofix and LTT combinations for accumulation of degree-days in peach orchard in central Alabama. However, the results from the models, particularly the three-parameter Weibull model, showed that January 1 at LTT of 10°C was the most accurate to accumulate degree-day for modeling both the first and peak trap captures. The practicality of this combination (January 1 at LTT of 10°C) is also an important factor, since the January 1 biofix is a relatively easier date to remember by growers who are the ultimate users of the degree-day model. Furthermore, growers can obtain degree-day values at this biofix and LTT from several weather service providers within their locality at reasonable or no cost (Flint and Gouveia 2001). In addition, some studies have already used this biofix with some degree of accuracy and thus will allow comparison of our results with other studies (Reissig et al. 1998, Piñero and Prokopy 2006). Among the polynomial models evaluated the six-order polynomial function predicted three major population peaks for plum curculio. Although the R^2 values reported by the polynomial functions are low, such values are typical of most field-collected data, particularly, those involving insects like plum curculio whose activity in the field is affected not only by temperature but also other environmental conditions such as precipitation, humidity, wind speed etc. Although the relationship between plum curculio behaviors, particularly, flight behavior and abiotic factors such as temperature, humidity, wind speed are relatively well studied (Whitcomb 1932, Dixon et al. 1999, Lan et al. 2004), there is a conspicuous lack of information on how all these factors work together to affect plum curculio activity in the field and a multivariate analysis of all the factors which influence plum curculio in the field is lacking. In spite of this, our model supports the conjecture that plum curculio is multivoltine in central Alabama because of the usually high temperatures recorded in Alabama throughout the peach growing season. The multivoltine populations found in central Alabama is contrary to the popular notion that plum curculio is bivoltine in most parts of the southeastern U.S. (Lan et al. 2004). Field conditions such as temperature, humidity, precipitation, and host availability were adequate to support multiple generations in central Alabama and perhaps most other parts of the region.

The predicted cumulative degree-day for first and peak trap captures using the 3-parameter Weibull functions are 108.0 ± 10 and 220.1 ± 16, respectively (Biofix of January 1 at LTT 10°C). The validation tests confirmed the accuracy of the model in general. However, the variable conditions that could occur from year to year in Alabama during spring may impact the accuracy of the model. For example, an intense freeze event early in the season in 2007 coupled with drought conditions resulted in the loss of many fruits and as a result contributed to the failure of the model to predict the first and peak trap capture of the spring generation. Our data on degree-day requirement for the first and peak trap captures are consistent with previous reports which showed that plum curculio's first (onset of immigration) capture in baited traps can be predicted using degree-days (Piñero and Prokopy 2006). However, it is difficult to completely compare our data with those reported by the above authors since a LTT or base temperature of 6.1°C was used to calculate degree-days. Furthermore, the difference between our data and those of Piñero and Prokopy (2006) may be related to differences in microclimate experienced in the orchards, population genetics (different strains), and host quality, all of which have been reported to affect developmental requirements of insects (Pitcairn et al. 1992).

In conclusion, our results showed that the first and peak trap captures of the spring generation of plum curculio in central Alabama can be predicted using a degree-day model. The model is intended to be used as a guide in timing the first insecticide application in order to kill the majority of the early spring migrant population. Thus, application of the first insecticide spray should occur within cumulative degree-day not exceeding 220 ± 16 DD_{10} (Biofix of January 1) to coincide with the first peak trap capture of the spring generation of plum curculio and also to ensure that spraying is not done during bloom. It is hoped that the use of this model will result in reduced insecticide use and more effective control of plum curculio in peaches in central Alabama and other parts of the southeastern U.S.

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Table 1. Akaike information criteria (AIC) for assessment and selection of the best model

Equation	LTT 7.2°C				LTT 10°C			
	Biofix Jan 1		Biofix Jan 15		Biofix Jan 1		Biofix Jan 15	
	AIC	R^2	AIC	R^2	AIC	R^2	AIC	R^2
Linear	1256.1	0.006	1256.0	0.006	1256.1	0.005	1256.0	0.006
Quadratic	1257.4	0.009	1257.6	0.008	1257.4	0.009	1257.5	0.008
Cubic	1256.3	0.027	1255.3	0.032	1255.9	0.029	1255.0	0.034
4th order polynomial	1258.2	0.027	1257.2	0.033	1257.4	0.031	1256.1	0.040
5th order polynomial	1251.7	0.074	1251.8	0.074	1253.5	0.065	1253.4	0.066
6th order polynomial	1245.2	0.119	1244.5	0.118	**1244.3**	**0.122**	1246.0	0.116

Bold indicates model with the smallest AIC and coefficient of determination (R^2) selected for all analyses

Table 2. Parameter estimates for fit of sixth-order polynomial model to determine peaks of plum curculio trap capture during 2000-2008 peach seasons in an unmanaged peach orchard in Clanton, AL

Order of term in equation	Parameter estimate	Standard error	t-ratio	Prob.> |t|
Intercept	-106.4	44.0	-2.40	0.0178
DD_{10}	1.75	0.5	3.56	0.0005
DD_{10}^{2}	-6.98×10^{-03}	1.86×10^{-03}	-3.76	0.0002
DD_{10}^{3}	1.21×10^{-05}	3.23×10^{-06}	3.74	0.0003
DD_{10}^{4}	-1.01×10^{-08}	2.79×10^{-09}	-3.61	0.0004
DD_{10}^{5}	4.03×10^{-12}	1.170×10^{-12}	3.44	0.0008
DD_{10}^{6}	-6.18×10^{-16}	1.90×10^{-16}	-3.21	0.0014

$Y = -106.4 + 1.66DD_{10} - 6.98x10^{-03}DD_{10}^{2} + 1.21x10^{-05}DD_{10}^{3} - 1.01x10^{-08}DD_{10}^{4} + 4.03x10^{-12}DD_{10}^{5} - 6.18x10^{-16}DD_{10}^{6}$

DD_{10} is degree-day calculated using biofix of January 1 and a base temperature of 10°C

Table 3. Best fit and mean (± SE) parameter estimates of the three-parameter Weibull model for predicting first and peak trap captures of spring population of plum curculio at different lower temperature thresholds (LTTs) and biofixes

	LTT 7.2°C		LTT 10°C	
Parameter	Biofix Jan 1	Biofix Jan 15	Biofix Jan 1	Biofix Jan 15
α	210.3 ± 14	222.1 ± 12	108.0 ± 10	78.0 ± 9
β	339.8 ± 23	244.9 ± 20	220.1 ± 16	171.0 ± 14
γ	1.0 ± 0.5	1.0 ± 0.2	1.0 ± 0.5	1.0 ± 0.8
R^2	*83.3*	*84.1*	*84.1*	*79.6*
F	*243.5*	*302.8*	*255.7*	*189.9*
$P\alpha\&\beta$	<0.0001	<0.0001	<0.0001	<0.0001

α is predicted cumulative degree-day at first plum curculio trap capture for each year from 2000-2008; β is predicted cumulative degree-day at peak trap capture, γ is the rate of trap capture ($P = 0.05$), R^2 coefficient of determination; LTT is lower temperature threshold. *df* = 2, 113

$Y = 1 - exp (-DD-\gamma)/\beta)^{\mu})$

Table 4. Comparison of observed and predicted first and peak plum curculio trap captures using the three-parameter Weibull model

Year	First trap capture				Peak trap capture			
	LTT 7.2°C		LTT 10°C		LTT 7.2°C		LTT 10°C	
	Obs.-Pred. Jan 1	Obs.-Pred. Jan 15	Obs.-Pred. Jan 1	Obs.-Pred. Jan 15	Obs.-Pred. Jan 1	Obs.-Pred. Jan 15	Obs.-Pred. Jan 1	Obs.-Pred. Jan 15
2000	99.2	**12.2**	81.9	66.0	**11.72**	**31.48**	**-3.00**	**0.14**
2001	**32.8**	**14.7**	**13.7**	**43.7**	138.38	227.10	68.89	117.96
2002	**-30.9**	**-53.1**	**-19.7**	**6.7**	-72.74	**11.81**	-62.26	**-16.80**
2003	**19.1**	**-6.1**	**9.0**	**35.6**	**48.53**	130.04	**7.48**	**53.22**
2004	**-33.8**	-88.7	**-14.7**	**-10.6**	**-8.68**	**43.19**	**-24.04**	**-0.85**
2005	**34.4**	-78.3	**22.6**	**-9.9**	73.79	67.81	**22.77**	**9.32**
2006	**36.0**	**-39.5**	**19.8**	**16.2**	317.61	348.81	210.30	225.75
2007	70.3	**-2.9**	64.9	56.5	171.79	205.33	125.36	136.01
2008	**13.3**	**-36.5**	**7.0**	**14.0**	**-13.11**	**43.83**	**-34.47**	**-8.46**
Predicted (2000-2008)[a]	210. 3 ± 14	222.1 ± 12	108.0 ± 10	78. 0 ± 9	339.8 ± 23	244.9 ± 20	220.1 ± 16	171.0 ± 14
%	*77.7*	*77.7*	*77.7*	*77.7*	*50.0*	*50.0*	*62.5*	*75.0*

[a] Overall prediction using the 2000-2008 data (number of sampling weeks =171). Bold indicates the difference between observed and predicted cumulative degree-days showing the ability of model to predict the first and peak plum curculio trap captures within ± 7 days window. This was based on daily average degree-day accumulation of ca 8 during spring migration.

[b] Percentage of number of years in which observed did not differ from predicted within ± 7 days when daily average degree-day accumulation was ca 8.

LTT is lower temperature threshold

$Y = 1 - exp\ (-DD-\gamma)/\beta)^{\mu})$

Table 5. Validation of the Weibull model in two unmanaged peach orchards in Clanton, AL in 2009 and 2010

Observed event (plum curculio)	January 1 @ base 10°C		
	First unmanaged orchard		Second unmanaged orchard
	2009	2010	2009
1st trap capture	183.9 (75.9)	96.4 (-11.6)*	183.9 (75.9)
Peak trap capture	226.4 (-39.2)*	163.7 (-56.4)*	265.6 (45.6)*

Numbers in parentheses indicate difference between observed and mean predicted degree-day (2000-2008); Differences less than or equal to 56 DD are judged to be acceptable. LTT is lower temperature threshold. * Represents the events that were predicted well by the model

Figure legend

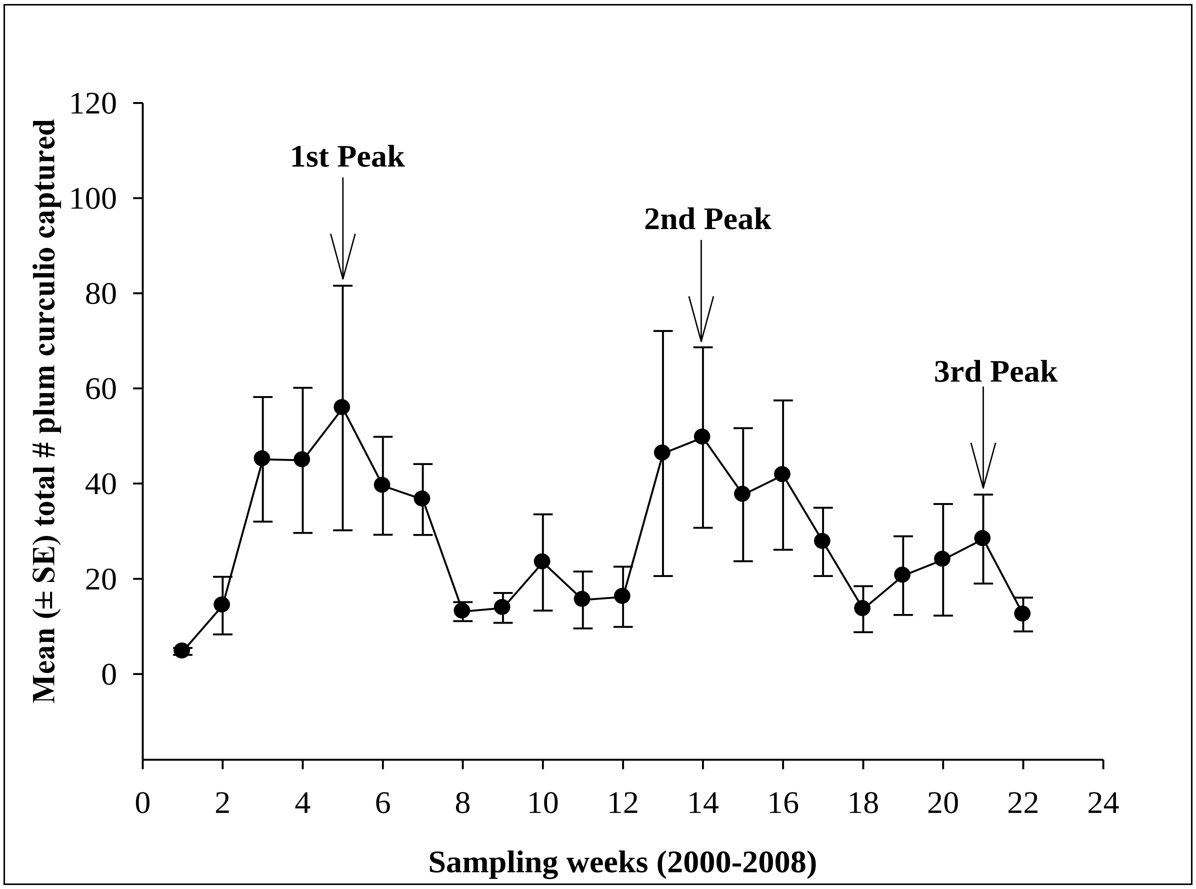
Figure 1. Seasonal abundance of plum curculio in an unmanaged peach orchard in Clanton, AL during the 2000-2008 peach growing seasons. Figure shows combined mean (± SE) for all nine years.

Figure 2. Coefficient of variation calculated for different biofixes and lower temperature thresholds (LTT) for (A) first plum curculio trap capture and (B) first peak trap capture of the spring population during 2000-2008. * denotes values with low coefficient of variations chosen to calculate all degree-days used for the degree-day model

Figure 3. Polynomial fit of the seasonal peak trap captures of plum curculio vs. cumulative degree-day at base 10°C using Jan 1 as biofix (2000-2008; n = 171) in an unmanaged peach orchard in Clanton, AL. Dark trend line indicates predicted seasonal trend. Arrows show predicted peaks.

Figure 4. Three-parameter Weibull model relating the proportion of adult plum curculio (spring generation) captured in traps vs. cumulative degree-day at base 10°C and biofix of January 1 during the 2000-2008 peach growing seasons.

Figure 1



Mean (± SE) total # plum curculio captured
120
100
80
60
40
20
0
1st Peak
2nd Peak
3rd Peak
0
2
4
6
8
10
12
14
16
18
20
22
24
Sampling weeks (2000-2008)

Figure 2

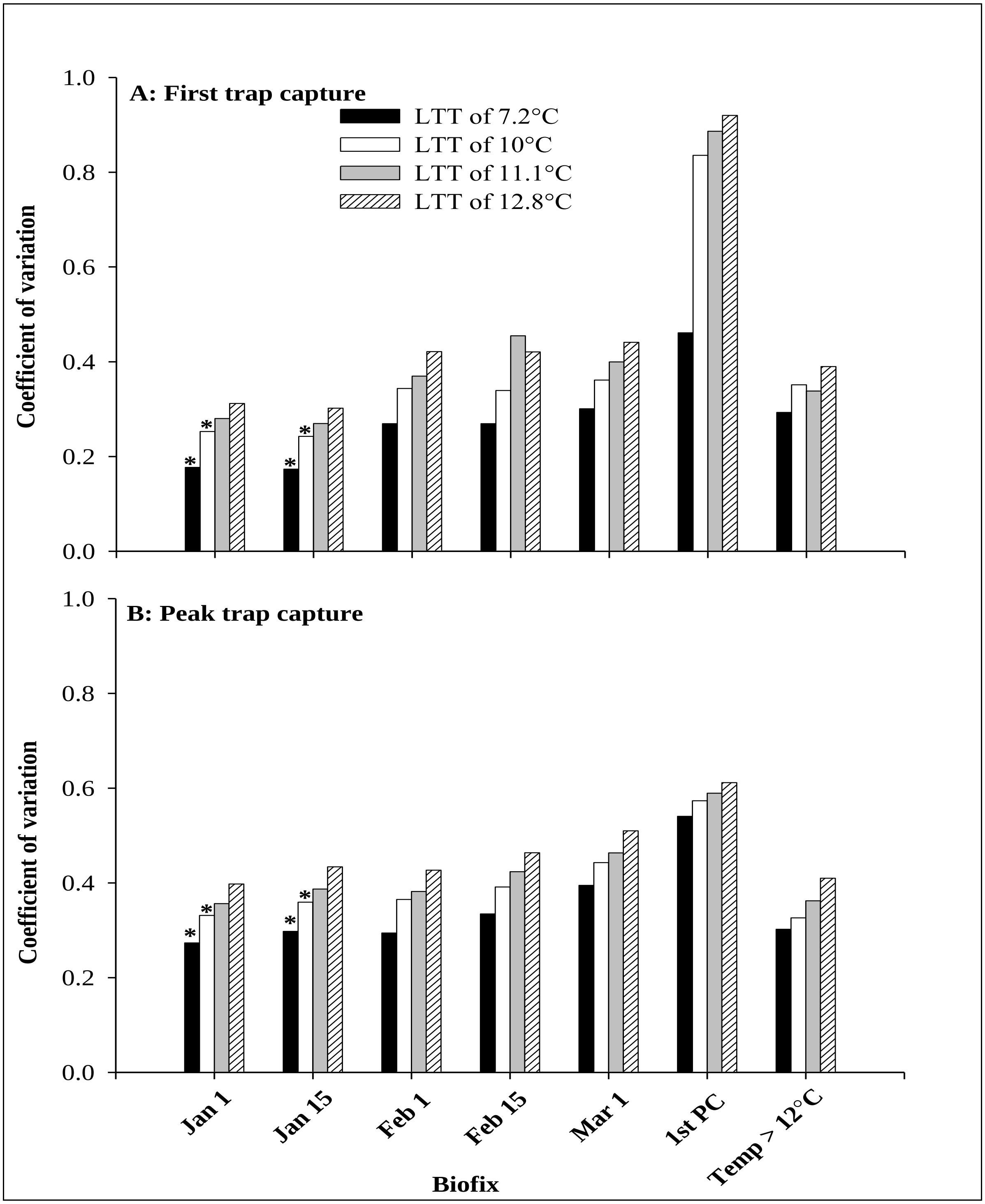
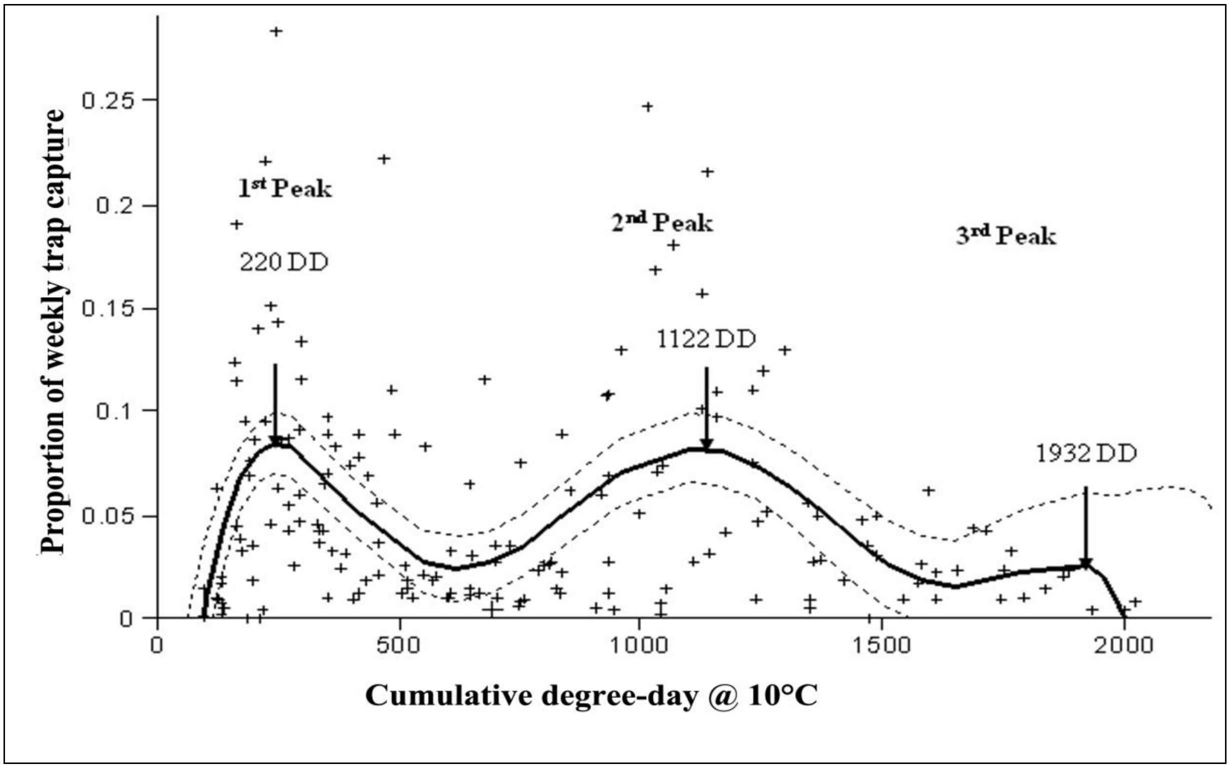
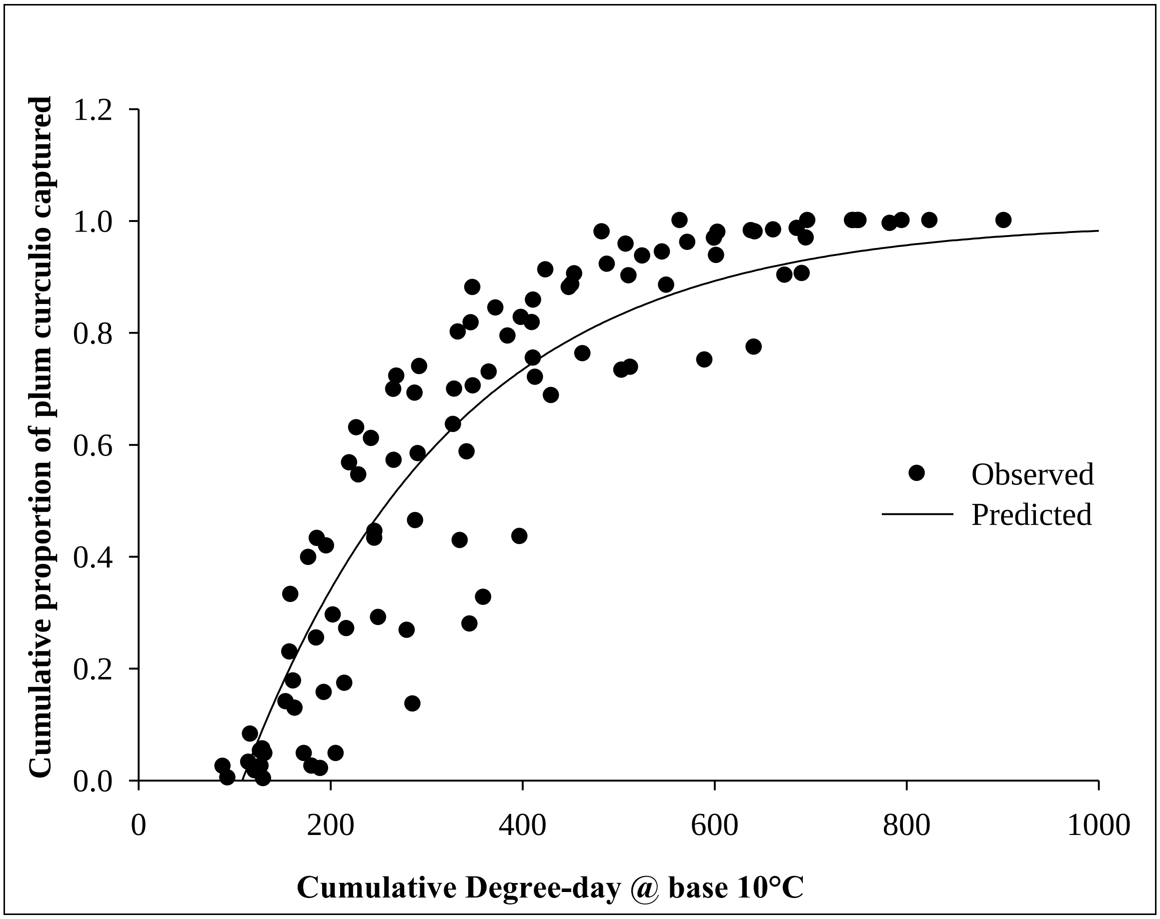

A: First trap capture
LTT of 7.2°C
LTT of 10°C
LTT of 11.1°C
LTT of 12.8°C
B: Peak trap capture
Coefficient of variation
0.0
0.2
0.4
0.6
0.8
1.0
*
Jan 1
Jan 15
Feb 1
Feb 15
Mar 1
1st PC
Temp > 12°C
Biofix

Figure 3



Proportion of weekly trap capture
0.25
0.2
0.15
0.1
0.05
0
1st Peak
220 DD
2nd Peak
1122 DD
3rd Peak
1932 DD
0
500
1000
1500
2000
Cumulative degree-day @ 10°C

Figure 4


Cumulative proportion of plum curculio captured
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
200
400
600
800
1000
Cumulative Degree-day @ base 10°C
● Observed
Predicted

CHAPTER 5

FIELD EVALUATION OF TARGETED INSECTICIDE SPRAYS AGAINST PLUM CURCULIO *CONOTRACHELUS NENUPHAR* (COLEOPTERA: CURCULIONIDAE) IN ALABAMA PEACHES

5.1 Introduction

The plum curculio, *Conotrachelus nenuphar*, is a serious pest of stone and pome fruits in continental North America (Chapman 1938, Vincent et al. 1999) and is ranked the number one pest of peaches in the southeastern United States (U.S.) (Horton and Ellis 1989, Horton 1998, Johnson et al. 2002, Lan et al. 2004). The primary damage by plum curculio is caused by the females during feeding and oviposition into young developing fruits, although the males also cause damage through their feeding. To protect their eggs, the females partially cut a slit in the fruit skin a little below the oviposition site (Chapman 1938, Armstrong 1958). The larva feeds into the fruit resulting in fruit abscission by the time the larva is ready to enter the soil to pupate. New generation adults emerge about 21-24 days after larva had entered the soil and continue their feeding and oviposition at the time fruit are close to maturity. This behavior makes the control of plum curculio very difficult because the immature stages develop and are concealed within fruits or soil.

In the U.S., broad-spectrum insecticides, in particular organophosphates such as phosmet (Imidan®), carbamates, and to lesser degree pyrethroids such as permethrin (Arctic®) and esfenvalerate (Asana® XL) are among insecticides that have been reported to provide commercially acceptable control of plum curculio in peaches and apples (Smith and Fiori 1959, Forsythe and Rings 1965, Hagley and Chiba 1980). Some of these insecticides, in particular the organophosphates, also provide broad-spectrum control of other key fruit pests, including internal feeding lepidopteran (Leakey and Wright 2004, Croft and Hoyt 1983, Bancroft et al. 1974, Agnello et al. 2000).

In Alabama, harvested fruit is devoted to extensive fresh marketing efforts and wholesale shipping of high quality fruit that meet consumer standard is required. However, the challenge posed by plum curculio and other pests in achieving the consumer standard has provided the basis for a predetermined intensive calendar-based spray program practiced by peach growers. In this conventional spray program, insecticides (i.e. phosmet) are applied on a weekly/biweekly basis from petal fall to harvest resulting in 6-12 sprays per year, depending on the variety (Foshee et al. 2008). Very few peach growers use the current management recommendations, which include combining scheduled, crop stage-based pesticide applications and economic threshold levels for plum curculio and other insect pests of peaches in the southeastern U.S (Horton and Ellis 1989, Foshee et al. 2008). In response to growing public concerns over food safety and the environment, the U.S. Environmental Protection Agency (EPA), through the Food Quality Protection Act (FQPA 1992), recently announced cancellations or restrictions of several major broad-spectrum insecticides used for peach production. These include the loss of the organophosphates, methyl parathion (Penncap-M®) and azinphos-methyl (Guthion®), the dominant peach insecticides in Alabama and much of the southeastern U.S. The remaining organophosphate labeled for peach production in the southeastern U.S., phosmet (Imidan®), is currently restricted to six or seven sprays per season. Thus, peach pest management practitioners and growers are now challenged more than ever before to develop and implement effective alternative management strategies for plum curculio and other key pests to mitigate the potential impact of the FQPA.

Several new insecticides, notably thiamethoxam (Actara®), indoxacarb (Avaunt®) and kaolin clay (Surround®) are also labeled for use against plum curculio with different degrees of efficacy (Leskey and Wright 2004, Foshee et al. 2006). Although these new insecticides could offer an alternative to the use of broad-spectrum insecticides for plum curculio control in many stone and pome crops (Wise et al. 2006, 2007; Hoffmann et al. 2008, 2009), most are restricted in use and are more costly than the traditional insecticides. A targeted insecticide spray strategy in which insecticide applications are appropriately timed to coincide with the period when plum curculio activity is high represents an ecological friendly alternative approach for managing plum curculio, and may also result in lower production costs. Targeted insecticide spray (TIS) programs involving alternation of some of new insecticides such as thiamethoxam or indoxacarb with phosmet (Imidan®) could provide an effective management strategy for plum curculio and reduce the likelihood of insecticide resistance.

Studies conducted in the North East suggest that plum curculio can indeed be effectively managed in apples with reduced insecticide sprays (Wise et al. 2007, Wright et al. 2000) however this strategy was only evaluated in peaches using organophosphate insecticides in the late 1990's. A recent study in Alabama peaches showed that an integrated pest management (IPM) approach in which insecticides were applied based on the crop's developmental stages resulting in a total of 4-6 sprays per season was effective in reducing plum curculio damage (Foshee et al. 2008). Data collected on trap captures of plum curculio in an unmanaged peach orchard in Alabama from 2000-2008 suggest that plum curculio seasonal activities such as first trap capture, first peak capture of the spring generation and peak trap capture of the summer generation is predictable (Johnson et al. 2004, Akotsen-Mensah et al. unpublished data), and can be used to properly time insecticide applications to achieve acceptable control in peaches.

The objective of this study was to compare the current grower standard pest management program involving calendar-based weekly insecticide spray program to three different reduced spray programs involving targeted (well timed) applications of insecticides for managing plum curculio. The aim was to reduce insecticide use in peaches and associated costs while providing effective control of plum curculio. We hypothesized that reduced spray programs involving three targeted sprays of insecticides will be as effective as the current grower standard conventional calendar-based spray program. Data were also collected on the effect of the different sprays programs on reducing fruit damage due to stink bugs (Hemiptera: Pentatomidae), since these insects have recently risen in status as serious pests of peaches in the southeastern U.S. (Foshee et al. 2008, Mizell et al. 2008). Thus, stink bugs must be considered in the development of reduced spray programs for peach production.

5.2 Materials and Methods

5.2.1 Evaluation of Spray Programs. This study was conducted at the Chilton Research and Extension Centre (CREC), Clanton, Chilton County, Alabama during 2007-2009. The experimental peach orchard was an 8-yr old mature Ruston red, a mid-season peach variety covering 0.688 ha (1.7 acres), with fruits maturing in mid-July. The orchard had been used for insecticide trials in previous years and typically has plum curculio infestation levels similar to commercial orchards. The orchard consisted of 12 rows of 20 trees with row spacing of 6.1 m (20 ft) and tree spacing of 4.57 m (15 ft). In all years, three insecticides were evaluated namely: phosmet (Imidan® 70 WP, Gowan, Co., Yuma, AZ), thiamethoxam (Actara® 25 WG, Syngenta, Greensboro, NC), and permethrin (Arctic® 3.2 EC, Winfield Solutions Inc, St Paul, MN). Phosmet is an organophosphate insecticide that functions by inhibiting acetylcholine esterase, while thiamethoxam is a neonicotinoid with a broad-spectrum systemic activity which acts as acetycholine receptor agonist. Permethrin is a pyrethroid with contact activity, which functions as axonic poison by modulating the sodium channel gateway. All three insecticides are currently labeled for use in peaches and were selected based on their present and projected future importance as peach insecticides (Tillman et al. 2009, EPA 2007).

In the 2007 trial, the following five treatments (spray programs) were evaluated: Program 1: Weekly spray of phosmet (Imidan® 70W at rate of 2.24 kg/ha or 2.0 lb/acre): total of 9 sprays per season; Program 2: Three targeted sprays of phosmet (Imidan® 70W at rate similar to treatment 1) applied at the following plum curculio phenological stages: 1st plum curculio peak trap capture, pre-hand thinning, and 1st peak of the second (summer) generation; Program 3: Two targeted sprays of phosmet (Imidan® 70W at rate similar to treatment 1) and 1 targeted spray of thiamethoxam (Actara® 25 WG rate of 0.58 l/ha or 8.0 fl. oz/acre) applied in an alternated fashion (i.e. phosmet followed by thiamethoxam) at plum curculio phenological stages similar to treatment 2 above; Program 4: Two targeted sprays permethrin (Arctic® 3.2 EC at rate of 0.25 l/ha or 3.4 fl. oz/acre) and 1 targeted spray of thiamethoxam (Actara® 25 WG at rate similar to treatment 3) applied in an alternated fashion at plum curculio phenological stages similar to treatment 2 above; and Control: Untreated (water) . Each treatment was applied to experimental plots consisting of 3 rows of 4 trees (12 trees total) and replicated four times. All spray treatments were applied with an air-blast sprayer mounted on a tractor and calibrated to deliver at 150 gallons per acre. The decision to apply the first and subsequent sprays was based on captures of plum curculio in 4 unbaited pyramid traps deployed in an unmanaged peach block located about 1.5 km (0.93 mile) north-east of the orchard. The orchard was managed using standard disease (fungicides) and weed management (herbicides) practices throughout the study. To assess fruit damage due to plum curculio and other insects such as stink bugs, three trees in the innermost rows in each plot were randomly selected and tagged from which 60 fruits were randomly harvested (20 fruits per tree) every month starting at fruit set. At harvest, 150 (50 fruit per tree) were randomly harvested from the tagged trees and assessed for insect damage. To assess the damage on the fruit, the pubescence of each harvested fruit when present, was first removed by gently scrapping them using a small cutting knife and inspected for plum curculio oviposition and stink bug feeding punctures. Each fruit was subsequently cut opened with a knife to check for egg or larval presence. The damage due to plum curculio and stink bugs were separated and used to calculate the percentage damage by dividing the total number of fruit harvested by the number of fruits with plum curculio or stink bug damage.

Similar methodologies were used for the 2008 and 2009 trials but with some modifications to modestly increase the scale of the experiment. In 2008 and 2009 the weekly insecticide application treatment was excluded because it has historically resulted in <1% fruit damage in peaches. In both years the experimental plot size was also increased to 4 rows of 5 trees (20 trees total). Each treatment was replicated three times instead of four replications in 2007. Also the number of fruit harvested for damage assessment at harvest was increased to 600 per plot (200 per tagged tree).

5.2.2 Statistical Analyses. Monthly percentage plum curculio damage calculated for each replicate was analyzed by using one-way analysis of variance (ANOVA) (JMP version 7.0.1 2007) to test for significant effects of the treatments (spray programs) at each sampling date, as well as block (replicates) effect. Similar analyses were conducted for fruit damage due to stink bugs. In all cases percentage damage data were tested for assumptions of ANOVA. Where any of the assumptions were not met an appropriate transformation was performed on the data for further analysis. Percentage damage data was transformed by arcsine ($\sqrt{X} + 0.1$) prior to analysis. In all tests, significant differences between means were established by using Tukey-Kramer honesty significant difference (HSD) comparison test. Significant differences were established at $P = 0.05$. Untransformed data were presented as means in the results.

5.3 Results

5.3.1 Phenology of Plum curculio and Peach Trees

5.3.1 Phenology of Plum curculio and Peach Trees. Throughout the 3-yr period the phenology of plum curculio such as first trap capture, peak trap capture in the unmanaged peach block was consistent and also synchronized well with the phenology of the peach tree (Fig. 1A-C). The first trap capture was recorded during 40-90% bloom (range: 3-21 March). The range of the bloom period was wide because the unmanaged peach block consisted of different rootstock varieties (e.g., Hagler and Nemaguard) and commercial cultivars (e.g., Loring and Elberta) that required different chill hours to bloom. The peak trap capture occurred during petal fall (range: 2-11 April) or when fruit had attained an appropriate size of about 5 mm diameter for oviposition. Overall, the use of plum curculio phenology as determined by captures in pyramid traps allowed us to properly time insecticide applications for effective control of plum curculio and stink bugs.

5.3.2 Evaluation of Spray Programs. In 2007, percentage of fruit damage due to plum curculio was not significantly different among the spray program treatments on 17 April (F =

0.33, df = 4, *P* = 0.8551), 9 May (*F* = 1.36, df = 4, *P* = 0.3036), and 5 June (*F* = 0.60, df = 4, *P* = 0.6698). However, significant differences were recorded at harvest on 6 July (*F* = 3.18, df = 4, *P* = 0.0197). Percentage of fruit damage due to plum curculio was significantly lower in the standard conventional calendar-based spray program which resulted in 9 weekly applications of phosmet (Program 1) and in the TIS program in which 2 sprays of permethrin were alternated with 1 spray of thiamethoxam (Program 4) compared to the untreated control (Fig. 2A). Fruit damage due to plum curculio was also numerically lower in the other two TIS program than in the control, and this trend was true for most of the sampling dates (Fig. 2A). No significant block (replicate) effect was recorded on any of the sampling dates (17 April: *F* = 0.29, df = 3, *P* = 0.8294; 9 May: *F* = 0.90, df = 3, *P* = 0.4718; 5 June: *F* = 1.75, df = 3, *P* = 0.2101 and 6 July: *F* = 3.00, df = 3, *P* = 0.0724).

In 2008, no significant differences were recorded among the treatments in percentage fruit damage due to plum curculio on 18 April (*F* = 0.39, df = 3, *P* = 0.7639) and on 12 June (*F* = 0.48, df = 3, *P* = 0.7096), but significant differences were recorded on 16 May (*F* = 4.97, df = 3, *P* = 0.0457) and at harvest on 9 July (*F* = 107.79, df = 3, *P* < 0.0001). Sampling on 16 May showed significantly lower fruit damage in the two alternated TIS programs (Programs 3 and 4) than in the untreated control. On 9 July percentage fruit damage due to plum curculio was significantly lower in all three TIS programs (Programs 2-4) compared to the control (Fig. 2B). No significant block (replicate) effect was recorded on 18 April (*F* = 1.00, df = 2, *P* = 0.4219), 16 May (*F* = 0.43, df = 2, *P* = 0.6686), and 12 June (*F* = 1.35, df = 2, *P* = 0.3286). However, a marginally significant block effect was recorded at harvest on 9 July (*F* = 5.48, df = 2, *P* = 0.0443).

Similar results were recorded in 2009. Percentage fruit damage due to plum curculio was not significantly different among the treatments on 18 April (*F* = 0.56, df = 3, *P* = 0.6623) and 19 June (*F* = 1.85, df = 3, *P* = 0.2388). However, significant differences were recorded among the treatments on 21 May (*F* = 9.31, df = 3, *P* = 0.0113) and at harvest on 9 July (*F* = 19.49, df = 3, *P* = 0.0017). On both sampling dates, all the three TIS programs (Programs 2-4) provided significant reductions in plum curculio damage relative to the control (Fig. 2C). No significant block (replicate) effect was recorded on any of the sampling dates (18 April: *F* = 0.57, df = 2, *P* = 0.5947; 21 May: *F* = 1.00, df = 2, *P* = 0.4219; 19 June: *F* = 0.09, df = 2, *P* = 0.9170 and 9 July: *F* = 0.92, df = 2, *P* = 0.4470).

The effect of the spray program treatments on stinkbug damage was evaluated at harvest in 2008 and 2009. The key stink bug species recorded in this study were the southern green stink bug, *Nezara viridula* (Linnaeus); adult brown stink bug, *Euschistus servus* (Say), dusky stink bug, *E. tristigmus* (Say); and green stink bug, *Acrosternum hilare* (Say). In 2008, percentage fruit damage due to stink bugs was significantly reduced (*F* = 58.91, df = 3, *P* < 0.0001) in all the three TIS programs compared to the control (Fig. 3). Significant differences were also recorded in 2009 (*F* = 9.63, df = 3, *P* = 0.0104): stink bug damage was significantly reduced in the two alternated TIS treatments (Programs 3 and 4) than in the control (Fig. 3). The mean percentage fruit damage due to stink bugs in control plot was > 12% in both years, indicative of high population pressure. No significant block effect was recorded on stink bug damage in both years (2008: *F* = 0.07, df = 2, *P* = 0.933; 2009: *F* = 0.46, df = 2, *P* = 0.649).

5.4 Discussion

The reduced, targeted (timed) insecticide spray (TIS) programs provided significant reduction in fruit damage due to plum curculio (< 1%) comparable to the conventional weekly spray program, particularly during harvest in 2008 and 2009. The damage levels recorded monthly in many cases did not show significant differences among the treatments, especially during the early stages of fruit development. However, treatment effects were mostly significant after the May drop and at harvest.

Overall, the results showed that the two alternated TIS programs (Program 3: 2 phosmet sprays alternated with 1 spray of thiamethoxam and Program 4: 2 sprays of permethrin alternated with 1 spray of thiamethoxam) provided acceptable control of plum curculio damage in this study. Our results are similar to those from previous studies which showed that plum curculio can be effectively managed in apples with reduced insecticide applications (Wise et al. 2007, Wright et al. 2000). Specifically, a study conducted in Massachusetts reported that three applications of phosmet, thiamethoxam and surround provided significant reduction of plum curculio damage on apples compared to the untreated control, however, damage levels were still too high to be acceptable by fresh market consumers (Wright et al. 2000).

The damage levels reported in this study are similar to those reported in related previous studies (Foshee et al. 2008, Wright et al. 2000). For instance a recent study in three separate peach cultivars orchards in Alabama found that damage levels of plum curculio and other insects were reduced significantly when phosmet was applied based on specific peach plant phenology and economic threshold levels of plum curculio and other pests (Foshee et al. 2008).

Although we did not determine the actual quantity of the active ingredient translocated into the fruit, the positive results obtained for the treatments suggests some systemic effect of the new insecticides (Wise at al. 2007, Hoffmann et al. 2008, 2009). Thiamethoxam, which was applied as second cover, has indeed been reported to show systemic activity against plum curculio in apples, peaches and tart cherries Hoffmann et al. 2008, 2009).

Comparing the years, damage at harvest in 2007 was higher than in 2008 and 2009 (Figs 1 A-C). Other authors have also reported similar results in which higher fruit damage due to plum curculio was recorded in the first year than in subsequent years (Prokopy et al. 1996, 1990). It is likely that the decline in fruit damage during the second and third years of the study was related to treatment applications in the test orchard during the first year, although environmental factors may also play a role.

Although the main focus of this study was plum curculio, our results indicated that the *N. viridula*, *E. servus*, *E. tristigmus*, and *A. hilare* are becoming very serious pests of peaches in most of the southeast, including Alabama (Foshee et al. 2008, Mizell et al. 2008). Increased pressure by stink bugs could pose a challenge to the adoption of insecticide spray programs targeted against plum curculio, particularly for late season cultivars. Aside from stink bugs, none of the other insect pests of peaches reported in other regions (e.g., Oriental fruit moth, mites, aphids, and scale insects) were recorded in significant numbers in control and treatment plots in the test orchard, and thus no damage was attributed to these insects. This finding may suggests the possible effect of abiotic and biotic natural mortality factors in maintaining populations of these secondary insect pests below economic injury levels.

In summary, our results showed that plum curculio can be effectively managed using three targeted (timed) applications of the insecticides evaluated in this study in an alternated fashion. Monitoring will be required to determine peak plum curculio activity in the orchard because the performance of the spray programs depends largely on the precise detection of the seasonal activity and phenology of plum curculio in an orchard. The results also suggest that permethrin and thiamethoxam are effective alternatives to phosmet in peach production. A reduced, targeted spray program in which both insecticides are applied in an alternated fashion begs for evaluation, since insecticide rotation is an effective insecticide resistance management strategy. Further studies are also necessary to replicate this study in larger peach blocks at multiple sites and over several years, prior to recommending the TIS programs to peach growers.

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Figure Legends

Figure 1. Seasonal phenology of plum curculio in an unmanaged peach orchard in Clanton, Alabama in (A) 2007, (B) 2008, and (C) 2009. Figure shows number of plum curculio captured in unbaited pyramid traps (n = 4). Data was used to time application of insecticides in the test orchard. Arrows indicate dates when insecticide treatments were applied in the test orchard.

Figure 2. Mean (± SE) monthly percentage peach fruit damage due to plum curculio recorded at different sampling dates at the Ruston red peach orchard in Clanton, Alabama in (A) 2007, (B) 2008, and (C) 2009. Note: the conventional spray program (weekly spray of phosmet) was not evaluated in 2008 and 2009. Means within each date having no letter in common are significantly different (ANOVA, Tukey-Kramer HSD, $P < 0.05$).

Figure 3. Mean (± SE) percentage peach fruit damage due to stink bugs recorded at harvest at the Ruston red peach orchard in Clanton, Alabama during 2008-2009. Means within each year having no letter in common are significantly different (ANOVA, Tukey-Kramer HSD, $P < 0.05$).

Figure 1

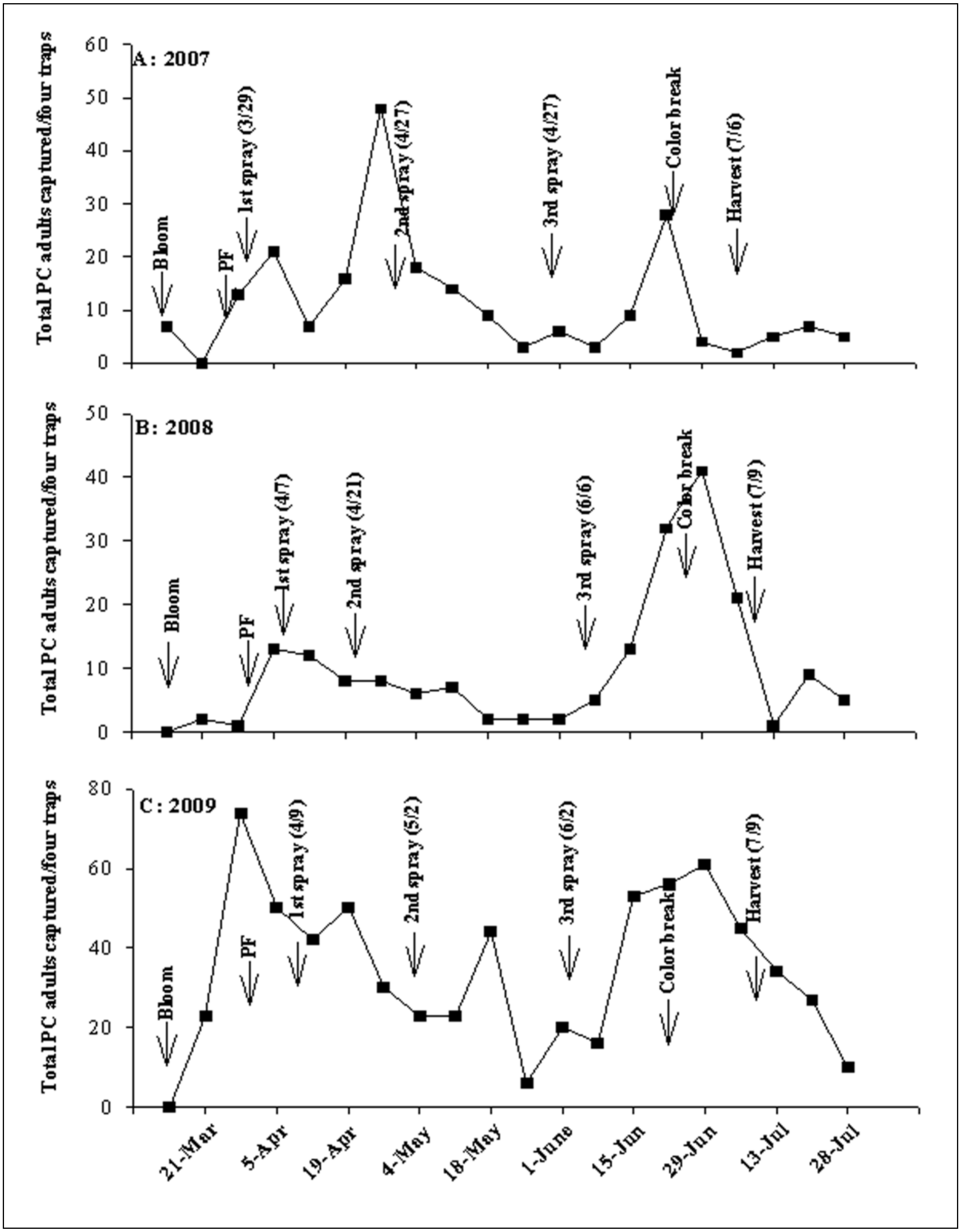

A: 2007
Total PC adults captured/four traps
60
50
40
30
20
10
0
Bloom
PF
1st spray (3/29)
2nd spray (4/27)
3rd spray (4/27)
Color break
Harvest (7/6)
B: 2008
Total PC adults captured/four traps
50
40
30
20
10
0
Bloom
PF
1st spray (4/7)
2nd spray (4/21)
3rd spray (6/6)
Color break
Harvest (7/9)
C: 2009
Total PC adults captured/four traps
80
60
40
20
0
Bloom
PF
1st spray (4/9)
2nd spray (5/2)
3rd spray (6/2)
Color break
Harvest (7/9)
21-Mar
5-Apr
19-Apr
4-May
18-May
1-June
15-Jun
29-Jun
13-Jul
28-Jul

Figure 2

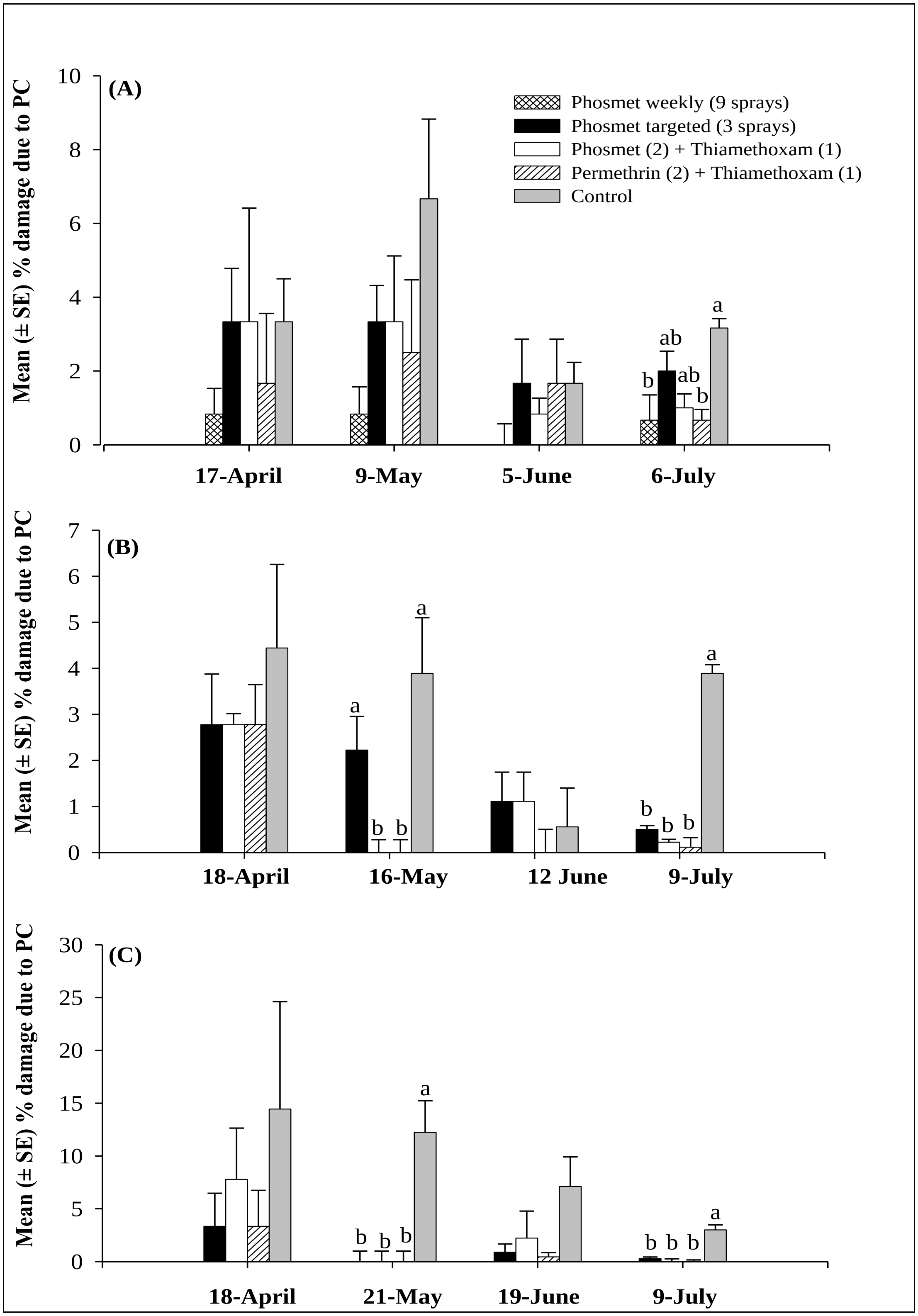
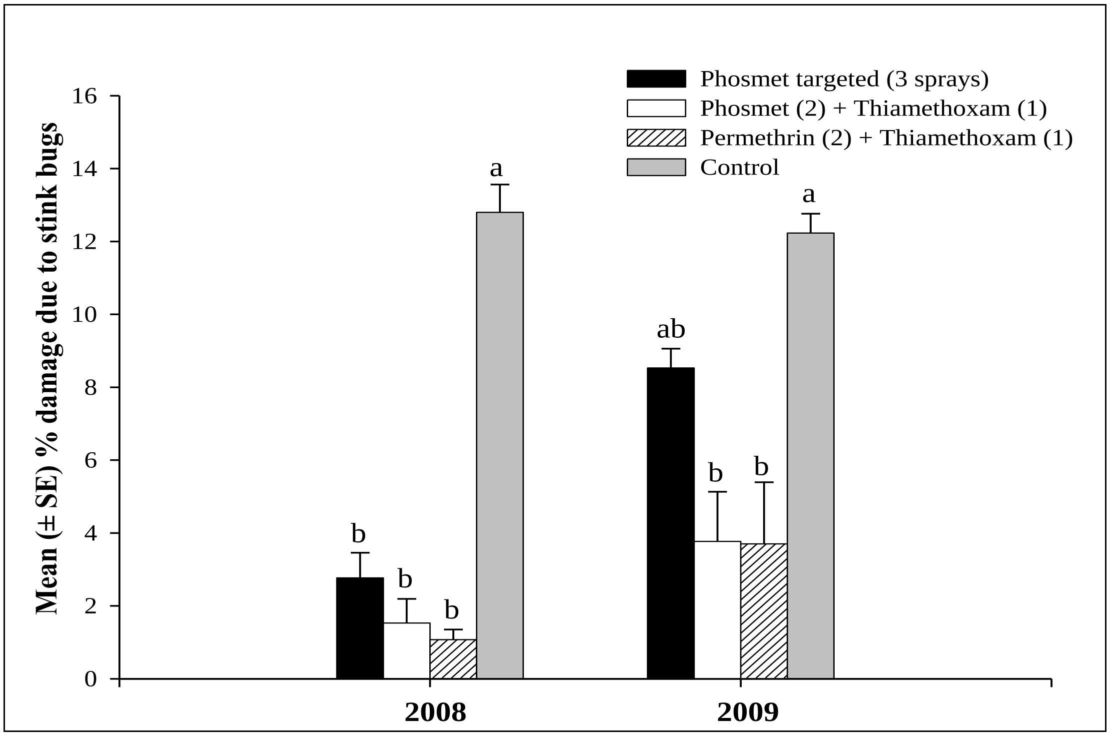

(A)
Mean (± SE) % damage due to PC
10
8
6
4
2
0
Phosmet weekly (9 sprays)
Phosmet targeted (3 sprays)
Phosmet (2) + Thiamethoxam (1)
Permethrin (2) + Thiamethoxam (1)
Control
17-April
9-May
5-June
6-July
a
ab
ab
b
b
(B)
Mean (± SE) % damage due to PC
7
6
5
4
3
2
1
0
a
a
b
b
a
b
b
b
18-April
16-May
12 June
9-July
(C)
Mean (± SE) % damage due to PC
30
25
20
15
10
5
0
a
b
b
b
a
b
b
b
18-April
21-May
19-June
9-July

Figure 3


Phosmet targeted (3 sprays)
Phosmet (2) + Thiamethoxam (1)
Permethrin (2) + Thiamethoxam (1)
Control
Mean (± SE) % damage due to stink bugs
0
2
4
6
8
10
12
14
16
b
b
b
a
ab
b
b
a
2008
2009

CHAPTER 6

EFFECT OF SOIL AND WEED MANAGEMENT PRACTICES ON PLUM CURCULIO *CONOTRACHELUS NENUPHAR* HERBST (COLEOPTERA: CURCULIONIDAE) LARVAL DEVELOPMENT AND ADULT EMERGENCE IN ALABAMA PEACHES

6.1 Introduction

Soil and weed management practices maintained by most peach growers in the southeastern U.S., particularly, in Alabama are diverse (Horton and Ellis 1989, Horton and Johnson 2005). The diversity seen in the different orchard floor maintained by growers is because each orchard floor management practice has its own advantages and disadvantages depending on the type of crop (Parker and Meyer 1996). While some peach growers maintain grasses or sods and herbicides strips (Mitchem http://www.ent.uga.edu/peach/peachhbk/pdf, Horton and Johnson 2005), other orchards are allowed to become weedy with very minimal weed management, particularly, during off season. Alternatively, mulching is one practice adopted by majority of growers involved in small fruit production such as blueberries and strawberries and other fruits (Foshee et al., 1999) for managing weeds and moisture conservation. The primary goal of any orchard floor management practice is to optimize yield by minimizing weed competition, reducing presence and damage of insect pests. Orchard floors can be grouped into those that involve the use of live materials such as planting of leguminous plants to improve fertility, maintenance of weeds to control erosion, removal of weed using herbicides and in some case the use of synthetic materials to cover the weeds. Each of these has different effect on the orchard floor ecosystem. Notably among these effects are alteration of nutrient availability, soil moisture and physical properties, prevalence of weeds, plant pathogens and insect pests (Altieri and Schmidt 1986).

To date investigation carried out in orchards relating to soil and weeds have stressed on competition of weeds with peach plants in relation to nutrients and moisture. However, presence of weeds can have direct impact on insect pest population and damage they cause to crop plants (Norris and Kogan 2000, 2005). Whereas plant growth and yield may be adversely affected by weed interference through competition, the vegetational diversity provided by weeds may also improve yields of some crops by suppressing damage from key pests (Altieri and Whitcomb 1980, Showler and Reagan 1991, Qureshi et al. 2007).

Plum curculio spends part of its life cycle, specifically, the pre-pupal, pupal and post-moulting stages in the soil (Stedman 1904). The adult after emerging from overwintering sites, usually during spring when environmental conditions such as temperature, humidity, precipitation are favorable, mate and migrate to find feeding and oviposition sites within nearby orchards. The female adult after locating a good developing fruit feed and lays its egg in the fruit. The egg hatch and the larva eat its way into the developing fruit. Premature fruit abortion occurs either before or during the time the fully grown 4th instar larva is about to pupate. The larva exits the aborted fruit and pupates in soil. It spends varying amount of time in the pre-pupal stage in the soil within a depth of about 3-8 cm before final pupation (Garman and Zappe 1929, Quaintance and Jenne 1912, Horton and Ellis 1989). The time spent in the soil before the adult finally emerges depends greatly on soil conditions (Armstrong 1958).

Given this activity level of plum curculio, we hypothesized that different soil and weed management practices will maintain different ecosystem consisting of both biological biotic and abiotic mortality factors such as predatory arthropod community, soil temperature etc that can reduce plum curculio pupal activity in the soil and thus contribute to control of plum curculio in the peach orchards.

This study was therefore undertaken to evaluate the effect of some commonly used soil and weed management practices on the development of the pre-pupae and pupae of plum curculio in the soil. Ultimately, we hope to identify and recommend to commercial peach growers soil and weed management practices with negative impacts on the development of this pest.

6.2 Materials and Methods

6.2.1 Field Study. The study was conducted in two peach orchards at Chilton Research and Extension Centre (CREC) Clanton, AL., during 2007-2009 peach seasons. The 2007 (preliminary trial) study was done in an unmanaged peach orchard that was planted in 1985 and has since not been sprayed with any insecticide or fungicide. Eight small plots (3 m × 3 m) were established at both sides of three randomly selected peach trees appearing in a row (four plots on each side) to which four soil and weed management treatments were applied. The treatments per established in each plot are: (1) Centipede grass [(*Eremochloa ophiuroides* (Munro) Hack.)] understory (soil covered with centipede grass); (2) Weed free understory (bare soil, weeds removed with herbicide sprays); (3) Weedy (natural weeds) understory; and (4) Pine bark understory (soil covered with pine bark). Treatments consisting of plants (centipede grass) were established 3-4 weeks before larvae were ready to be introduced. Field infested fruits were collected and placed in a wooden larval emergence trays designed to capture emerging larvae. Twenty newly emerged plum curculio larvae were collected such fruits and placed in the soil in each treatment plot. In each plot, one cone emergence trap was set up over the introduced larvae to monitor the adult emergence. Traps were checked daily for three days after the introduction of the larvae and daily after until the first adult emerged. The traps were monitored for 30 days after emergence of the first adult. The number of emerged adults that were captured in the cone traps were counted and recorded.

Also pitfall traps were deployed in the treatment plots outside the cone traps as additional sampling method to determine the presence and abundance of other ground dwelling arthropods.

The sampling dates which pitfall traps were collected were 10 April, 16 May and 22 June. Although several arthropods were found in pitfall traps in orchards, the following were reported based on their importance reported in a previous study: ground beetles (*sensu lato* Carabidae and Cicindelidae), ants (Formicidae) and Spiders. Similar methods were used in 2008 and 2009 except that an additional orchard was included in the study to increase the number of replications and locations. Also 10 larvae were used in both years instead of the 20 larvae used in 2007. The sampling dates which pitfall traps were installed in 2008 were 23 May, 30 May, 13 June and 4 July and 14 May, 21 May, 11 June and 18 June in 2009. Plot maintenance was done by using a hand held mower to cut overgrown weeds in the weedy and centipede grass orchards to a height of ~10 cm. Also hand-picking of undesirable plants from the centipede grass and pine bark treated plots was done when necessary.

6.2.2 Greenhouse Study

Development of plum curculio pre-pupa and pupa in different soil and weed management practice treatments was evaluated under greenhouse conditions [25 ± 2 ºC, 50 ± 10% RH, and a photoperiod of 12:12 (L: D) h] during fall 2007, and spring 2009. The goal of this study was to confirm the results obtained from the field study in a much controlled environment than the variable conditions experienced in the field. The field treatments in addition to two other treatments were replicated in 100 x 40 x 40 cm Rubbermaid® plastic containers (herein refer to as container) in the greenhouse. Prior to the main experiment, a preliminary test (2007) was done to determine the frequency and amount of water that will provide optimal growth of the plants and at the same time providing better development of the insects in the containers. The outcome of this preliminary study was used to apply at weekly intervals for the treatment that had plants and every 10 days with treatments without plants. Thus, in all cases adequate moisture was maintained for both plants and insects to develop. In addition to the four treatments used in the field, two additional treatments were included. The treatments evaluated in 2007 were (1) Centipede-grass (CPG) growing on autoclaved field soil (AFS); (2) Weeds growing on AFS; (3) Pine bark (PB) spread on AFS; (4) Orchards weeds on untreated soil (5) AFS only representing bare soil; and (6) Mixture of vermiculite and AFS (1:2) (positive control). The centipede grass (treatment1) was obtained as sod from Beck's Turf, Tuskegee, AL. Each sod was cut to fit the size of the container used for the experiment. The sod was grown on AFS which had a depth of about 15 cm. The depth of soil was chosen based on the fact that plum curculio pupates within 3-8 cm in the field and also to provide a good estimation of the rooting zone of the plants used. The sod was allowed to break dormancy and also establish well on the soil for about 2 months before the start of the experiment. Similar procedures were used to establish treatment 2 (weedy understory). Weeds growing in the unmanaged peach orchard in Clanton were picked at random and brought to the greenhouse to establish this treatment. The soil on the roots was washed before plants were planted in autoclaved field soil. The pine bark (treatment 3) was obtained from Home Depot Stores, Opelika, AL., and a local producer at Auburn; AL. Herbicides treatments were not included in the 2007 study due to our inability to secure proper permission to allow for the application of herbicides in the greenhouse which was also used for other ornamental plants. All treatments were established on autoclaved soil in order to eliminate the effects of predatory insects such as ants, ground beetles and other arthropods like spiders which have been reported to prey on developing larvae (Jenkins et al. 2006). After treatments involving plants have established well in the containers, four polyvinyl chloride (PVC) pipes of diameter 12 cm which were cut to a height of ~25 cm were inserted (Fig. 1) in the soil in the container. Each PVC pipe was considered a plot to which 20 fourth instar larvae of plum curculio (~15–18 mg each) removed from the laboratory colony immediately after emergence were introduced. A boll weevil trap top was used to cover the PVC pipes to ensure that emerging adults did not escape. Adults that emerged into the boll weevil trap top were collected and recorded daily until 21 days after first adult appeared in the trap top. Similar procedures were used in 2009 except that 10 larvae were used instead of twenty in 2007. The number of days taken for all adults to emerge was also recorded. The number of adults that emergend were converted to percentage emergence by dividing the total number that emerged by the total number of larvae that were introduced into the PVC pipes.

6.2.3 Statistical Analyses. For the field study percentage adult emergence data were analyzed using one-way analysis of variance (ANOVA). Pitfall trap data for other arthropods were combined for all sampling dates and the total used to perform the ANOVA. In the green house study similar analyses were done for all data involving larval and adult survival. All data obtained were tested for the assumptions of ANOVA. Data points that recorded zeros were transformed using $\sqrt{x} + 0.5$. For all ANOVA performed means were separated using Tukey-Kramer honesty significant difference (HSD) test ($P < 0.05$; JMP version 7.0, SAS Inc. Cary, NC, 2007).

6.3 Results

6.3.1 Field study. In the unmanaged peach block, the percentage of adults that emerged from the soil and weed management practice treatments was significant in 2007 ($F = 3.29$, df = 3, $P = 0.0371$) but not in 2008 ($F = 0.61$, df = 3, $P = 0.6168$) and 2009 ($F = 0.84$, df = 3, $P = 0.4872$). In 2007 the percentage emergence was significantly lower in the centipede grass treated plots compared to weed free understory (Fig. 1A). Although not significant, the percentage emergence of plum curculio was also numerically lower in the centipede grass treated plots than the other three soil and weed management treatments in 2008 and 2009 (Fig. 1A). Location of the plots (replicates) within the unmanaged orchard was not significant in 2007 ($F = 1.64$, df = 3, $P = 0.2057$), 2008 ($F = 0.88$, df = 3, $P = 0.4668$) and 2009 ($F = 2.51$, df = 3, $P = 0.0815$).

Similarly in the Loring orchard in 2008 no significant difference was observed among the treatments ($F = 0.70$, df = 3, $P = 0.5615$). However, treatments effect was significant in 2009 ($F = 3.13$, df = 3, $P = 0.0514$). The mean percentage emergence showed that emergence of the adults in the weed free orchard was higher than the other treatments (Fig 1B). This was true for most of the years in both orchards (Fig 1 A & B). The location of the treatment plots within the orchard was also not significant in 2008 ($F = 0.88$, df = 2, $P = 0.0654$) and 2009 ($F = 2.37$, df = 2, $P = 0.1216$).

Overall the emergence patterns of the adults were similar in both orchards and during the three seasons of the study (Fig. 2 A-C). The peak emergence of the adults for all the years occurred during the week of June 10-18 in all the years which is consistent with natural field emergence in the orchards at the study area.

Although the pitfall trap caught several arthropods, we report here those which have previously been reported to be important predators of plum curculio and other insects (Jenkins et al. 2006). Thus we analyzed data on ground beetles (*sensu lato* Carabidae and Cicindellidae), ants (including fire ants) and spiders. In all the years in both orchards there was varying number of captures of these arthropods in the soil and weed management treatments (Tables 1-4). In 2007 there was generally no significant effect of the pitfall trap captures for ground beetles, ants and spiders (Table 1). However, weedy (natural weeds) treatment had numerically more ground beetles (mean ± SE: 31.33 ± 4.8) than the other treatments. Also, centipede grass and weed free had more ants than the other treatments (Table 1). In 2008, there was significant difference among the treatments in the unmanaged peach orchard for ground beetles and ants (Table 2). Also significant difference among the treatments was observed for ants in the Loring orchard. In general the pitfall traps captured more of the other arthropods in the weedy (natural weeds) than the other treatments (Table 2). Similar pitfall trap capture numbers were observed in 2009 in both orchards although there were generally no significant differences among the treatments for all the insects (Table 3). In general the pitfall traps recorded numerically more insects in the weedy (natural weeds) treatments. Also there were more ants recorded in the pine bark treatment for ants in both orchards in 2009 (Table 3).

6.3.2 Greenhouse study. The results in 2007, showed that all five soil and weed management treatments had significant effects on the development of the larvae and subsequent emergence of adults ($F = 2.60$, df = 4, $P = 0.0462$). All the treatment tested in the field did not differ from each other (Table 4). However, centipede grass treated soil consistently showed numerically fewer numbers of plum curculio emergence compared with the other treatments used in the field study (natural weeds and pine bark) (Table 4).

In 2007, the number of days taken for adults to develop and emerge was not significantly different among the soil and weed management practice used (F = 0.1.86, df = 4, P = 0.1303). However, in 2009 there was significant difference in the number of days for adults to appear in the traps (F = 18.97, df = 6, P < 0.0001) (Table 5). Weather conditions during the three years that the study was conducted varied considerably (Table 6). There were generally drought conditions in 2007 during the period (April-June) the larvae and pupae were developing in the soil (Table 6). In general 2008 and 2009 were wet seasons during the same time that larvae and pupae developed in the soil.

6.4 Discussion

This research provides data from three years of field studies combined with two greenhouse experiments which, together suggest that plum curculio pre-pupal and pupal development in the soil can be influenced by the type of soil and weed management practice adopted in peach orchards. The soil and weed management treatments used in this study are those commonly used by commercial peach and small fruit growers in Alabama and other parts of the southeastern U.S. The results suggest that all treatments (centipede grass, weed free, weedy and pine bark understories) had effect on plum curculio development but each treatment showed varying effects on the emergence of plum curculio adults. The greenhouse results confirmed the field results which showed that fewer plum curculio adults developed in centipede grass compared with some of the commonly used orchard understories such as bare soil and weedy (natural weeds) although this was not always significant.

The performance of centipede grass in reducing the emergence of plum curculio adults was surprising. Although supporting literature is lacking, we believe that the performance of centipede grass in reducing the emergence of the adults was possibly due to obstruction provided by the increased biomass of the rooting systems. The rooting system of grasses as a physical barrier to development has been observed in other insects. For example, Wood et al. (2009) found that oviposition of the Japanese beetles, *Popillia japonica* on a hybrid bermudagrass [C. *dactylon* (L.) Pers. X *C. transvaalensis* Burtt-Davy, ‘Tifway] was deterred. They attributed this to the physical barrier provided by the root mat of the grass species since they found that it took nearly twice as long to break apart the roots of bermudagrass than other turfgrasses. Also Bao and Hirata (2006) reported that centipede grass has a high ability to develop tillers and stolons (roots) and are superior to other species of grasses. This superiority of centipede grass in producing increased stolon and tiller formation possibility suggests that it contributed to the emergence level recorded in the centipede grass treated plots.

Our expectation that weed free (bare soil) treatment which is the commonly used orchard floor management practice recommended and used by growers in Alabama and many parts of the southeast will produce fewer adults was not achieved in the field study. While in the greenhouse house, weed free (autoclaved field soil) had significantly fewer adults emerging than all the other treatments, this was not the case in the field. This was surprising because we expected the bare soil in orchard to expose the insect to extreme inclement conditions such as sunlight, low and high soil moisture etc and as a result produce fewer adults. This was, however, not the case; instead more adults emerged from the bare soil (herbicide treated soil) compared with other treatments. Comparing our results with a similar study in a blueberry orchard, Szendrei and Isaacs (2006) found fewer larvae of Japanese beetle surviving in a bare soil. We explain this difference to be possibly due to difference in the tree canopy of peaches and blueberry plants and also the possible differences in the biology of plum curculio and June beetles. It was possible that the peach tree canopy provided enough shade to the plum curculio development in the soil and thus prevented the exposure of the insects in the soil to any of the inclement weather conditions usually experience during the period the prepupae and pupae develop in the soil in the study area. This however, needs further investigation.

Although the presence of other predatory arthropods in the field varied among the treatments, particularly in 2008, majority of the treatments showed no significant effect on the number of ground beetles, ants and spiders and results were not very consistent. The inconsistence results in, for example, ground beetles numbers in cropping system is not peculiar to this study only since different soil and weed management practice depends on the crop or the insect species (Cárcamo 1995, Cárcamo et al. 1995, Andersen 1999). Because the focus of the study was not mainly on arthropods, we were unable to investigate in detail the contributions of each of the groups of arthropods reported. However, we cannot rule out the contribution of these arthropods and other abiotic factors because most of them have previously been reported to have effect on plum curculio survival in the peach and apple orchards (Armstrong 1958, Mampe and Neunzig 1967, Jenkins et al. 2006) and other insects. For example, carabids (ground beetles) have been reported to be natural enemies of above ground pests such as aphid (Hagley and Allen 1990), and apple maggot (Hagley et al. 1982), and those that have a life stage in below-ground such as apple sawfly, apple twig cutter or apple leaf midge (Miñarro and Dapena 2003).

In the greenhouse the treatment with no weeds growing showed the lowest number of days for the adults to appear in the trap tops used. The difference could have been because the insect possibly spent time on the leaves after emergence before they moved to the trap set over the PVC pipe to capture emerging adults. The presence of other arthropods captured by pitfall traps suggests the importance of these arthropods in the treatments used. On the whole the average percentage survival regardless of treatment was in the range of 32-46% in the greenhouse, 13-32% in the Loring orchard in 2008 and 22-27% in the unmanaged peach block. In general survival of adults was high in 2009 than in 2007 and 2008. The difference between the overall emergence of plum curculio in the greenhouse and laboratory could be attributed to other factors such as temperature and moisture. In particular, examination of the temperature, humidity and precipitation records during the period when larvae were in the soil revealed that the low numbers recorded in the field could also be explained by drought condition experienced particularly in 2007.

In summary this study suggests a previously undocumented effect of soil and weed management practice in peach orchards in Alabama. Most previous studies in orchards have focused on abiotic factors such as temperature, moisture, etc and cover crops and their effect of insects (Altieri and Schmidt 1986). We are currently not aware of any study that has specifically looked at the effects soil and weed management practices have on pre-pupal and pupal development of plum curculio in peach orchards. This study has therefore demonstrated for the first time the potential utility of soil and weed management practice to reduce plum curculio emergence in peach orchards in Alabama and other parts of the southeastern region. The results which showed that centipede grass can reduce the emergence of the adults suggest a role for centipede grass in the development of an IPM program for plum curculio particularly as key insecticides used to control plum curculio are being lost due to Environmental Protection Agency’s (EPA) restrictions and that as pest management systems become less reliant on the use of broad spectrum insecticides, research into the enhancement of cultural practices through the manipulation of the orchard habitat and natural enemies populations such as those reported in this study will be much needed. A greater understanding of the mechanisms involved in the soil and weed management practice will offer better improvement and also offer advancement in the management of plum curculio in peach orchards. Future studies are required to elucidate the mechanism involved in centipede grass performance in reducing larval and pupal development in the soil.

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Table 1. Mean (± SE) total number of other arthropods recorded in treated soil and weed management plots in an unmanaged peach orchard (Clanton, AL) in 2007

Treatments(Type of understory)	Ground beetles	Ants	Spiders
Centipede grass	20.33 ± 2.11	78.67 ± 7.47	22.00 ± 0.51
Weed free	17.00 ± 2.50	70.33 ± 3.29	13.33 ± 3.99
Weedy	31.33 ± 4.84	59.67 ± 7.07	23.00 ± 4.56
Pine bark	26.33 ± 3.58	49.33 ± 4.07	16.00 ± 4.93
P	0.1484	0.0825	0.4311
F	2.59	3.66	1.07

Means within the same column having no letter are not significantly different (ANOVA, Tukey-Kramer HSD, $P < 0.05$, df = 3, 6)

Table 2. Mean (± SE) total number of other arthropods recorded in soil and weed management in unmanaged and Loring peach orchards (Clanton, AL) in 2008

Treatments (Type of understory)	Mean (± SE) per sampling date					
	Unmanaged orchard			Loring		
	Ground beetles	Ants	Spiders	Ground beetles	Ants	Spiders
Centipede grass	41.25 ± 2.96b	93.5 ± 6.49ab	17.00 ± 2.75	13.75 ± 3.32	45.75 ± 1.82bc	15.50 ± 2.09
Weed free	27.00 ± 4.04b	45.75 ± 8.63b	17.00 ± 1.35	19.75 ± 4.38	31.75 ± 5.46c	15.25 ± 0.27
Weedy	60.75 ± 4.99a	113.50 ± 13.76a	20.25 ± 2.20	37.75 ± 7.86	86.50 ± 3.15a	24.75 ± 2.56
Pine bark	31.00 ± 1.89b	95.00 ± 6.98a	23.50 ± 1.40	21.25 ± 3.60	57.00 ± 4.57b	16.50 ± 2.50
P	0.0014	0.0098	0.2179	0.0869	<0.0001	0.0600
F (df = 3, 9)	12.75	7.05	1.80	3.01	25.44	3.57

Means within the same column having no letter in common are significantly different (ANOVA, Tukey-Kramer HSD, $P < 0.05$)

Table 3. Mean (± SE) total number of other arthropods recorded in soil and weed management in unmanaged and Loring peach orchards (Clanton, AL) in 2009

Treatment (Type of understory)	Mean total (± SE) of arthropods recorded					
	Unmanaged orchard			Loring		
	Ground beetles	Ants	Spiders	Ground beetles	Ants	Spiders
Centipede grass	19.75 ± 1.86	49.5 ± 5.65	5.25 ± 2.91	19.25 ± 1.20	53.25 ± 13.35	2.50 ± 1.31
Weed free	17.00 ± 1.86	29.00 ± 10.73	4.25 ± 2.03	15.50 ± 3.48	24.00 ± 6.03	3.00 ± 2.89
Weedy	16.50 ± 3.48	25.00 ± 4.05	8.75 ± 2.31	19.25 ± 1.37	48.00 ± 11.01	13.25 ± 3.26
Pine bark	18.75 ± 3.50	52.00 ± 9.35	1.50 ± 1.14	18.00 ± 1.74	75.25 ± 10.74	4.5 ± 1.88
P	0.8805	0.1491	0.3055	0.6866	0.0912	0.0799
F (*df* =3, 9)	0.22	2.27	1.39	0.51	2.94	3.14

Means within the same column having no letter are not significantly different (ANOVA, Tukey-Kramer HSD, $P < 0.05$)

Table 4. Mean (± SE) percent emergence of plum curculio adults in treated soil and weed management in the greenhouse

Soil and weed practice treatment	Mean (± SE) % emergence	
	2007	2009
Autoclaved field soil (AFS)	35.83 ± 6.74b	50.00 ± 6.10
Centipede grass	40.42 ± 5.72ab	30.00 ± 6.66
Weedy (natural weeds)	41.67 ± 7.57ab	42.50 ± 11.62
Orchards weeds on untreated soil	N/A	57.50 ± 8.56
Weed free (Herbicide)	N/A	55.00 ± 7.58
Pine bark	51.25 ± 6.23ab	45.00 ± 6.57
2AFS + 1 vermiculite	62.08 ± 5.39a	45.00 ± 6.01
P	0.0462	0.3361
F	2.60	1.17
df	4, 53	6, 18

Means within the same column having no letter in common are significantly different (ANOVA, Tukey-Kramer HSD, $P < 0.05$).

N/A not included in treatments.

Table 5. Mean (± SE) number of days for adult plum curculio to emerge from soil and weed management treatments in the greenhouse

Soil and weed treatment	Mean (± SE) number of days to emerge	
	2007	2009
Autoclaved field soil (AFS)	27.42 ± 1.76	21.25 ± 0.24b
Centipede grass on AFS	30.08 ± 1.15	27.00 ± 0.24a
Weedy on AFS	31.25 ± 0.92	28.85 ± 0.50a
Weedy on untreated field soil	N/A	27.50 ± 1.21a
Weed free AFS	N/A	22.50 ± 0.47b
Pine bark AFS	28.83 ± 0.55	22.00 ± 0.65b
2AFS + 1Vermiculite	27.67 ± 1.12	21.00 ± 0.57b
P	0.1303	<0.0001
F	1.186	18.97
df	4, 53	6, 18

Means followed by the same letter in the same column are not significant different from each other at P = 0.05.

N/A not included in treatments.

Table 6. Weather conditions within the months that plum curculio larvae were in soil at Clanton, AL

	Maximum temperature (°C)	Minimum temperature (°C)	Maximum RH (%)	Minimum RH (%)	Total precipitation (cm)	# rain days
2007						
April	22.50	10.12	87.87	36.30	5.38	8
May	30.09	17.07	80.87	29.26	0.03	1
June	34.64	21.12	88.00	31.80	3.33	6
2008						
April	23.99	12.24	90.77	43.43	7.34	9
May	28.10	16.78	89.65	43.19	7.67	8
June	32.94	20.59	92.23	42.03	9.42	9
2009						
April	23.23	10.18	91.20	39.60	6.20	9
May	27.09	18.08	92.00	55.60	16.92	16
June	30.88	19.72	92.90	45.00	0.86	2

Figure Legends

Figure 1. Mean (± SE) number of plum curculio emerging from soil and weed management treatments in (A) an unmanaged peach block and (B) Loring at CREC, Clanton, AL., during 2007-2009 peach season

Figure 2. Seasonal trend of emergence of plum curculio adults from soil and weed management treatments in unmanaged peach and Loring peach orchards at CREC, Clanton, AL., in 2008-2009 peach crop seasons

Figure 1

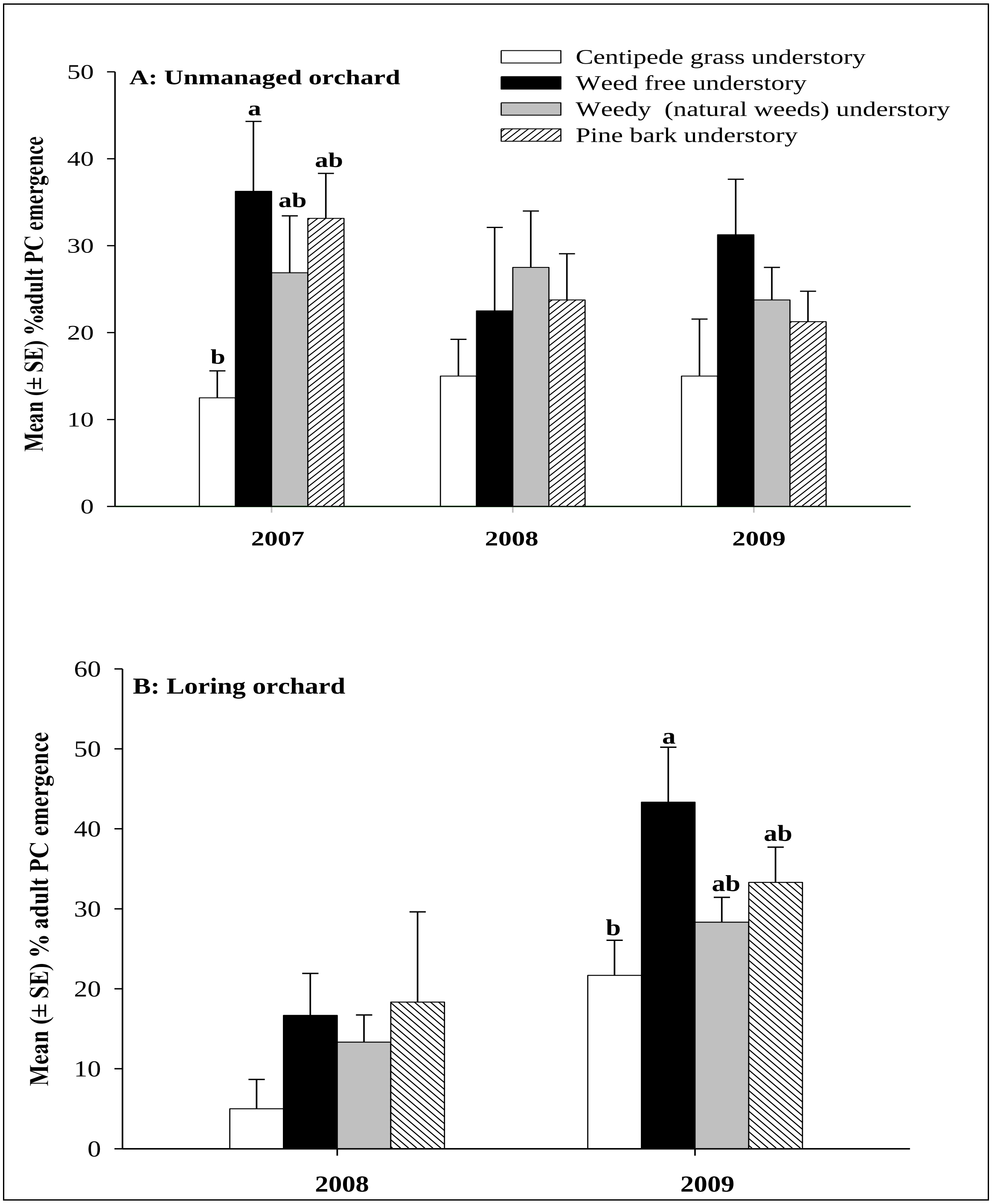
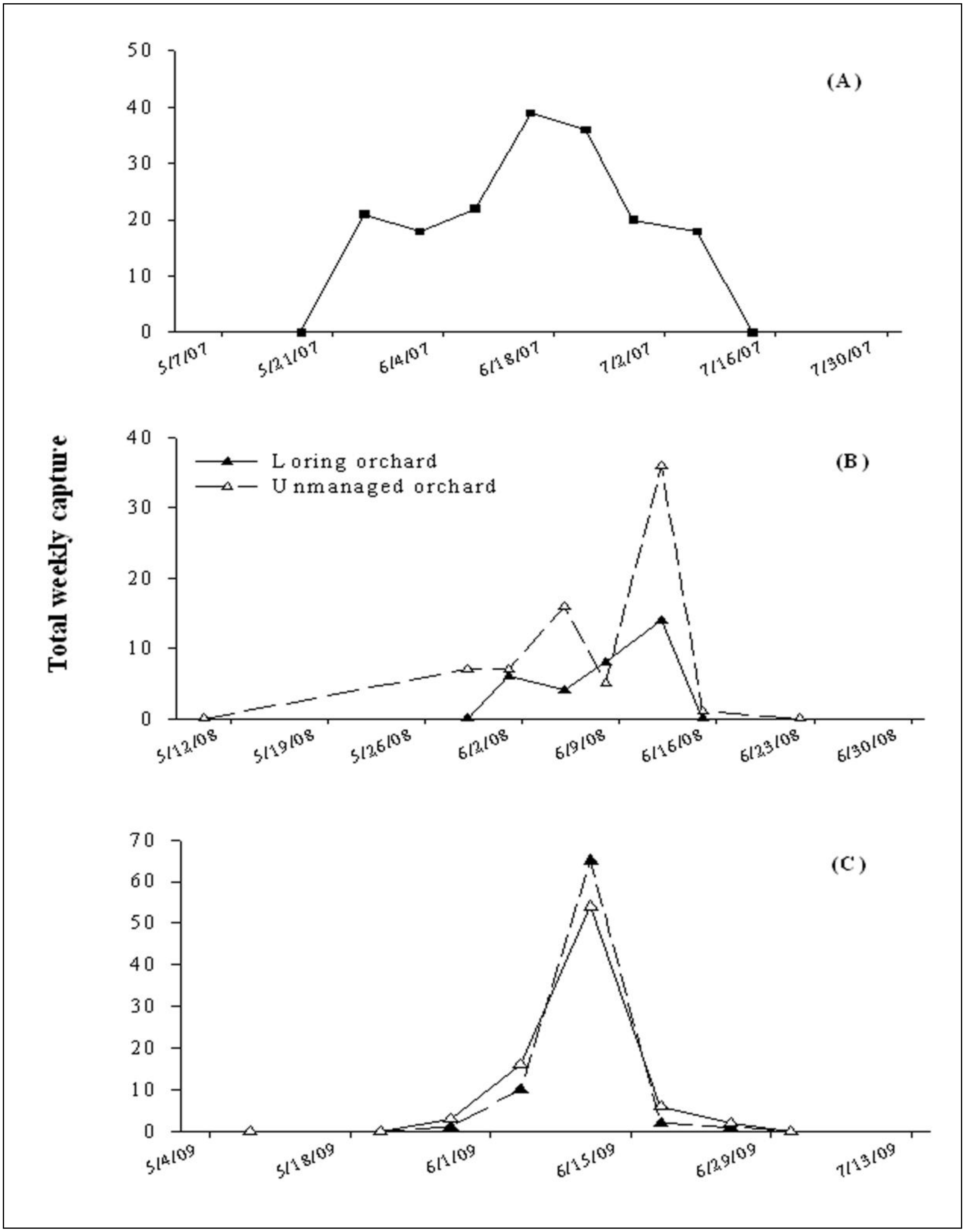

A: Unmanaged orchard
Centipede grass understory
Weed free understory
Weedy (natural weeds) understory
Pine bark understory
Mean (± SE) %adult PC emergence
0
10
20
30
40
50
b
a
ab
ab
2007
2008
2009
B: Loring orchard
Mean (± SE) % adult PC emergence
0
10
20
30
40
50
60
b
a
ab
ab
2008
2009

Figure 2


Total weekly capture
(A)
50
40
30
20
10
0
5/7/07
5/21/07
6/4/07
6/18/07
7/2/07
7/16/07
7/30/07
(B)
Loring orchard
Unmanaged orchard
40
30
20
10
0
5/12/08
5/19/08
5/26/08
6/2/08
6/9/08
6/16/08
6/23/08
6/30/08
(C)
70
60
50
40
30
20
10
0
5/4/09
5/18/09
6/1/09
6/15/09
6/29/09
7/13/09