STUDIES ON SEX PHEROMONES AND BIOLOGY OF THE PECAN WEEVIL, *CURCULIO CARYAE* (COLEOPTERA: CURCULIONIDAE), AND THE SEX PHEROMONE OF THE HICKORY SHUCKWORM, *CYDIA CARYANA*, (LEPIDOPTERA: TORTRICIDAE)

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CHAPTER 1

MATING BEHAVIOR AND PEAK MATING ACTIVITY OF THE PECAN WEEVIL *CURCULIO CARYAE* (HORN)

ABSTRACT

Mating behavior of the pecan weevil, *Curculio caryae* (Horn), was observed in the laboratory to characterize the mating behavior of this insect, and to elucidate peak activity. Mating increased beginning at 900 hrs and peaked from 1400-1459 hrs, then decreased until 1700 hrs. In the laboratory during the 24 hour period, mating occurred at other times, but was not significant. No mating occurred in the field trial. This study also determined that pecan weevil males tapped mesothoracic legs against lateral margins of the female prior to insertion of the aedeagus and not the metathoracic legs as previously published.

INTRODUCTION

The pecan weevil causes significant reductions in yields throughout the pecan *(Caryae illinoinensis* (Wangenh) C. Koch) growing regions of the U.S. Damage is caused by feeding of male and female weevils on fruit during the liquid endosperm, oviposition by females during the dough stage (deposition of carbohydrates in the kernel), and feeding in developing fruit by the larva (Calcote, 1975). Larva, upon completing development in the fruit, exit through a small hole cut in the shell and shuck, and burrow into the soil. Prepupae remain in the soil for a period of 1-2 years before pupating into a pharate adult that remains in the soil before emerging (Raney & Eikenbary, 1968; Van Cleave & Harp, 1971).

Plant volatiles have been investigated as attractants for pecan weevil. In addition, some of these same materials may serve as constituents in sex pheromones of pecan weevil (Mody et al. 1976). In attempts to modify the adult behavior patterns, sex pheromones of the pecan weevil have been investigated by several researchers (Van Cleave & Harp, 1971; Mody et al., 1973; Polles et al., 1977; Hedin et al., 1979). To effectively modify adult behavior with plant volatiles and/or pheromones, adult behavior of the pecan weevil must be known.

Evidence of precopulatory behavior or courtship in Coleoptera is limited (Engleman, 1970). August (1971) observed that male *Tenebrio molitor* (Coleoptera: Tenebrionidae) exhibited a courtship ritual. Males exhibited locomotor activity with vibration of antennae, followed by extension of the prothoracic legs resulting in a raising and lowering of the anterior portion of the body. As locomotion activity increased, males

would usually attempt to copulate immediately upon contact with a female, and the male tenebrionid strokes the elytra and pronotum of the female with his antennae. The male would the rapidly climb up on the female while stroking the lateral margins of the abdomen with his prothoracic legs. Genitalia are then extended under the female and genital contact is made with copulation lasting from three to ten minutes, and female locomotion at this time generally declines.

In contrast to the tenebrionid, pecan weevils exhibit similar leg tapping and copulation patterns. There is rapid locomotion orientation of males to females with the male mounting as soon as reaching the female. If the female is receptive, the male taps his metathoracic leg on the lateral margins of the thorax for 3-5 seconds, males the extend the genitalia until contact is made. Pecan weevil has a mating period of 3 minutes up to 9.02hrs, and there is a decline in female locomotion once genital contact is made. If the female is unreceptive, locomotion increases with rapid swaying movements of the body.(Hatfield et al., 1982).

The boll weevil, *Anthomonus grandis grandis*, mating behavior differs from that of the tenebrionid and the pecan weevil. Boll weevil females actively seek the males when released in the field. Females fly toward the male weevils, landing with inches of the males. Females circle the males or in some cases crawl over the males before copulation. Copulation may last only minutes, and multiple copula are seen (Cross & Mitchell, 1966). Leg tapping prior to copulation has not been reported for the boll weevil.

Other curculionids exhibit some precopulatory behavior. The American palm weevil, *Rhynchophorus palmarum,* exhibits jerky, swinging motions after antennating

females (Rochet & Zagatti, 1993). Giblin-Davis (1996) reported that the palmetto weevil, *Rhynchophorus cruentatus*, attennated the pronotum of live conspecifics and mount males and females with equal affinity.

Hatfield et al. (1982) indicated that during mating male pecan weevils assume the mating posture and begin tapping their metathoracic legs upon lateral margins of the females' thorax. In their study, 15 mating pairs were observed in four, four hour blocks. Once mating was initiated the pairs were separated and placed back into holding containers. Hatfield et al. (1982) reported 1800 hrs as peak mating activity in the laboratory.

In our laboratory observations of pecan weevil mating for another behavior study on this insect, males were found to tap mesothoracic legs on lateral margins of female abdomens. Our preliminary observations did not agree with the results. Therefore we designed a study to elucidate peak mating periods for the pecan weevil.

MATERIALS AND METHODS

Pecan weevils used in this study were collected from the OSU Horticulture research station in Stillwater, OK. Using 150 cone traps (Raney & Eikenbary, 1968) from 25 July-October 15 1995, 1800 adult weevils were captured.

To determine which legs were used to tap females and the time of peak mating, weevil pairs were observed for all mating periods studied. Day old male and female pecan weevils were collected, separated, and fed for 24 hours with immature pecan fruit. Each mating pair of weevils were placed in a 100 cm Petri dish. Fifty mating pairs were used for each one hour time block in a completely randomized design. Petri dishes were placed

in front of a window on a dark counter top. The window simulated natural lighting and the dark table top mimicked tree bark. Temperature was 25°C ± 5. For each one hour time interval during the 24 hour period of this study, 50 mating pairs were used for a total of 1200 mating pairs. Beginning at 0800 hrs, weevils were observed for one hour intervals and when mating began the time was recorded. If no mating occurred within one hour, weevils were removed, the pairs separated, and weevils were the placed in holding chambers for subsequent studies. Weevils were not used again in this mating study. This process allowed determination of the exact time when mating frequency peaked. If mating occurred, data on leg tapping were recorded to determine if male weevils tapped prothoracic, mesothoracic, or metathoracic leg prior to insertion of the aedeagus. Data on mating behavior were analyzed using ANOVA and separated with an LSD (SAS Inc., 1987).

Similar mating studies were conducted in a pecan grove for the same 24 hrs period to provide the natural temperature fluctuations and wind variations found in the field using a completely randomized design. Mating chambers were 5 cm tall and 8 cm in diameter, constructed of wire mesh and Mason® rings. For each time period (1 hr), ten pairs were used for a total of 240 mating pairs. A virgin male and female pecan weevil were placed in each trap with a immature pecan. Mating cages were suspended from a pecan branch. Data were recorded as in the laboratory study. Data were analyzed using SAS and separated with an LSD (SAS Inc., 1987).

RESULTS AND DISCUSSION

In the laboratory, the peak mating period was 1400-1459 hrs with 82% of weevil mating occurring at this time (LSD = 12.2, P=0.05). Percent mating response from 1000 hrs till 1359 hrs and from 1500 hrs until 1659 hrs were similar, but were significantly lower from 1400-1459 hrs and other time periods (Figure 1). Mating activity sharply decreased from 1600-1659 hrs (54%) to 1700-1759 hrs (12%) with activity continuing to decline after 1759 hrs. Data indicates that mating behavior of pecan weevils is diurnal under these controlled conditions. Schroeder (1981) reported that mating of the sugarcane rootstalk borer weevil, *Diaprepes abbreviatus*, was highest at 1400-1600 hrs and differed significantly than the morning period (0900-1100 hrs) and the evening period (1800-2000 hrs) differed significantly from the morning period. Thus mating of *D. abbreviatus* is a diurnal behavior pattern.

Mating trials in the field were unsuccessful. During the 24hr time periods, pecan weevils did not mate. Both males and females spent the time trying to find a way out of the mating chamber and exhibited no interest in the other gender or the pecan. These results were typical of all mating pairs (240) over the 24hr period. The observed behavior may have been a consequence of volatile compounds found in pecan leaves and fruit (Mody et al., 1976) or the negative geotropic behavior of pecan weevils for upward movement into the trees (Raney & Eikenbary, 1968).

Results from observations of 209 mating pairs indicated males tap the mesothoracic leg against the lateral margins of female pecan weevils during mating. The male approaches the female from behind, assumes a mating posture, if the female is

receptive, and taps his mesothoracic legs for 3-5 seconds before inserting the aedeagus. In each observed mating, the male tapped the female with his mesothoracic legs, and in all matings if leg tapping behavior was exhibited, copulation did occur. Males did not tap metathoracic or prothoracic legs at any time prior to or during mating.

Results from this study broaden the knowledge of pecan weevil biology and provides a background for testing pecan weevil pheromones in the laboratory and field by determining peak weevil activity. Additional work on the biology of this pest to determine requirements for oviposition and feeding preferences are needed.

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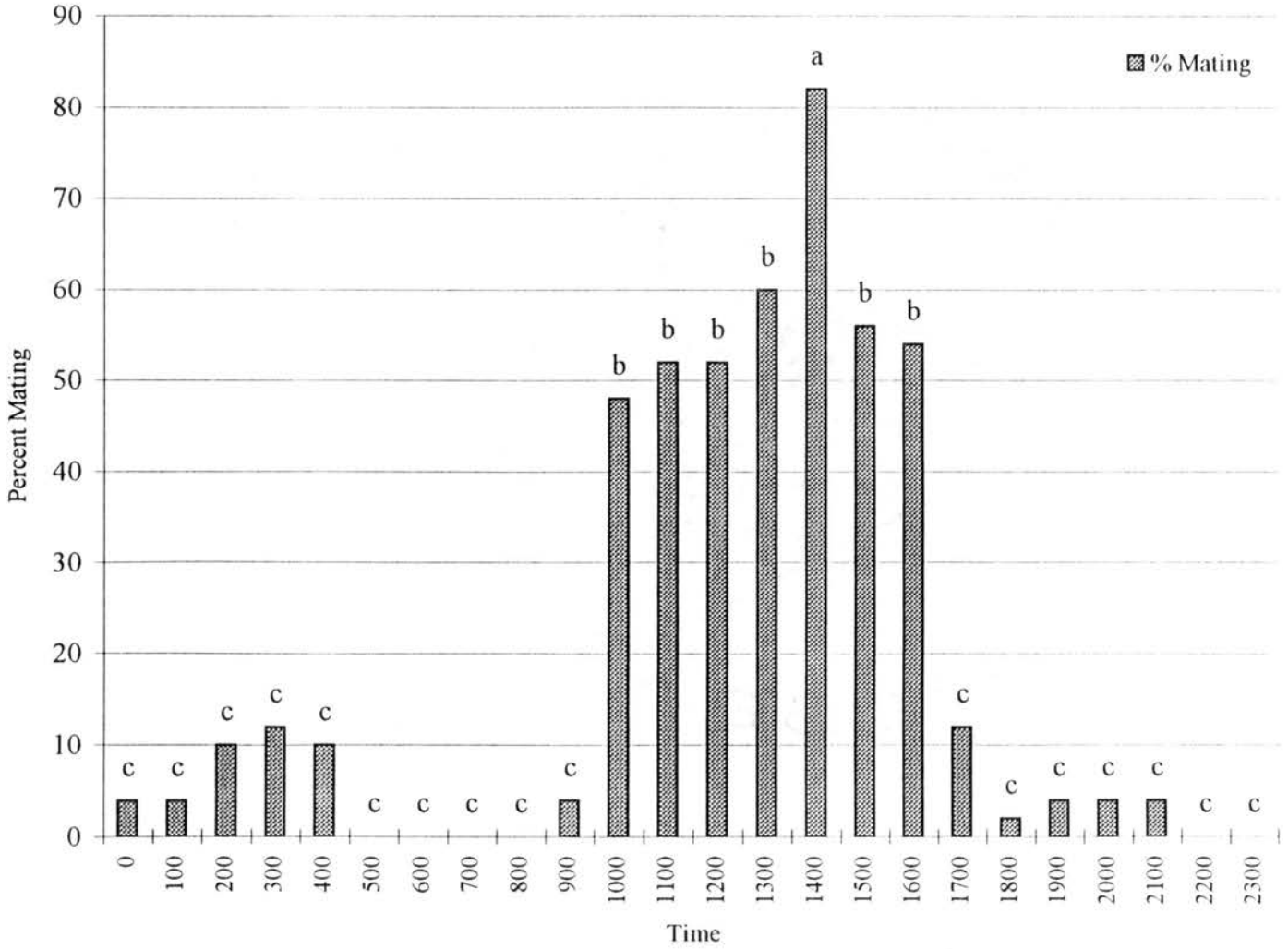
Figure 1. Percentage of pecan weevil mating over a 24 hour time course



CHAPTER 2

IDENTIFICATION OF THE MALE PECAN WEEVIL PHEROMONE WITH NOTES ON LABORATORY BIOASSAYS

ABSTRACT

A pecan weevil, *Curculio caryae* (Horn), pheromone was identified as a mixture of 4 components; (I) as both the cis and trans isomers of 2-isopropenyl-1-methyl-cyclobutaneethanol (also identified as 1R, 2S-(+ and -) Grandisol), (II) [(Z)-3,3-dimethylcyclohexane-$\Delta^{1,\beta}$-ethanol], (III) [(Z)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$ -acetaldehyde], and (IV) [(E)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$ -acetaldehyde]. These compounds are biosynthesized by the male pecan weevil, but not the female, in a ratio of 7/16/3/3: I/II/III/IV. These same compounds were identified earlier as the pheromone of the boll weevil, *Anthonomus grandis grandis* (Boh.) isolated from the frass in a ratio of 6/6/1.5/1.5: I/II/III/IV, but only the (+) isomer of grandisol was biosynthesized by the male pecan weevil.

Laboratory tests showed 80% of female pecan weevils were attracted to the synthetic formulation based on the ratio in male pecan weevils while 60% of female pecan weevils were attracted to the commercial boll weevil formulation, and 28% to synthetic formulation based on boll weevil frass. When males pecan weevils were tested against these synthetic formulations, attraction was minimal (14, 4, and 2%, respectively). Live males and their extracts were also attractive to females. Preliminary field tests demonstrated that females were more attracted to the synthetic pecan weevil formulation, than to the synthetic boll weevil formulation.

INTRODUCTION

The pecan weevil attacks maturing pecan *(Caryae illinoinensis* (Wangenh) C. Koch) fruit in late summer and damages the fruit by making feeding and oviposition. Larva, upon completing development in the fruit, leave through a small hole cut in the shell (pericarp) and burrow into the soil. The weevil larvae remain there for a period of 2-3 years before emerging as adults (Raney & Eikenbary, 1968; Van Cleave & Harp, 1971).

Van Cleave and Harp (1971) reported that field-caged female pecan weevils attracted more weevils of both sexes than did male weevils. Polles et al. (1977) reported on wing-type traps baited with 6 live females and pecan fruit for food, captured 85 pecan weevils, 73% of them males. The same number of traps baited with males captured 56 weevils, 66% males; and blank traps capture 55 weevils, 65% males. Polles et al. (1977) also baited traps with one or more of the pheromone components of the boll weevil, (Tumlinson et al., 1969) on the premise that related insects may biosynthesize and respond to similar compounds. A total of 23 pecan weevils, 87% of them male, were captured with the 4-component mixture, grandlure (Hercon® Environmental, Inc.). With (+)-cis-2-isopropenyl-1-methylcyclobutaneethanol (Component I), the capture totaled 7 (86% males) with (Z)-3,3-dimethyl-) ^{1,b}-cyclohexaneethanol (II) 15 were captured (100% males); and with (Z)-and (e)-3,3-dimethyl -) $^{1,\mu}$-cyclohexaneacetylaldehyde (III and IV) there were 10 (90% males). The blank captured 10 weevils (70% males). These tests were not sufficiently comprehensive to permit statistical evaluations and further work was not reported.

Other beetle species exhibit attraction to sex pheromones of other beetles within

the same family. White & Birch (1987) reported that two species of Anobiids, *Anobium punctatum* and *Stegobium panicium*, use the same female produced sex pheromone in attracting males. It has been reported that several species within the family Dermestidae respond to the same female produced sex pheromone. *Trogoderma inclusium*, *T. simplex*, and *T. variabile* all exhibited positive response to the sex pheromone of *T. grassmani* in the laboratory (Vick et al., 1970; Greenblatt et al. 1977).

Mody et al. (1976) bioassayed fractions obtained from volatile oils of each sex of the pecan weevil in field tests. Primarily males were trapped with female fractions, and primarily females were trapped with male fractions. Volatile components from the male and female oils were identified, but on the basis of their structure, none appeared capable of accounting for the attraction of either sex.

Hedin et al. (1979) showed that live male and female pecan weevils were attractive to the respective opposite sex using a laboratory bioassay. Extracts of males attracted females and vice versa. (Z)-3,3-dimethyl-)[1,b]-cyclohexaneethanol (component II) was isolated from a active extract of weevils and was shown to have some attractiveness to both sexes of pecan weevil in preliminary field bioassays.

In contrast to the male produced sex pheromones of the boll weevil, the sex pheromone of the sweetpotato weevil, *Cylas formicarius elegantulus,* is produced by the female and attracts males (Heath et al., 1986). Female produced sex pheromones have also been isolated from the large pine weevil, *Hylobius abietis* (Kalo, 1979).

Reports on male produced aggregations pheromones are more common in Curculionidae than male or female sex pheromones. Male aggregation pheromones have

been reported for four species of palm weevil, subfamily Rhynochophorinae (Rochet et al.1993; Gries & Gries, 1993; Giblin-Davis et al., 1994). Male produced aggregation pheromones have also been reported for red sunflower seed weevil *Smicronyx fulvus* that attracts primarily females (Roseland et al. 1990), pea and bean weevil, *Sitona lineatus*, that attracts both sexes equally (Blight & Waddhams 1987).

The objectives of this study were to identify the chemical components of the pecan weevil sex pheromone, and to test these compounds for activity under laboratory and field conditions.

MATERIALS AND METHODS

Procurement of Insects and Collection of Extracts and Washings

Two trapping sites were established to obtain weevils for isolation of the weevil pheromone. The first site was located near the Samuel Robert Noble Foundation Red River Demonstration Farm, Burneyville, OK. The second site was located on the Horticultural Research Station, Stillwater, OK. Weevils were collected by placing cone emergence traps under infested pecan trees (Raney & Eikenbary, 1968, Boethel et al., 1976, Eikenbary et al., 1978).

Insect extracts and washings for bioassay and chemical identification were obtained using the following procedures. After capture, individual weevils were placed in vials and transported to the laboratory, sexed, and then transferred to feeding chambers (5 oz glass jars) with immature pecans for five days. For collection male/females washing, five day old virgin male and female weevils were paired in 1 oz glass jars with teflon lids. After the males mounted and assumed mating posture, 1ml methylene chloride or hexane was added

to the chamber. The chamber was rotated to facilitate solution, and washings were transferred to an amber glass bottle, combined, and stored at -20°C. Individual washing of males and females were obtained by placing pairs in mating chambers and observed until excitation occurred. Males were not allowed to mount or assume a mating posture. Individuals were separated and washed with 1ml of methylene chloride or hexane. The washing were combined and stored at -20°C. Collection of insects for GLC-MS were: A. 68 M/F washing, 5-day-old, in methylene chloride; B. 68 M/F matings, 5-day-old, in hexane; C. 68 males, 5-day-old in methylene chloride; D. 68 males, 5-day-old in hexane; and E. 68 females, 5-day-old in methylene chloride.

Extractions, Fractionations, and Mass Spectral Analysis of Insects

Whole insects and extracts that were stored at -20°C under hexane or methylene chloride were ground with a Polytron homogenizer, and the entire contents were applied to the column, which was washed with 125 ml hexane followed by 125 ml of methylene chloride. The 1 x 30 cm column equipped with a bulb and frit was filled with a slurry of 6 gm of Baker silica gel (60-200 mesh, 3405-05) in hexane (Optima, Fisher Scientific). Fractions for GLC-MS were concentrated to 1 ml or less. The fractions were monitored using silica gel TLC that were chromatographed with 50% methylene chloride in hexane and visualized in an iodine chamber. It was determined, as expected, that the hexane eluate consisted mostly of hydrocarbons, and a separated column chromatographic test demonstrated that the boll weevil grandlure components were not eluted with hexane but with methylene chloride. GLC-MS analyses were performed on both hexane and methylene chloride fractions.

GLC-MS Procedures

Spectral interpretations were supported by the NIST/EPA/MSDC Mass Spectral Database 1A PC Version 3.0 (Lias & Stein, 1990), and the HP 59944C MS Chem System Version 8.05 (1992).

Synthetic Pheromone Formulations

Formulations were based on the following ratios. The ratio of the four boll weevil components (I) [cis-2-isopropenyl-1-methylcyclobutaneethanol], (II) [(Z)-3,3-dimethylcyclohexane-$\Delta^{1,\beta}$-ethanol], (III) [(Z)-3,3,-dimethylcyclohexane-$\Delta^{1,\beta}$-acetaldehyde], and (IV) [(E)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$ -acetaldehyde] in boll weevil frass were found to be 6/6/1.5/1.5: I/II/III/IV (Tumlinson et al., 1969, 1971). The relatively greater cost of I led to the use of the formulation 3/4/1.5/1.5: I/II/III/IV, which was found to be adequately attractive in field tests and has been used since as the commercial boll weevil sex pheromone formulation. In the male pecan weevil, these pheromone components were found to be present in a ratio of 7/16/3/3: I/II/III/IV. Formulations based on the ratios found by analyses of the previously described washes and extracts of live pecan weevils, and the previously published ratio of components found in boll weevil frass (Tumlinson et al., 1969, 1971) were dispersed in laminates prepared by Hercon Environmental Corporation, Emigsville, PA, so that 6.5 cm^2 sections contained 10 mg of the prescribed 4-component mixtures. Hercon® Luretape is a laminated, three-layered plastic dispenser; a reservoir layer contains active ingredients that are sandwiched between two outer permeable layers of polyvinyl chloride film. Laminates of the commercial boll weevil formulation were procured from Hercon^R. In the synthetic

formulation, both the (+) and (-) isomers of I are present. Pecan weevil biosynthesize both isomers (see Results and Discussion) while the boll weevil biosynthesize only the (+) isomer (Tumlinson et al., 1969, 1971).

Laboratory Bioassays

Laboratory tests to determine the attractiveness of the pecan weevil pheromone, boll weevil pheromone, boll weevil frass formulation, and live males to the female and male pecan weevils were carried out in two choice olfactometers with the airflow set at 25 ml/minute. The olfactometer (Figure 1) consists of an inverted glass funnel (30cm in diameter with a 12cm stem). Openings (2.5 x 2.5 x 1.9cm)at the base are at 180° angles with no. 5 stoppers with 125 ml suction flasks placed over the stoppers. These flask are for containment of synthetic pheromones or live insects. A 2.5 x 2.5 x 2.5 cm opening at the base of the apparatus provides an entrance for introducing the weevils into the apparatus. A 0.6cm piece of rubber tubing is attached to the top of the flask and attached to the laboratory bench vacuum. A mild vacuum facilitates the air movement across the synthetic pheromones or live insects into the apparatus and up to the top. Responding weevils enter the flasks on either side of the apparatus or remain in the center arena. (Hardee et al, 1967). Virgin, unfed, L:D 16:8, males or females were introduced into the olfactometer between 0900 and 1400 hrs, a time period determined to be peak mating activity of the pecan weevil (Collins, et al. 1996). A candidate pheromone lure was placed in one arm of the olfactometer and no lure was placed in the other arm. A female was introduced into the chamber and observed for one hour, and the response to the pheromone, control or no response was recorded. For each candidate pheromone tested,

a minimum of 100 replications were employed, except for the boll weevil frass formulation where only 50 replicates were used. Data were analyzed using Chi-square analysis (SAS, 1991).

Field Methods

In 1995, the effectiveness of the pecan weevil pheromone in the orchard was tested at the OSU Horticulture research station near Sparks, OK. Tests were conducted using weevil infested native pecan trees. Experimental design was a randomized complete block with a 2 X 3 factorial treatment. Two trap types, boll weevil traps and Tedder's traps (Tedders & Wood, 1995) were used in a factorial treatment combination with three pheromone treatments boll weevil pheromone, pecan weevil pheromone, or unbaited traps. Treatment combinations were replicated two times with four subsamples per replication. Each subsample occupied about 0.4 ha and treatments were separated by 0.4ha. The traps were checked every 3-4 days. Data were analyzed using ANOVA (SAS Inc., 1987) with a split plot design with time as the sub-plot.

RESULTS AND DISCUSSION

Chemical Analyses

GLC-MS analyses, conducted in 1993, of six collections of male, female, and mixed male/female washings and extracts gave 28 significant maxima, 19 for which structural assignments were made (Table 1). Collections made in 1992 did not contain any of the grandlure components (data not shown). Unambiguous mass spectral data for the presence of the four boll weevil pheromone components (Grandlure) (Figure 2) was

obtained from male collections C and D while male and female collections A and B (see Materials and Methods) contained the more prevalent components I and II only. Female collections E and F did not contain any of these constituents (Table 1).

Figure 3 is a chromatogram of male pecan weevil collection C. Mass Spectra of the four small maxima between 6.5 and 7.2 minutes were those of the four boll weevil sex pheromone components. From the total ion count, it was determined that the content from 68 males was 20mg or 0.29mg/insect of I+II+III+IV. The distribution was approximately 0.07mg I, 0.16mg II, 0.03mg III, and 0.03mg IV. On close examination of the chromatogram of male collection D, four maxima of the apparent masses 152-156 were found to be intermingled with those of the four Grandlure components. Two of these were also present in collection C, but not in either of the female collections E and F. Figures 4 and 5 give the mass spectra for these four maxima at I_k 1190, 1195, 1210, 1295. While these spectra could not be matched using the available databases, they have been included as a possible help to future investigations that other terpenoids may be necessary for a complete behavioral response by the pecan weevil. None of the components of Table 1 found in males or females appears to be particularly unique, and none were found in females but not in males.

Figure 6 represents chromatograms of grandlure (A), pecan weevil collection C (B), and boll weevil frass (C) as obtained with a chiral GLC column. Compound I exists in the boll weevil and its frass only as (1R, 2S)-(+)-Grandisol. We later showed that the antipode (-)-Grandisol is also active (Mori et al., 1978). The chromatogram for the boll weevil frass (Figure 6C) gives a maxima for the (+) isomer at 8.04 min. while both the (+)

and (-) isomers at 8.02-8.04 and 8.17-8.19 min. are present in the synthetic grandlure (Figure 6A) and male pecan weevil collection C (Figure 6B). While compounds II, III, and IV are poorly separated with this chiral column, the mass spectral data obtained with the DB-1 column (Figure 3) was definitive.

Results of Laboratory Experiments

Olfactometer data were analyzed using a Chi-square (SAS Institute, 1987) on all possible pairwise combinations and significant differences in percent attractiveness were seen between all combinations at $P > 0.05$, except one (Table 2).

Pecan weevil females (43%) responded to live males while males did not responded to live females. The response of females to the synthetic formulation based on the ration of components present in pecan weevil males (24/55/21: I/II/III+IV) was 80% while only 14% of males responded to this formulation (Table 2). The response of females to the commercial boll weevil pheromone (30/40/30: I/II/III+IV) was somewhat less with 60% of females and 4% of males responding. The response of females to the formulation based on boll weevil frass (40/40/20: I/II/III+IV) was significantly lower; 28% of females and 2% of males. Coudriet and Kishaba (1988) report similar results with the pepper weevil, *Anthonomus eugenii*, using a Hardee olfactometer. Female pepper weevils showed significantly greater attraction to live males or males extracts than to live females. Male pepper weevils did not show a significant attraction to either male, females, or male extracts.

Heath et al. (1986) reported that the female produced sex pheromone of the sweetpotato weevil, *Cylas formicarius elegantulus,* showed no significant differences in

crude extracts isolated from females or the synthetic in the ability of the compounds to attract male insects. Proshold et al. (1986) reported that the synthetic pheromone had an efficacy of > 90% in field trials. Extracts of the male, red sunflower seed weevil, attracted a mean of 8.93 females in the laboratory and only 0.21 males. Extracts from the female weevils attracted neither males nor females (Roseland et al., 1990). Jones and Schroeder (1984) reported that frass extracts from male sugarcane rootstalk borer weevil, *Diaprepes abbreviatus*, captured more males and females than frass extracts from females.

Chemical analysis demonstrated that components of the male boll weevil pheromone are the same as the male pecan weevil, but they are significantly different in component ratios. The bioassays showed the response of the female pecan weevils to the synthetic pecan weevil formulation is much stronger than to the formulation based on the ratio in boll weevil frass. Therefore, the pecan weevil pheromone, although consisting of the same four compounds, is unique because of the different ratios biosynthesized by males and responded to by females. The low response of the males to live females and formulations demonstrates that males are the primary attractive sex and the females are the primary responders (Table 2) as seen with the boll weevil (Hardee et al, 1967). Because male pecan weevils biosynthesizes both the (+) and (-) isomers of I, and females responded to the synthetic formulations that include both isomers, it is presumed that both isomers are either attractive, or at least not repellent to the female.

Results of Field Experiments

Preliminary results from field tests showed that synthetic formulations based on the male pecan weevil pheromone ratio were significantly more effective than synthetic

formulations based on the commercial boll weevil ratio or unbaited traps. A t-test grouped the control and the boll weevil pheromone together with a mean of 0.55 and 0.88 females per trap. Traps employing the pecan weevil pheromone captured a mean of 5.00 females per trap with an LSD of 1.67 ($\alpha > 0.05$). Additional field trials are planned to further evaluate the synthetic pecan weevil pheromone. Information from these trials may lead to the use of the pheromone to monitor pecan weevil in the field. In addition, if pheromone attractiveness and trap efficiency are sufficient, their combination may provide a possible control alternative in insecticide sensitive urban environments.

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Figure 1. Bioassay apparatus for boll weevil sex attractants (Hardee et al. 1967).

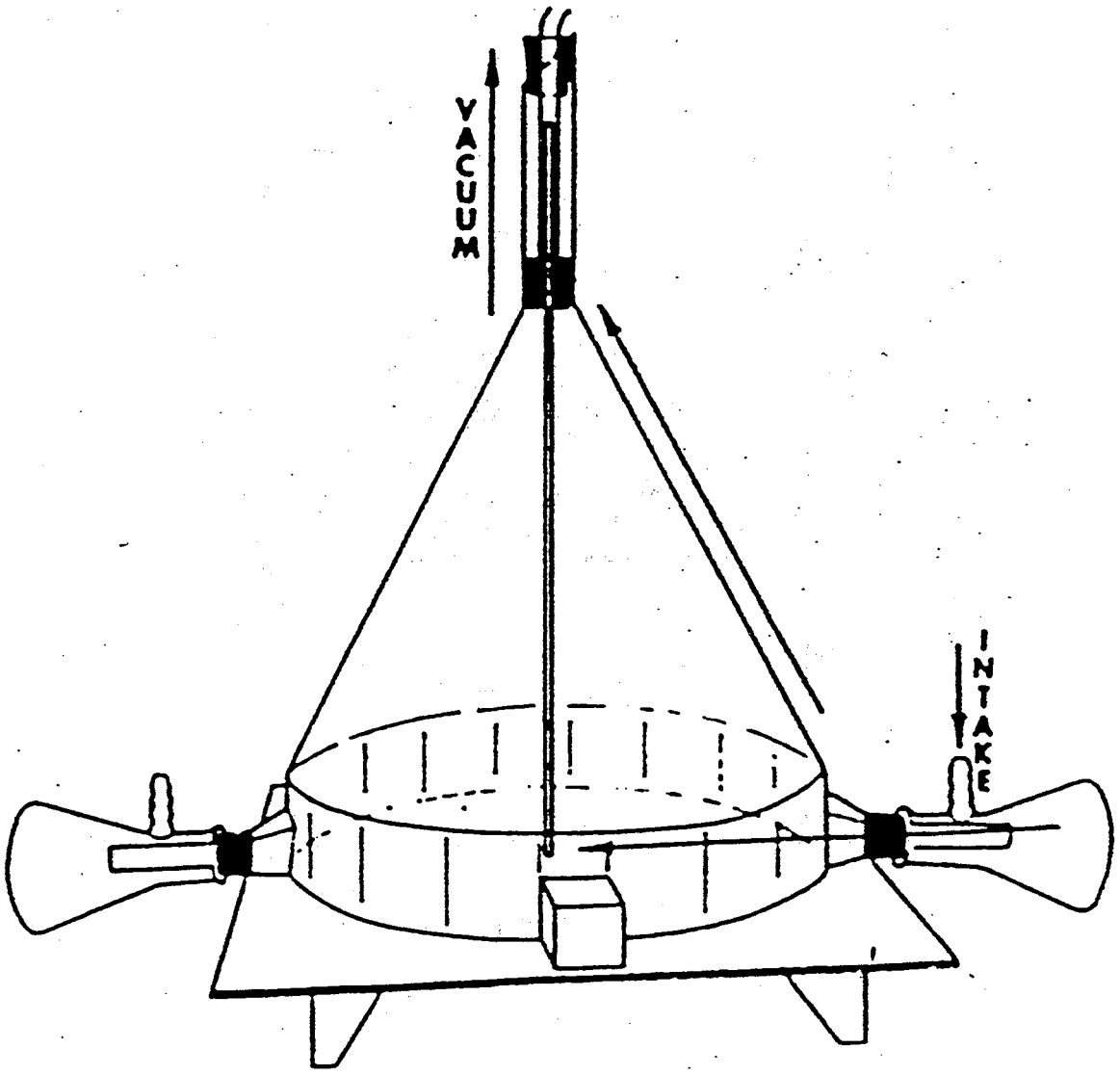
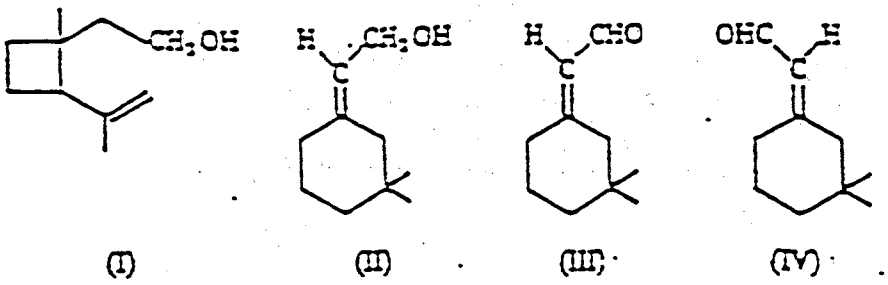


Figure 2. Structure of the four boll weevil sex pheromone components. See table 1 for nomenclature.



(I) (II) (III) (IV)

Figure 3. GLC-MS of male pecan weevil collection C. Note the four boll weevil pheromone components at 6.5-7.2 minutes, their total content is 0.29mg/insect.

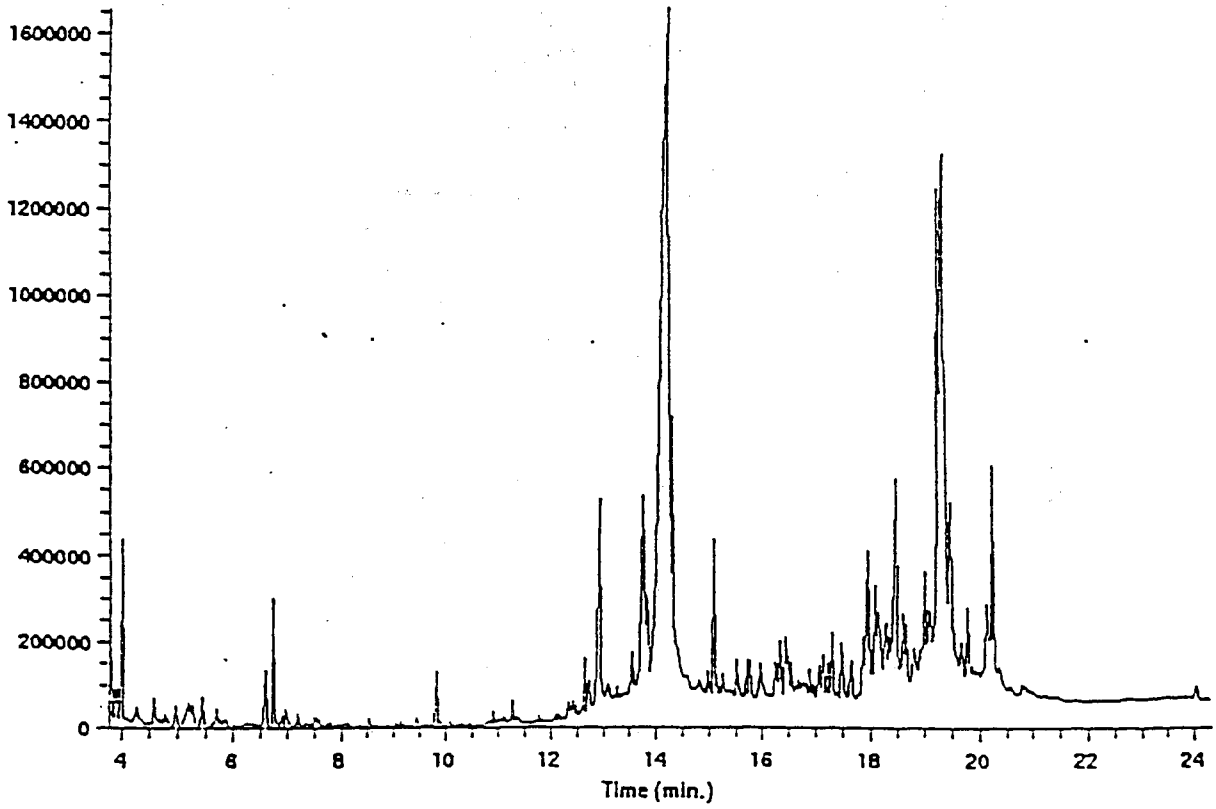


Figure 4. Mass spectral fragmentation patterns for male pecan weevil maxima at I_k 1190 (A) and I_k 1195(B); see Table 1.

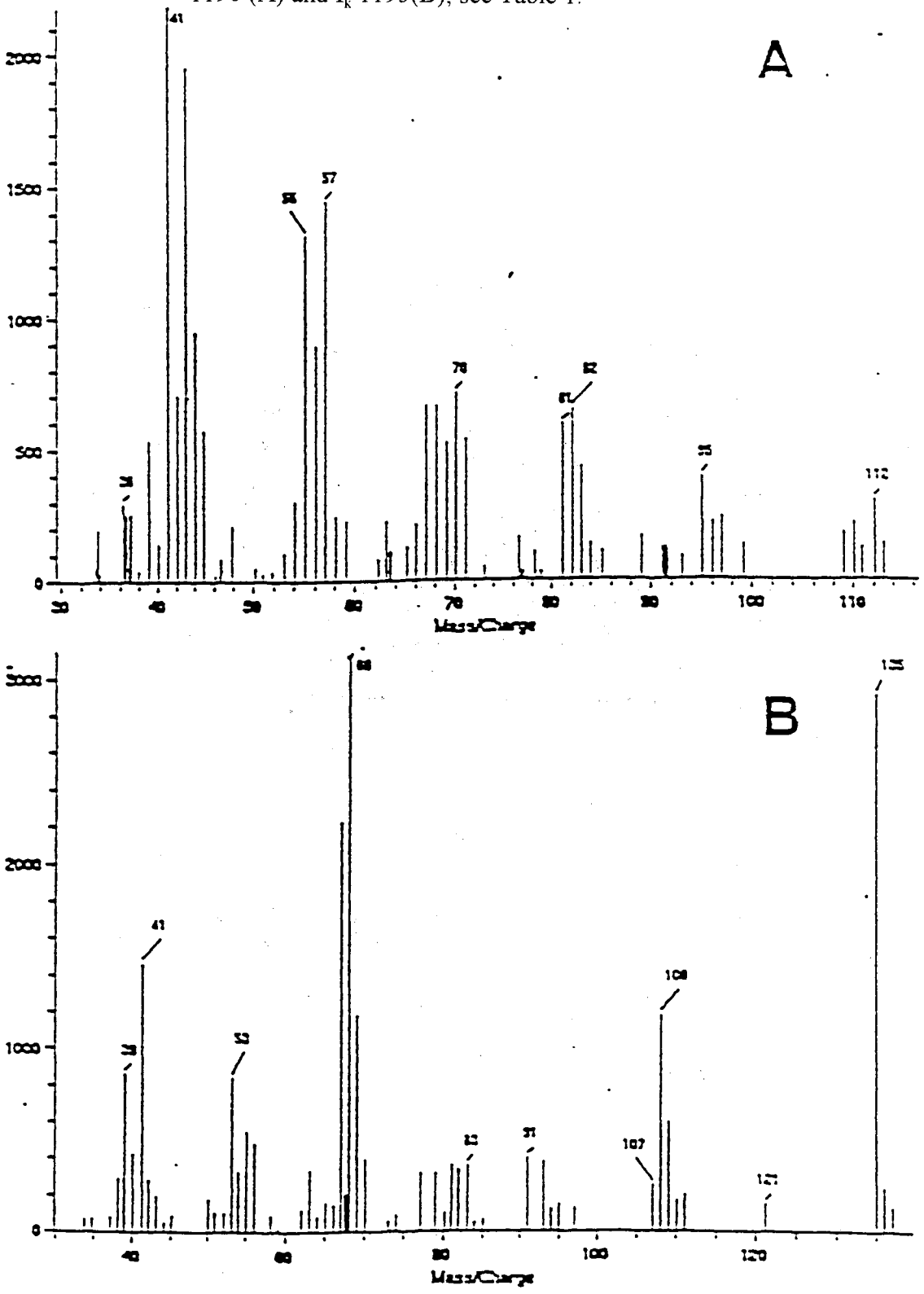


Figure 5. Mass spectral fragmentation patterns (see Table 1) for male pecan weevil maxima at I_k 1210 (A) and I_k 1295 (B).

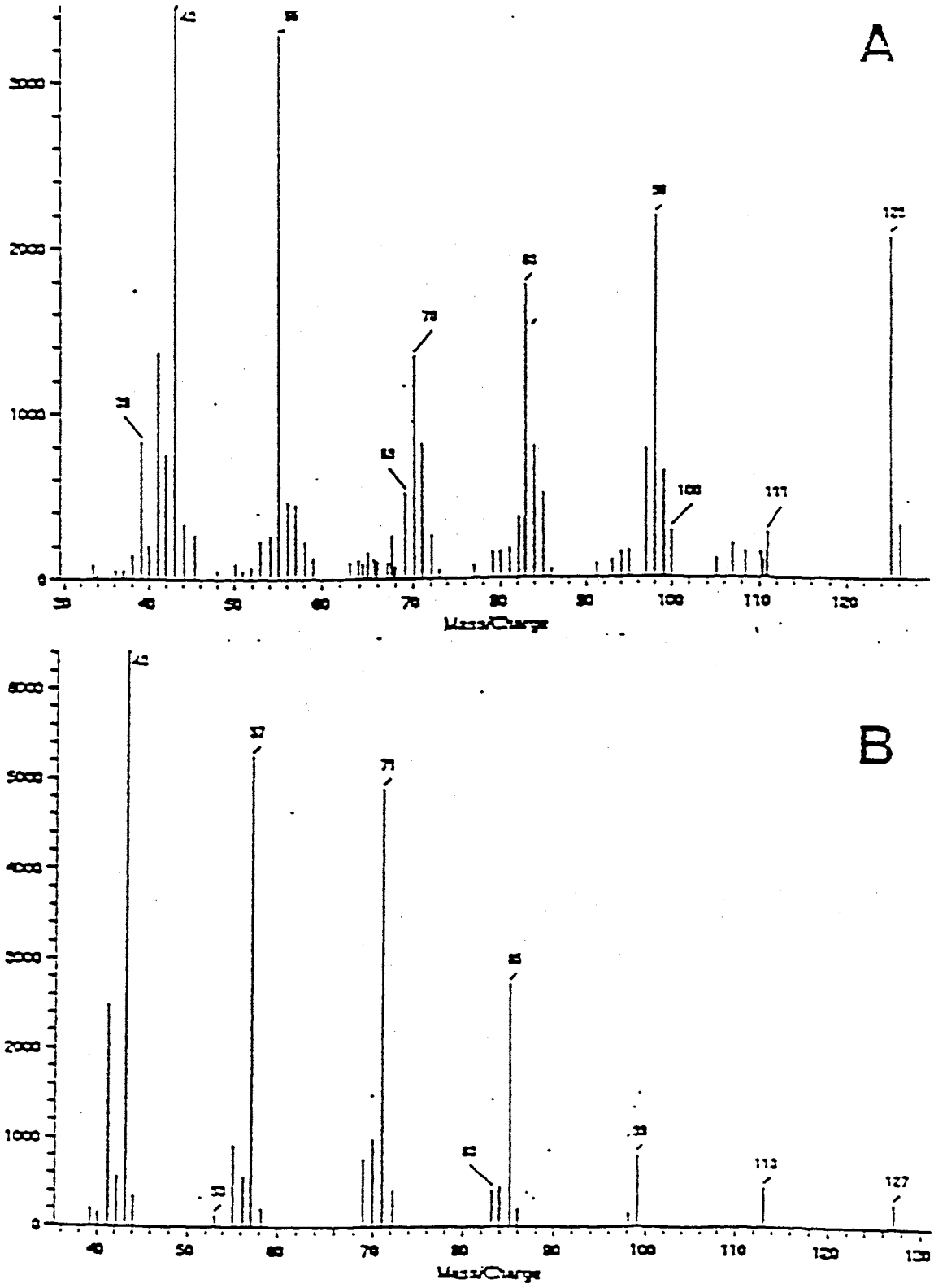


Figure 6. GLC separation of synthetic grandlure (A), male pecan weevil collection C (B), and boll weevil frass (C). Note that only (+)-grandisol (8.04 min) is present in boll weevil frass © while both (+) and (-) - grandisol are present in male pecan weevil and synthetic grandisol.

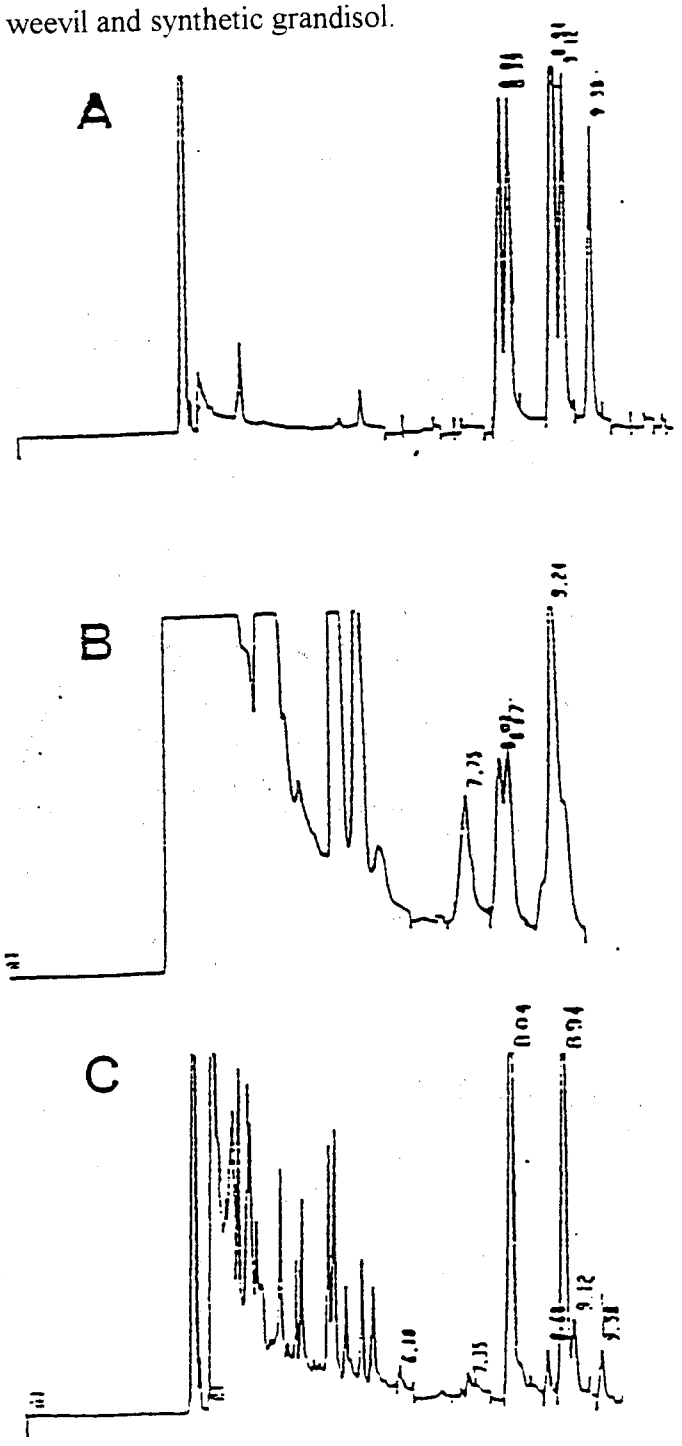


Table 1. GLC-MS Analysis of Methylene Chloride Fractions from Washing and Extractions of Pecan Weevils.

I_k	MW	Compound	A	B	C	D	E	F
			Washings					
815	100	----------	X	X	X	X	X	X
850	100	1-Methyl-cyclopentanol	X	X	X			
865	100	3-Hexanol	X	X	X	X	X	X
880	94	Phenol	X	X	X	X	X	X
955	100	3-Hexane	X		X			X
962	98	2-Hexenal						X
1005	142	n-decane	X	X	X	X	X	X
1080	142	n-nonanal	X	X	X	X		X
1190	---	M/e 112			X	X		
1195	154	M/e 135				X		
1200	154	Compd I[b]	X	X	X	X		
1210	152/154	M/e 125					X	
1215	154	Compd Ii[b]	X	X	X	X		
1225	152	Compd III[b]			X	X		
1235	152	Compd Iv[b]			X	X		
1240	154	(Z)-2-decenal				X		
1250	---	M/e 142	X		X			
1281	152	Thujone				X	X	
1282	158	1-Decanal				X		
1295	---	M/e 127			X	X		
1295	156	M/e 113	--	--	--	--	--	--
1350	182	2-Dodecenal						
1425	220	∝-Caryophyllene oxide				X		
1510	250	---	X	X	X	X		X
1520	222	---				X		
1610	222	Bulnesol			X		X	
1690	228	Myristic acid				X	X	
2010	268	Oleyl alcohol					X	X

[a]A male/female washings (methylene chloride), B male/female washings (hexane), C male washings (methylene chloride), D male washings (hexane), E female washings (hexane), and F female washings (methylene chloride).

[b] (I) [cis-2-isopropenyl-1-methylcyclobutaneethanol], (II) [(Z)-3,3-dimethylcyclohexane-$\Delta^{1,\beta}$-ethanol], (III) [(Z)-3,3,-dimethylcyclohexane-$\Delta^{1,\beta}$-acetaldehyde], and (IV) [(E)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$ -acetaldehyde]

Table 2. Olfactometer Tests of the Percent Relative Attractiveness of Three Synthetic Pheromone Formulations and Pecan Weevil Males to Virgin Females and Males.

Sex	Pecan Weevil Pheromone	Boll Weevil Pheromone	Boll Weevil Frass	Live Pecan Weevils
Female	80a	60b	28c	43d (males)
Male	14e	4f	2f	0f (females)

[1] Numbers followed by the same letter significantly are non significant (Chi-square P=0.05)

CHAPTER 3

FIELD EVALUATION OF PECAN WEEVIL PHEROMONE AND BOLL WEEVIL PHEROMONE IN PYRAMIDAL AND BOLL WEEVIL TRAPS IN MONITORING PECAN WEEVIL POPULATIONS IN THE FIELD

ABSTRACT

Many field studies monitoring the pecan weevil, *Curculio caryae* (Horn), have been conducted, but monitoring techniques have not been widely accepted and used by pecan producers. This study evaluated an inexpensive trap with a pheromone designed for the boll weevil in cotton, and the pyramidal trap with experimental formulation of the pecan weevil pheromone. This experiment was designed to evaluate the boll weevil pheromone against live pecan weevil in the field using only boll weevil traps, and evaluate the boll weevil pheromone with the experimental pecan weevil pheromone and the two trap types. The results indicate significant differences in attraction of pecan weevils to traps containing boll weevil pheromone when compared to unbaited traps boll weevil traps, but in experiments with the two pheromones and two trap types, the boll weevil trap was found to be less efficient when compared to the pyramidal traps. Data from the boll weevil pheromone test alone, indicated the boll weevil pheromone baited traps were significantly more attractive to the pecan weevil than unbaited traps. In the split plot design with a 2X3 factorial in 1995, the pecan weevil pheromone was significantly more effective than the boll weevil pheromone or untreated control, but due to possible inconsistencies in the pheromone formulation, the 1996 data showed no significant differences between pheromones.

INTRODUCTION

The pecan weevil is one of the most damaging pests to pecan production. During the liquid endosperm stage of fruit development, feeding by the males and females causes the fruit to abort, feeding by the adult during the dough stage causes discoloration of the kernel, and with oviposition occurring in dough stages of pecan development, larva feeding causes fruit loss (Calcote, 1975). Depending on levels of infestation, losses in pecans can range from a few fruit per tree to the entire pecan crop.

Traditional methods of monitoring pecan weevil emergence have involved using jarring tree limbs (Raney & Eikenbary, 1968), cone traps (Boethel et al., 1976, Eikenbary et al., 1978) following a soaking rain ≥ 1" (Dupree & Bissell, 1965, Tedders & Osburn, 1971, Nash & Thomas, 1972, Harris & Ring, 1980), and tagging fruit clusters to determine feeding and ovipositional damage (Hall et al, 1979). While these methods are viable, none have been well received by Oklahoma growers. Cone traps are labor intensive to build, monitor, and require substantial storage space. These traps are also expensive (ca. $24.00) to build and normally 50% of the traps used must be repaired extensively at the end of the growing season. Growers frequently begin spraying pecan trees for weevils based on rainfall or the spray schedules of their neighbors. This approach leads to unnecessary sprays and insufficient control resulting in crop damage.

In 1994, Tedders & Woods introduced a new method for monitoring pecan weevils known as the pyramidal trap, though more commonly referred to as tedders' trap. These traps are easier to construct, deploy in the orchard, and fewer traps are needed to monitor pecan weevil emergence. In addition, cost is only one-fourth that of the cone trap. Field

trials have suggested that these traps achieve better results than cone traps (Tedders & Woods, 1994; Tedders & Woods, 1995). Pyramidal traps rely on the visual cues used by the pecan weevil in orienting itself to the pecan tree. Pecan tree trunks are painted white up to a height of 2m, and the traps placed around the tree at the drip line. The pyramidal traps were originally painted brown, but Tedders et al. (1995) revised paint color to black. Pyramidal traps attract weevils emerging from around the tree, and probably weevils migrating into the orchard as well.

Laboratory tests using a Hardee olfactometer have shown that the boll weevil pheromone attracted 60% of female pecan weevils (Collins, et al. 1996). Other researchers have reported similar results with boll weevil pheromone components, live pecan weevils, and pecan weevil extracts (Mody et al., 1973; Polles et al., 1977; Hedin et al., 1979). Polles et al. (1977) reported on wing-type traps baited with males captured, 66% males while blank traps capture, 65% males. The same number of traps baited with six live females and pecan fruit for food, captured, 73% of them males.

Fractions were obtained from volatile oils of each sex of the pecan weevil in field tests (Mody et al., 1973). Primarily males were trapped with female fractions, and primarily females were trapped with male fractions. Volatile components from the male and female oils were identified, but on the basis of their structure, none appeared capable of accounting for the attraction of either sex.

Laboratory bioassays showed live male and female pecan weevils were attractive to the opposite sex. The compound, (Z)-3,3-dimethyl-)[1,b]-cyclohexaneethanol (component II of the boll weevil pheromone), was isolated from active extracts of weevils and was

shown to posses some attractiveness to both sexes of pecan weevil (Hedin et al., 1979).

This study was designed to evaluate the response of pecan weevils to boll weevil pheromone in boll weevil traps. Boll weevil traps cost significantly less ($2.00/trap) than pyramidal traps or cone traps, thus if the boll weevil traps are an efficient means in monitoring pecan weevil densities in the field with the pheromone, monitoring would be more cost efficient for the producer. Other studies were conducted to compare the effectiveness of the synthetic pecan weevil pheromone with the boll weevil pheromone in the field with different trapping systems.

MATERIALS AND METHODS

Two studies were conducted at the OSU horticulture research station in Stillwater, OK, and the OSU Horticulture Research Station in Sparks, OK. At the Stillwater site, unbaited boll weevil traps and boll weevil traps baited with boll weevil pheromone were placed in a completely randomized design with a 2X2 factorial treatment. Two treatments (pheromone or no pheromone), two cultivars (Gormely or native), and six replications per treatment (Figure 1). Even numbered traps contained the pheromone and odd numbered traps were unbaited. Commercial boll weevil pheromone (Hercon® Environmental) was obtained from Southeast Boll Weevil Eradication Foundation (Montgomery, AL).

Traps were separated by 25 m to reduce competition between traps. Traps were placed at a height of .75 m from the ground on .50cm wooden dowels. Traps were monitored daily from 25 July - 31 October 1995, and the number of weevils captured were recorded. Data were analyzed using ANOVA (SAS, 1987).

At the site near Sparks in 1995, the traps were arranged in a split plot design with a 2X3 factorial treatment. Two trap types (pyramidal trap and boll weevil trap) and three treatments (pecan weevil pheromone, boll weevil pheromone, and unbaited control) with four subsamples and replicated twice. Traps were monitored every 2-3 days from 1 August 1995-11 October 1995. Data were analyzed using ANOVA (SAS, 1987).

In 1996, the boll weevil trap was eliminated from the experiment due to poor performance in 1995. A randomized complete block design was used in 1996 with pyramidal traps and three treatments (pecan weevil pheromone, boll weevil pheromone, and unbaited control with four subsamples and replicated five times. Traps were monitored every 2-3 days from 27 July 1996 - 11 October 1996. Data were analyzed using ANOVA (SAS, 1987).

RESULTS AND DISCUSSION

Pheromone baited traps caught 4.67 weevils compared to 1.41 in traps without pheromone with LSD = 2.12 ($\propto = 0.05$). There was no significant difference between cultivars at the Stillwater site.

Data from this study using boll weevil traps only in 1995 indicates that boll weevil pheromone was significantly better than no pheromone in attracting weevils to the traps. Although the number of weevils caught in the traps was significantly different between pheromone and no pheromone, the combined mean number of 5.6 weevils per trap were captured from 25 July - 31 October. Data indicates that while boll weevil pheromone attracts female pecan weevils, trap design does not estimate weevil density effectively when compared to data from pyramidal traps in the Sparks trial.

Data from the Sparks site in 1995 indicated that the Tedders trap was superior to boll weevil traps in attracting pecan weevils with or without pheromone. Pyramidal traps caught a mean of 2.11 weevil per trap per day compared to 0.273 weevils per trap per day in the boll weevil traps [LSD = 0.9233($\propto$ = 0.05)]. Data clearly indicated the pyramidal trap to be a more efficient trapping method than the boll weevil traps. Thus in 1996 field trials, the boll weevil trap was eliminated from the experimental design.

Results from 1995 field evaluation in Sparks showed that synthetic formulations based on the male pecan weevil pheromone ratio were significantly more effective than synthetic formulations based on the commercial boll weevil ratio or unbaited traps. A t-test grouped the control and the boll weevil pheromone together with a mean of 0.55 and 0.88 females per trap. Traps employing the pecan weevil pheromone captured a mean of 5.00 females per trap with an LSD of 1.67 (α = 0.05). Coudriet and Kishaba (1988) reported that in field trapping that female pepper weevils, *Anthonomus eugenii*, showed a significantly greater attraction to traps baited with live males than the control, or traps baited with live females.

Male pepper weevils did not show an attraction for female baited traps, male baited traps or the control.

Initially the data in 1996 showed that the pecan weevil pheromone was performing below that of the boll weevil pheromone and untreated control. Laboratory bioassays showed that the pecan weevil pheromone formulation from 1995 was performing 64% better than the formulation received in 1996. The pheromone provide in 1996 was replaced by the pheromone provided in 1995 on 14 August 1996. Laboratory analysis of

the pheromone lures indicated that the ratio of the components were the same in both the 1995 and 1996 lures, but the 1995 lures contained 23% more of the pheromone components.

Data from the 1996 trial was divided into 2 separated groups and analyzed using ANOVA (SAS, 1987). The 1996 pheromone was placed in the field on 24 July 1996 until 16 August 1996, at that time the pheromone lures from 1995 replaced the 1996 lures. Data from the 1996 lures indicated there were no significant differences between the pecan weevil pheromone (0.85), boll weevil pheromone (1.50), or the unbaited control (0.45) with and (LSD =1.3289; $\propto$=0.05) for males caught in the traps per day. Females caught using the 1996 lures were also nonsignificant ($\propto$=0.05) pecan weevil pheromone caught a mean of 0.900, boll weevil pheromone caught 1.70, and the unbaited control caught 1.308 (LSD = 1.4569).

When the pecan weevil pheromone lures from 1995 were substituted for the 1996 lures from 16 August 1996 until 15 October 1996, and some significant differences were seen in mean number of weevils caught in the traps. Examining data on number of males caught during this portion of the study, the control caught 4.20, boll weevil pheromone 4.55, and pecan weevil 1.65 with an LSD 3.6728. Significance is seen between pecan weevil pheromone and the control for male capture. There were no significant differences seen even with the 1995 pecan weevil pheromone on mean number of females caught. Females attracted to the boll weevil pheromone with a mean of 6.70, pecan weevil pheromone 5.50, and control 3.55 (LSD= 4.724). Even with the replacement of the pheromone, field result were not as seen during the 1995 season.

Further studies need to be conducted on the release rates of pheromones and pheromones with trap types. Since the pecan weevil pheromone has been isolated, further testing should address if pecan weevil pheromone is significantly better than boll weevil pheromone, and methods to insure the quality of the pheromone.

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CHAPTER 4

ASSESSING FEEDING PREFERENCES OF PECAN WEEVIL ADULTS ON THREE *CARYA* SPECIES AND ONE *JUGLANS* SPECIES USING A HARDEE OLFACTOMETER

ABSTRACT

Pecan weevil, *Curculio caryae*, (Horn) is a serious pest of pecan and hickory in southern nut producing region of the United States. Pecan weevil is known to infest almost all hickory species and has been reported on Persian walnut in Canada. Experiments were conducted using a Hardee olfactometer, three species of *Carya* and one species of *Juglans*. Data indicated that when given a choice, pecan or bitternut hickory is the preferred host of this insect. Shellbark hickory was less preferred, and black walnut was not attractive to pecan weevil in any of the pairwise comparisons.

INTRODUCTION

The pecan weevil, *Curculio caryae* (Horn), causes significant reductions in yields throughout the pecan [*Caryae illinoinensis* (Wangengheim) K. Koch] growing regions of the U.S. Damage is caused by feeding of male and female weevils on fruit during the water and gel stages, oviposition by females during the dough stage, and feeding on developing pecan fruit by the larval stage (Calcote, 1975). Upon completing development in the fruit, larvae exit through a small hole bored through the shell and shuck, and burrow into the soil. Prepupae remain 1-2 years in the soil before pupating into a pharate adult that remains an additional year in the soil before emerging (Van Cleave & Harp, 1971).

Ring et al. (1991) investigated tree species used as hosts by the pecan weevil. This study included a broad range of host such as, 14 species of hickory (*Carya*), 8 species of walnut (*Juglans*), 4 species of chestnuts (*Castanea*), 1 species of tanbark oak (*Lithocarpus*), 2 species of oak (*Quercus*), 1 beech (*Fagus*), and 4 species of hazelnuts (*Corylus*). Testing involved caging 5 mating pairs on the fruit of trees in the field or introducing of 5 mating pairs into a chamber containing the fruit of a particular species in the laboratory. In these tests no choice to multiple hosts was provided. Laboratory and field studies indicated that pecan weevils prefer *Carya* sp. for feeding and oviposition (Ring et al., 1991). None of the other species tested were utilized by the weevils in the laboratory although Persian walnut, *Juglans regia*, was previously found to serve as a host for pecan weevil in Canada (Foott & Timmins, 1984).

The use of host kairomones in attraction of insects has been widely investigated. Giblin-Davis et al.(1996) found that the sugarcane weevil, *Metamasius hemipterus*

sericeus, were attracted to fermenting sugarcane, but that a mixture of molasses and water was also an effective attractant in the field. Butkewich & Prokopy (1993) reported that plum curculio, *Conotrachelus nenuphar*, showed a greater attraction to plum fruit than plum leaves, wax plum fruit, nonhost tomato fruit, or blanks in the laboratory. Giblin-Davis et al. (1994) reported that ethyl acetate, a palm produced kairomone synergized the attraction of the male synthesized aggregation pheromone of palmetto weevil, *Rhynchophorus cruentatus*. Jaffee et al. (1993) report that ethyl acetate with the aggregation pheromone increased capture of palm weevil, *Rhynchophorus palmarum,* in lethal and nonlethal traps. Gries et al. (1994) research indicated that ethyl propionate was a synergistic kairomone for the African palm weevil, *Rhynchophorus phoenicis*. Budenberg et al. (1993) reported that both male and female banana weevils, *Cosmopolites sordidus*, were attracted to freshly cut banana rhizomes and pseudostems and visits to volatiles from was significantly longer than to the fresh tissue. Further work with host kairomones with the use of aggregation or sex pheromones and different trapping systems may lead better monitoring of these pests.

The objective of this research was to determine if pecan weevils exhibit an odor preference to a particular species when multiple hosts were provided simultaneously.

MATERIALS AND METHODS

In 1996, two trapping sites were established to obtain weevils for this study. The first site was located at the OSU Horticulture Research Station near Sparks, OK. The second site was located on the Horticultural Research Station, Stillwater, OK. Weevils were collected from cone emergence traps under infested pecan trees (Raney & Eikenbary,

1968, Boethel et al., 1976, Eikenbary et al., 1978), and pyramidal traps (Tedders & Woods, 1995) baited with pecan weevil and boll weevil pheromone.

Laboratory tests to determine attractiveness of pecan (*Carya illinoinensis* (Wangenheim) K. Koch), black walnut (*Juglans nigra* L.), bitternut (*Carya cordiformis* (Wangenheim) K. Koch), and shellbark hickory (*Carya laciniosa* (Michaux f.) Loudon) to pecan weevil males and females were conducted in two choice olfactometers with the airflow set at 15 ml/minute (Hardee et al, 1967). Unfed adults were introduced into the olfactometer between 0900 and 1400 hrs, a period previously determined as the time of peak mating activity of pecan weevil in the laboratory (Collins et al. 1996). Pecan fruit in late gel stage were crushed and placed in one arm of the olfactometer and no fruit or another alternative fruit in late gel stage were crushed and placed in the other arm (Budenberg et al., 1993). Fruit was replaced with fresh ever two hours in clean flasks, and the olfactometer was cleaned every day with soap and water. A pecan weevil was introduced into the chamber and observed for two hours, and the response was recorded. A two hour time interval was chosen after preliminary trials with 15 replications and a one hour interval, no response was seen, but after two hours, 90% of the weevils responded. For each fruit species tested, a minimum of 50 replications per sex were employed for 100 replications per fruit. Data were analyzed using Chi-square analysis (SAS, 1991).

RESULTS AND DISCUSSION

Pairwise comparisons of responses by male versus female weevils to a particular fruit were nonsignificant (P=0.05) with all species of nuts tested in these experiments. In

all experiments, males and females were attracted to a particular fruit in similar ratios.

In the first comparison with pecan weevil with pecan fruit versus nothing in the opposite arm of the olfactometer revealed a marked preference by both sexes for pecan with 97.8% of 50 males and 95.9% of 50 females responding to the pecan fruit (P=0.05). Since pecan is the preferred host of the pecan weevil this response was expected (Table 1).

The second comparison was conducted using pecan and walnut. Male and female weevils were attracted to pecan 88% and 86% respectively, while only 12% and 14 % were attracted to the black walnut (P=0.05). This supports the findings of Ring et al. (1991) that pecan weevils would not oviposit on the black walnut. Likewise, these data indicate pecan weevils do not prefer feeding on black walnut if pecan is present.

Comparison of feeding preference on pecan versus bitternut hickory indicate that males were attracted to the pecan 44% and 56% to the bitternut hickory while females were equally 48% and 48% respectively (P=0.05). Data indicate that pecan and bitternut hickory are equally attractive host for the pecan weevil, and this supports previous findings on suitability for oviposition (Ring et al. 1991).

When feeding preferences of pecan weevil were tested between pecan and shellbark hickory, male weevils were attracted to pecan 86% of the time with only 14% attracted to shellbark. Female weevils preferred pecan, 86%, to shellbark, 12% (P=0.05) . Ring et al. (1991) found that shellbark hickory was suitable for oviposition by pecan weevils, but data from this study elucidate that, if given a choice, pecan weevils prefer pecan.

In comparing weevil response to bitternut hickory versus shellbark hickory, pecan weevils preferred the bitternut over the shellbark. Seventy-six per cent of the males tested chose bitternut while only 12% responded positively to the shellbark. Female response was similar, with 84% choosing bitternut hickory over shellbark hickory, 12%. While shellbark is a suitable host for feeding and oviposition, it appears that bitternut hickory is more attractive than shellbark hickory to weevils.

Weevil response to bitternut versus nothing and shellbark hickory versus nothing were similar to pecan versus nothing where 92% of males were attracted to the bitternut with 8% responding to the blank. Female response was 100% to the bitternut and no response to the blank. Response of male weevils to shellbark hickory was 93% with 7% to the blank, and female response was 100% to the shellbark and no response to the blank. Both test were conducted toward the end of the season, and only 15 replication per sex were used.

Response of weevils to walnut versus nothing were similar to walnut versus pecan in that only 10% of females and 6% of males were attracted to walnut. These data indicate that walnut, even when representing the only food source is unacceptable to pecan weevils.

Data from these tests indicate that bitternut hickory and pecan are the preferred hosts to male and female pecan weevils when given free opportunity. Data also confirm studies on ovipositional behavior by Ring et al.(1991). While shellbark hickory and black walnut are not preferred by pecan weevils, ovipositional studies indicate that shellbark hickory is preferred over black walnut (Ring et al. 1991).

Data from these studies indicate that black walnut producers should be less concerned with pecan weevils as a pest in production orchard, but evolutionary isolation of pecan weevils with this host could change and pose problems in the future. These data also indicate that shellbark and bitternut hickory should be removed from areas prior to planting a new orchard because use of these host, especially bitternut hickory, by pecan weevil populations may present and pose infestation problems as the new orchard matures.

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Table 1. Pecan weevil response to various tree fruit in a Hardee olfactometer.

	Male Response (N=50)		Female Response (N=50)	
Pecan vs Blank	97.8%a	2.2%b	95.9%a	4.1%b
Pecan vs Walnut	88.0%a	12.0%b	86.0%a	14.0%b
Pecan vs Bitternut	44.0%a	56.0%a	48.0%a	48.0%a
Pecan vs Shellbark	86.0%a	14.0%b	86.0%a	12.0%b
Bitternut vs Shellbark	76.0%a	12.0%b	84.0%a	12.0%b
Walnut vs Blank	06.0%a	23.0%b	10.0%a	20.0%b
Bitternut vs Blank	92.0%a	08.0%b	100%a	00.0%b
Shellbark vs Blank	93.0%a	07.0%b	100%a	00.0%b

Same letters is the same row between sexes are nonsignificant ($\propto$=0.05)

CHAPTER 5

MONITORING THE HICKORY SHUCKWORM *CYDIA CARYANA* WITH THREE PHEROMONE LURES IN AN ABANDONED PECAN ORCHARD

ABSTRACT

The hickory shuckworm is a serious pest of pecans in the southern United States. Efforts to effectively and economically monitor for this insect have been unsuccessful. The pheromone, although economical, has met with limited acceptance from growers and scientists. Research in Oklahoma has validated attractancy of the pheromone and shown to be an effective and economic method of monitoring this pest. Data obtained from pheromone trapping over three growing seasons also indicates that the hickory shuckworm has three generational flights in Oklahoma compared to five generational flights reported in Georgia, Alabama, and Louisiana. Data also indicates that pheromone traps follows similar trends as blacklight trap catches from 1984-89 and 1975-1978.

INTRODUCTION

The hickory shuckworm, *Cydia caryana* (Fitch) is a serious pests of pecans, *Carya illinoinensis* (Wangenh) Koch (Osburn et al. 1963; McQueen, 1973). This insect is considered a multivotine pest with three to five generations during the year in the pecan production regions of the southern United States (Calcote & Hyder 1979, Calcote & Hyder 1980, McVay et al. 1978; McVay et al. 1994). Emergence of hickory shuckworm from the overwintering state begins soon after 1 April and continues through May. The overwintering larvae pupate when relative humidity exceeds 62.5% (Gunasena & Harris, 1987), and the adults emerge from the shucks and oviposit in *Phylloxera* spp. galls (Boethel et al., 1974; Dinkins & Reid, 1988). Second generation begins emerging from the first of July until the end of July. Mated females oviposit in the developing fruit. Larval feeding on developing kernels results in fruit abortion. Growers are most concerned with the second generation due to the loss of fruit. The last generations begins emerging during the middle of August and continues until the latter part of October. These generations are also of concern due to the larvae boring through the shuck impeding shuck-split and staining fruit if shuck split does occur.

Scouting for hickory shuckworm consists of manually examining aborted fruit for hickory shuckworm larvae and examining pecan fruit on the tree for eggs. This method of scouting takes considerable labor and time. Experiments with blacklight traps, to determine infestation rates, began in the 1970's (Tedders & Edwards, 1970; Tedders & Edwards, 1974). Tedders et al. (1972) found blacklight traps were an effective means of monitoring for this insect in the field, and with enough traps in the orchard, hickory

shuckworm infestation decreased without using insecticides. However, researchers failed to recognize the weaknesses of blacklight traps: 1. electricity frequently lacking in the orchard, 2. cost to power the traps, 3. initial expense of the blacklight traps makes this method cost prohibitive, and 4. blacklight traps attract numerous species of insects, and the hickory shuckworm is a microlepidopteran thus, in order to determine trap catches, one must sort out these microlepidopteran insects and then be able to identify them. If trap captures are left unchecked for only a few days, the condition of specimens quickly deteriorates.

Anderson (1972) reported that female hickory shuckworm produced and emitted a substance that attracts male moths. In 1986, Smith et al. (1987) isolated sex pheromone components from the hickory shuckworm females and tested these components with electroantennagram and flight tunnel experiments. The most likely compound was E8, E10-12:acetate. McDonough et al. (1990) confirmed that E8,E10-12 acetate was the principle component of the sex pheromone in the hickory shuckworm. Additional research determined that the red rubber septa that was originally used to carry the sex pheromone, were ineffective, probably due to isomerization (Davis et al, 1984; Guerin et al., 1983;, Hoffman et al., 1983). Gray septa showed slower rates of isomerization and provided longer lasting lures (Brown & McDonough, 1986; McDonough et al., 1990). The sex pheromone of the hickory shuckworm is currently being produced and marketed commercially by Trecé®, Inc. (CA) and Scentry® (CA) for use with Pherocon® IC wing traps that were found to be the most effective for use with the sex pheromone lures (Hendricks & Calcote, 1991).

Many scientists have studied the effectiveness of the traps in commercial orchards with limited success (McVay et al. 1994). In these pheromone trials, little or no infestation levels were computed to compare trap catches. Some entomologists, working with pecan pest and specifically hickory shuckworm, have stated that blacklight traps are still the most effective method of determining infestation levels in pecan orchards (McVay et al. 1994) .

In Georgia from 1967-1969, Tedders et al. (1972) reported that blacklight traps placed in a managed orchard from 1 July - 21 July caught 1.4, 6.4, and 2.1 male moths per day respectively. During the second generation flight, Gentry et al. (1975) caught a mean of 5, 0, and 1 male moths per trap per day . In 1977, McVay and Ellis (1978) reported less than 2 male moths caught in blacklight traps per day.

In 1989, McVay et al. (1994) using pheromone traps in three orchards caught 3.57, 2.71, and 0.71 male moths per day respectively for second generation flights. In three other orchards monitored using pheromone traps in 1989, 0 and 0.75 male moths per trap per day were captured (McVay et al., 1994). In 1990, 0.75 and 0 male moths per trap per day were caught during the second generation (McVay et al., 1994). Data from the blacklight traps are similar to information obtained from pheromone traps.

Control of this pest has typically been achieved with insecticide application (Osburn & Tedders, 1969; Boethel et al.,1972). Some work with limited success has been conducted on biological control using parasitoids (Gunasena & Harris, 1988). Tedders et al. (1972) tried to suppress densities of hickory shuckworm in a pecan orchard with blacklight traps with some success. Suppression of the hickory shuckworm using

blacklights and limited success with releases of natural enemies makes management of this insect difficult without chemical usage.

Objectives in this study were: 1. to determine if the commercial pheromones performed differently in the field; 2. to determine if pheromone lures were effective in attracting male moths to the traps throughout the growing season particularly during the second generation; and 3. Determine the generational flight patterns of this pest in Oklahoma.

MATERIALS AND METHODS

For the study on pheromone trapping, two commercially available lures and a lure from the United States Department of Agriculture (USDA) E10-12 acetate, a concentration of 50 ug per septa at 99.9% purity were placed in the orchard with unbaited traps. Since the pheromones were chemically identical, with the same concentration, and purity, significant difference in catches was not expected between the lures, but the test was to confirm if the commercial formulations were effective in the field. A completely randomized design with 5 replicates of each pheromone were tested for three years (Figure 1). Pherocon IC® wing traps were used with the pheromone lures (Trecé, Inc. CA).

A five hectare orchard with both native and improved pecan cultivars with no chemical or other IPM practices have been used. This orchard belongs to the OSU Department of Horticulture and Landscape Architecture.

Pheromone traps were placed at a height of 9.1 m from the orchard floor where

possible (McVay et al., 1994). Traps were placed in the trees using a 2.5 cm X 5 m electrical conduit. A hook was fashioned from .50cm rebar with a hole drilled in the straight end to place #12 copper wire which served as a loop for the pulley system. Before the hook was placed in the tree, baling twine was run through the copper wire loop. The trap was attached to the baling twine and pulled into place.

Pheromone traps were monitored every 3 days from 1 April - 31 October. Trap bottoms and lures were changed every 28 days to insure effectiveness of the Pherocon® wing trap and lures. Analysis of the data were conducted using ANOVA (SAS, 1987).

RESULTS AND DISCUSSION

Mean captures for pheromone traps were separated with LSD at $\propto$=0.05 (Table 1). Pheromone trap catches were not significantly different each other ($P < 0.05$), but were significantly different than controls ($\propto = 0.05$).

The research from three growing seasons 1993-1995 indicate that the three pheromones tested were effective in attracting male hickory shuckworm moths to the traps throughout the growing season (Figure 1, 2, & 3). Some growers and entomologists insist that the pheromone is an ineffective tool in monitoring this pest. Studies conducted in Georgia and Alabama with the hickory shuckworm pheromone and blacklight trials do not support one monitoring method better than another. One possible conclusion is that Georgia and Alabama apply numerous pesticide applications for control of aphid species which do coincide with shuckworm flight during the second generation, thus shuckworm may or may not be present.

Data from this study clearly follows previous blacklight studies in this orchard on emergence of hickory shuckworm from 1984-1989 (Eikenbary, unpublished data), 1975-1978 (Hall unpublished data) and a previous study by Calcote and Hyder (1979). Data from the pheromone traps follows the same trends as the blacklight trap catches from 1984-1989 (Figure 4) and blacklight trap catches 1975-1978 (Figure 5). Most comparison of blacklight data with pheromone trap data on other insects is reported as following the same trend when plotted against time (Hendricks et al. 1973, Roach, 1975, Fletcher et al. 1983, & Herbert et al. 1991). Since the pheromone flight data follows similar trends when plotted against time as data from blacklight traps and is similar to natural emergence patterns (Calcote & Hyder, 1979), we feel the data obtained from these trials justifies the use of the pheromone in the field, and could provide overall cost reduction in monitoring this pest.

By comparing the emergence of the hickory shuckworm caught in pheromone traps and blacklight trap data, three generational flights are observed in Oklahoma. The first flight begins in early April and extends through the first of June. The second flight begins in late June and continues through the first of July. The last flight begins the first of August and continues through the first of October. There were fluctuations in populations throughout the growing season probably due to weather conditions especially during the third generation (Figure 1, 2, & 3). Knowledge of the flight patterns of this insect for Oklahoma will allow grower to begin monitoring traps so that more precise chemical applications can be made.

With validation of the pheromone, further trials might include looking at using

traps to time insecticide applications or mating disruption. Pheromones might also be used as a physical means of removal of the male insects from the orchard. Use of the pheromone with a degree day model, as seen with the pecan nut casebearer, *Acrobasis nuxvorella* would also prove useful in further control of this pest.

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Table 1. Mean trap catch of male hickory shuckworm in pheromone traps and blanks by year separated using LSD ($\propto$=0.05).

Year	Pheromone	Trap catch
1993	USDA	161.40a
	Trece	121.60a
	Scentry	210.20a
	Blank	000.00b
1994	USDA	431.00a
	Trece	376.80a
	Scentry	426.20a
	Blank	000.00b
1995	USDA	82.20a
	Trece	59.00a
	Scentry	63.00a
	Blank	00.00b

Numbers followed by different letters in columns indicate signficance at $\propto$=0.05

Figure 1. 1993 Hickory shuckworm flight pattern monitored with pheromone traps in Stillwater, OK

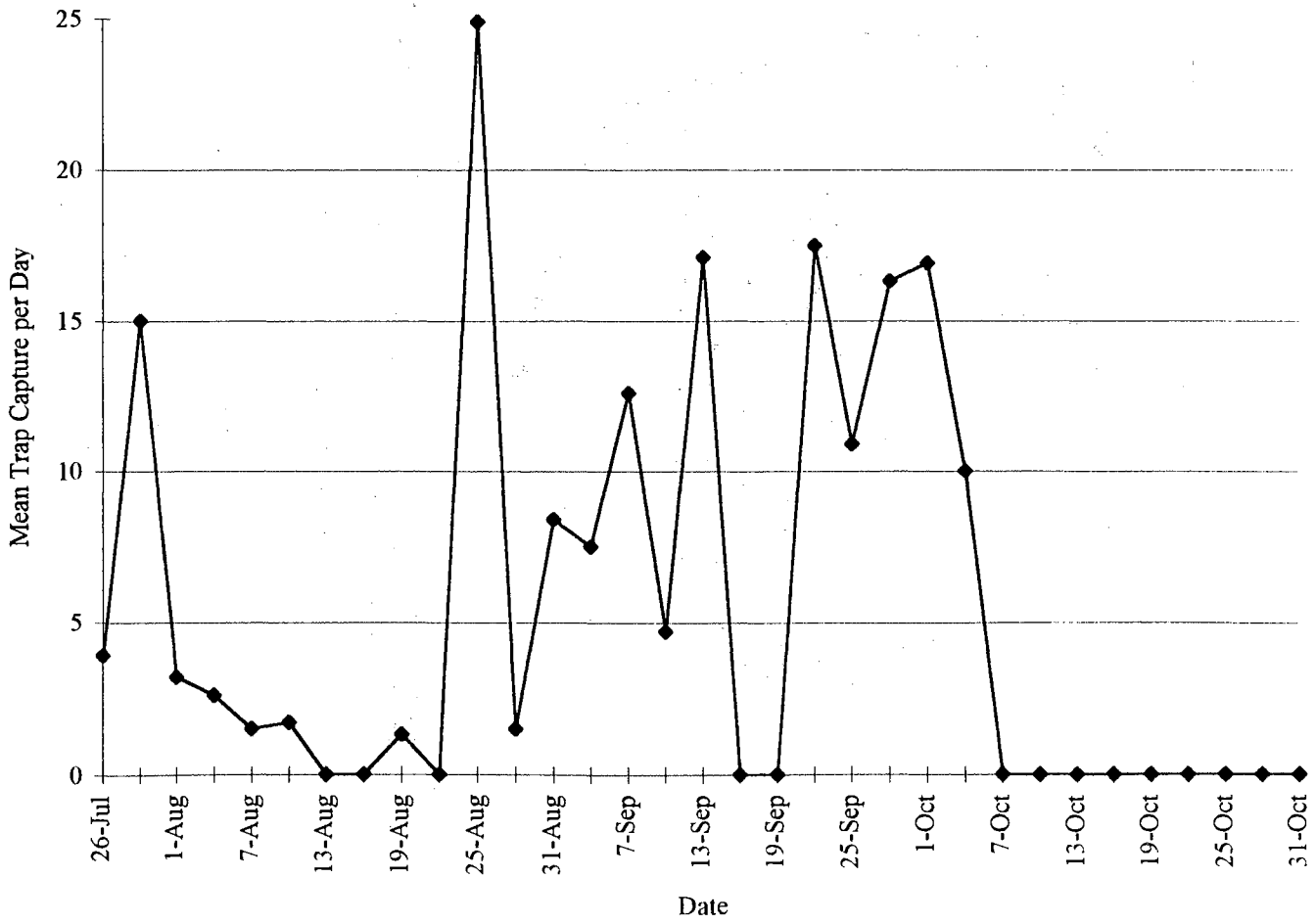
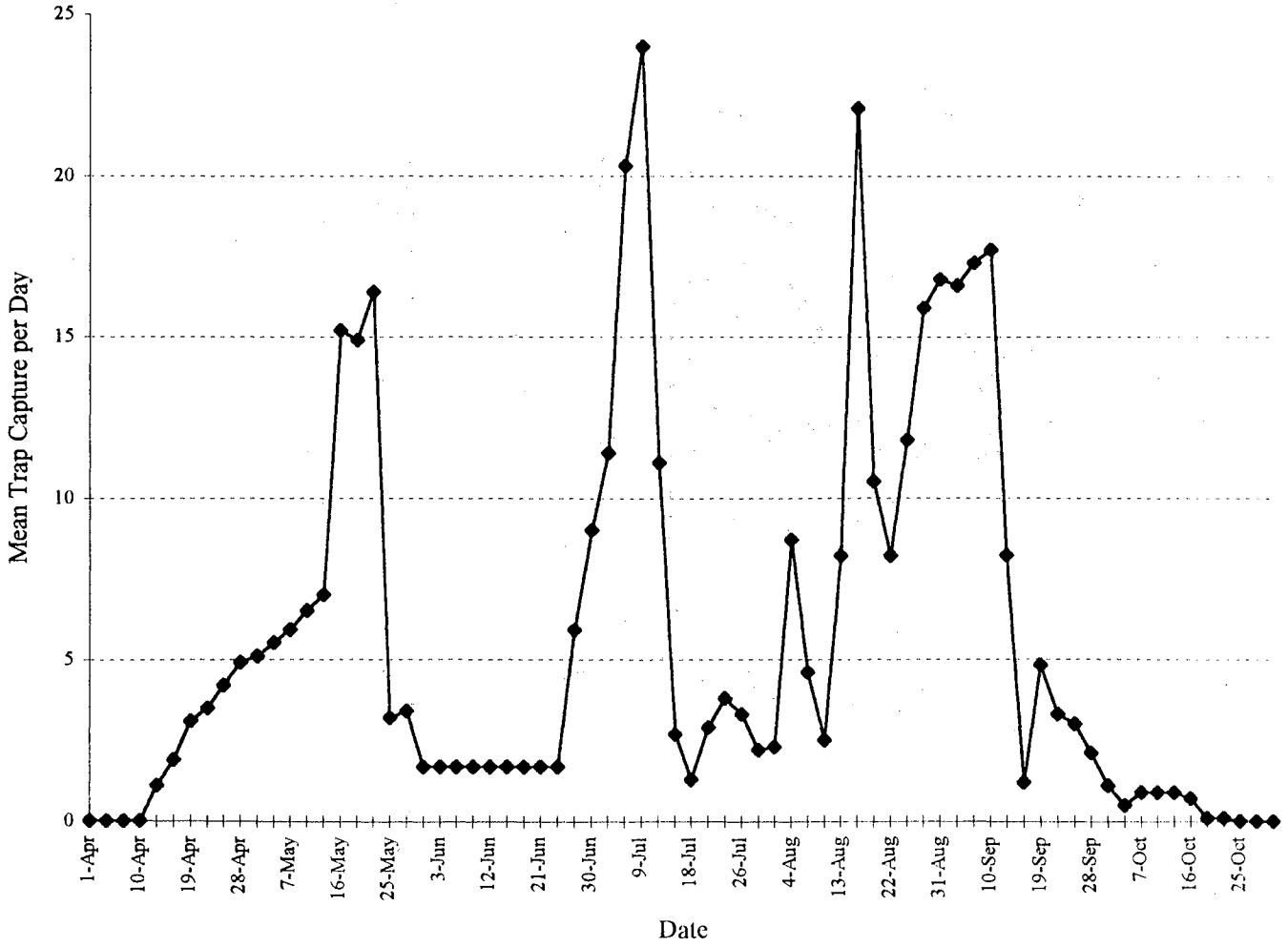


Figure 2. 1994 Hickroy shuckworm flight pattern monitored with pheromone traps in Stillwater, OK



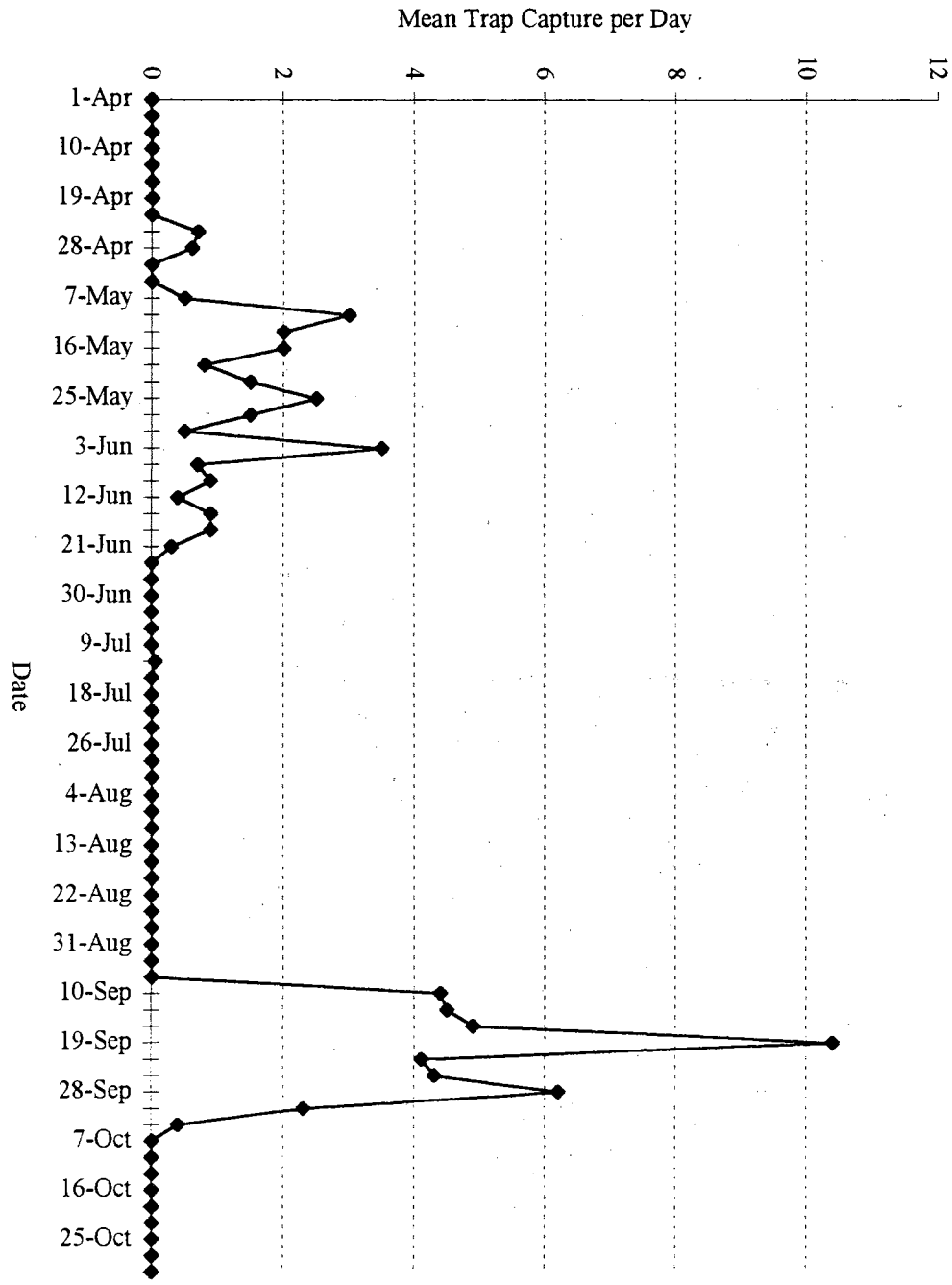


Figure 3. 1995 Hickory shuckworm flight pattern monitored with pheromone traps in Stillwater, OK

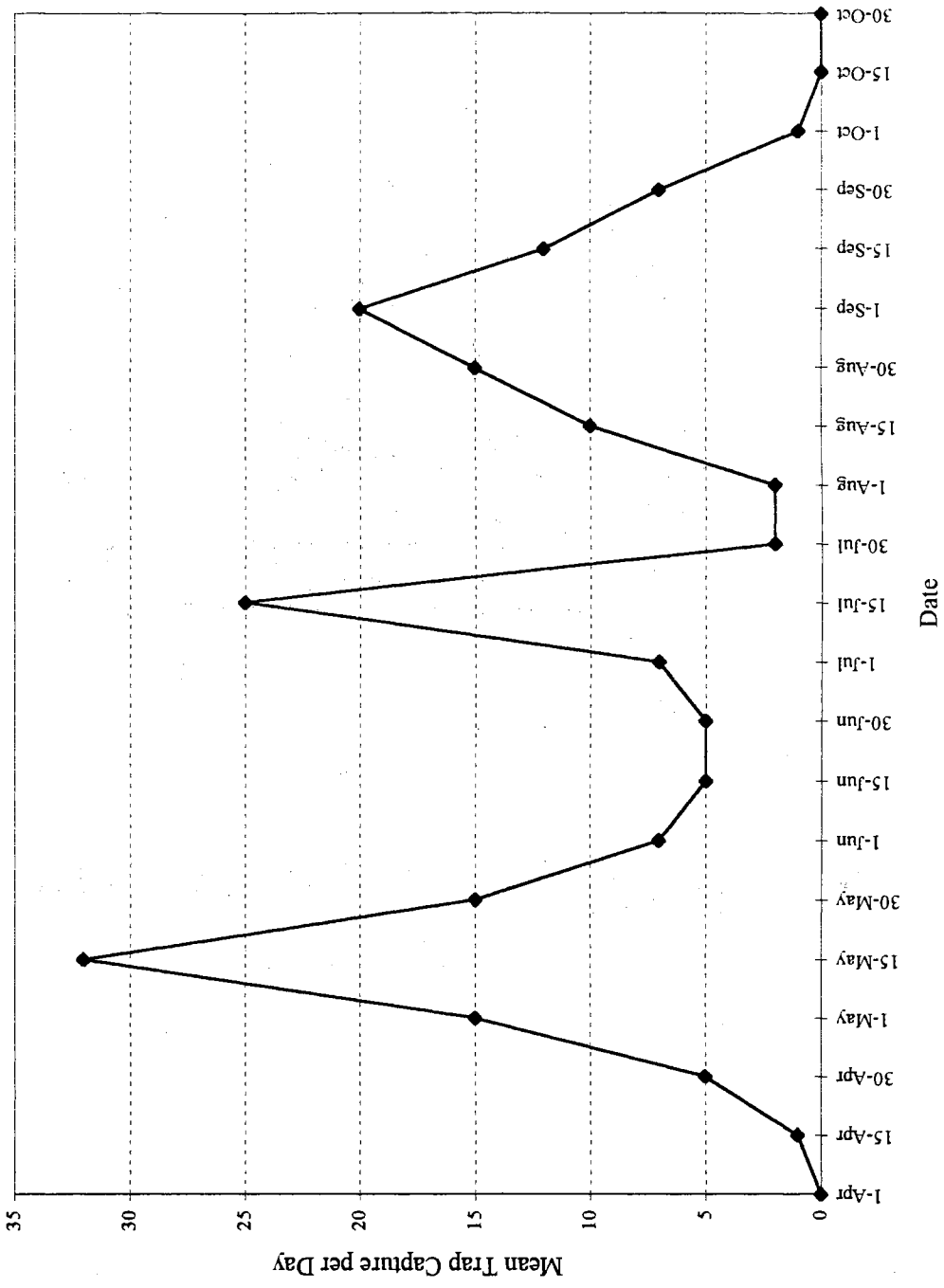


Figure 4. 1985-1989 Hickory shuckworm flight pattern monitored with blacklight in Stillwater, OK

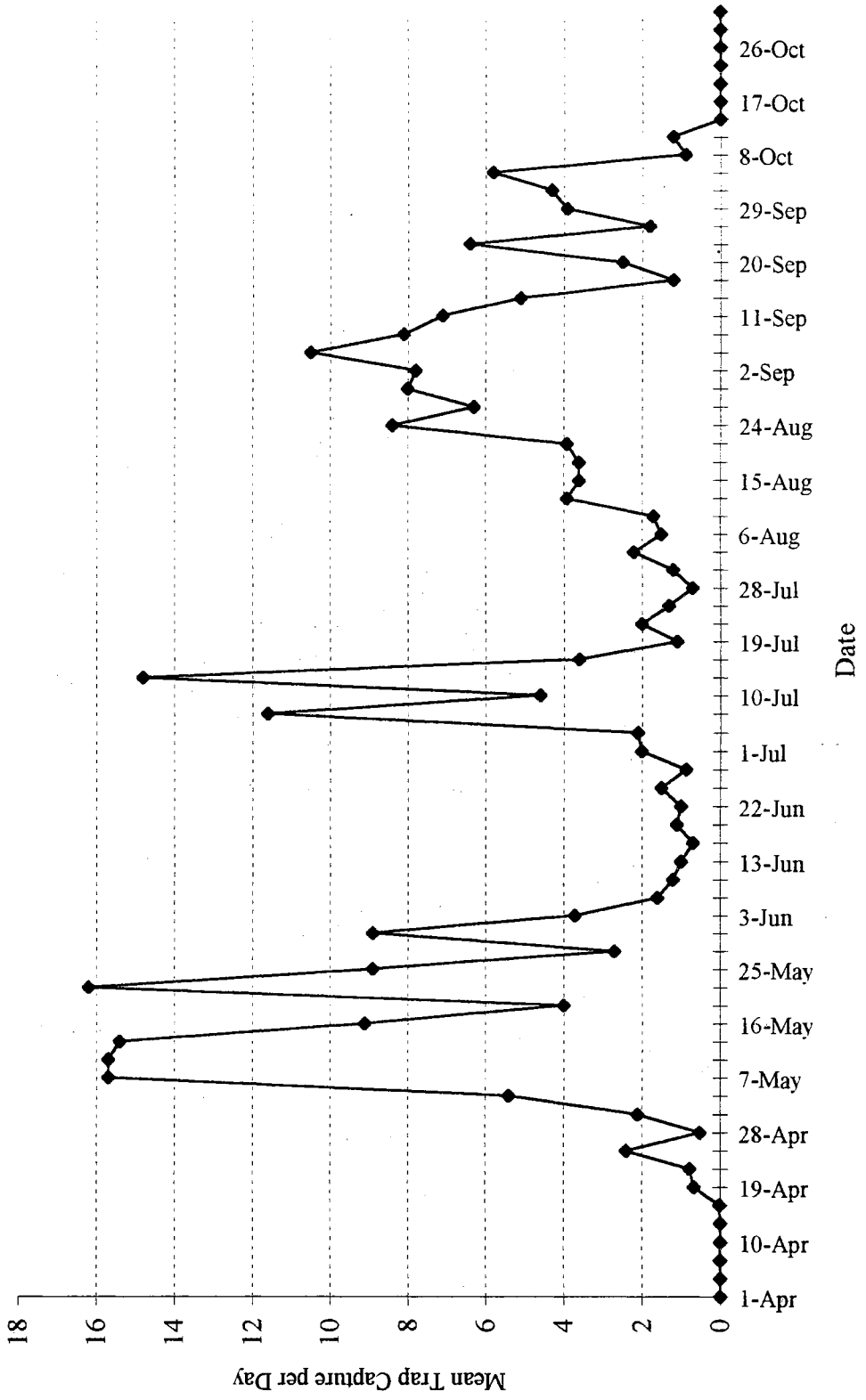


Figure 5. 1975-1978 Hickory shuckworm flight pattern monitored with blacklights in Stillwater, OK

VITA

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Candidate for the Degree of

Doctor of Philosophy

Thesis: STUDIES ON SEX PHEROMONES AND BIOLOGY OF THE PECAN WEEVIL, *CURCULIO CARYAE* (COLEOPTERA: CURCULIONIDAE), AND THE SEX PHEROMONE OF THE HICKORY SHUCKWORM, *CYDIA CARYANA*, (LEPIDOPTERA: TORTRICIDAE)

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