California Avocado Society 1981 Yearbook 65: 143-151

Field Development of the Sex Pheromone for the Western Avocado Leafroller, Amorbia cuneana

J. B. Bailey, M. P. Hoffman, L. M. McDonough
Principal investigator, Extension Entomologist, Department of Entomology, University of California, Riverside; Formerly Staff Research Associate, Cooperative Extension, University of California, Riverside. Present address, Department of Entomology, University of California, Davis; Research Leader, Yafeima Agricuitura! Research Laboratory, SEA, USDA, Yafeima, Washington, respectively.

Summary

Field trapping experiments were conducted to develop the sex attractant pheromone of Amorbia cuneana [Walsingham] for use as a monitoring tool. Optimum trap catches occurred with dose rates of 0.06-1.7 mg/septum and EZ/EE isomer ratios of 0.4:1-2.7:1. The average EZ/EE isomer ratio of native female pheromone extracts was found to be 1.31:1 [range 0.5:1 to 2.29:1]. Rubber septa were more attractive than polyethylene caps. A contaminant, E10-12: Ac, introduced during synthesis, inhibited trap catch, whereas E10-14: Ac, a component of the female pheromone extract, had no effect. Caged septa and single caged females were equally effective, but both caught significantly fewer males than uncaged pinned septa. Septa treated with 0.2 mg pheromone remained as attractive as fresh septa for 120 days. During this aging period, the isomer ratio [EZ/EE] shifted from 1.65:1 to 0.5:1.

Monitoring traps indicate that there are 3 distinct generations of A. cuneana per year in Orange and Ventura Counties.

Sex pheromones are chemicals or combinations of chemicals that are released by one organism to attract others of the same species for purposes of mating. Generally, females release the sex pheromone to attract males. Once identified and produced synthetically, pheromones can be placed in traps to attract and capture responding insects. Such traps are very useful in pest management for detection, population monitoring, and, under certain circumstances, for pest control.

The development of the western avocado leafroller (WAL), *Amorbia cuneana* (Walsingham), sex pheromone began in 1978 and 1979. At that time, we tested several pheromones of other insects for their ability to attract WAL males. None of the attractants was found to be effective.

Concurrently, we shipped female WAL to the U.S.D.A. Laboratory, Yakima, Washington, for extraction and identification of the pheromone. There, Dr. McDonough was able to extract, identify, and subsequently synthesize the pheromone. It consists of a combination of (E,E)-10,12-and (E,Z)-10,12 tetradecadien 1-01 acetates (1).

We report here the results of field and supportive laboratory tests to develop this new attractant for use as a monitoring tool for male moths of WAL. Because the WAL is a sporadic pest and because its seasonal cycles are not thoroughly understood, there is need for such a tool to predict population levels and so predict infestations and outbreaks. The current use of blacklight traps (2) is adequate but has the drawback of capturing hundreds of different species. Thus, a great deal of time must be spent in sorting through each catch for the WAL. On the other hand, other types of traps baited with the sex pheromone of the WAL will attract only males of the WAL; i.e., it is species specific.

Methods and Materials

All tests were conducted in commercial avocado groves hi Orange or Ventura Counties, California. Pherocon 1C®traps (Zoecon Corp.) were used in all tests and an extra coating of Stikem Special® was applied to trap bases to improve catch capacity. Traps were hung at a height of 5 to 7 feet above ground and spaced no closer than 90 feet in a trap row. Trap rows were no closer than 88 feet.

Traps were checked and serviced every 1 to 3 days. Traps were rotated at each observation to minimize bias in trap catch due to location within the grove. Unless otherwise stated, rubber septa dispensers were used in all tests and were treated with 0.2 mg pheromone in a 1.65:1 EZ/EE ratio. Septa were hung from the top inside center of the traps. All pheromone used in this work was synthesized and formulated at the U.S.D.A. facilities in Yakima.

The randomized block experimental design was used in all tests, with 5 replications of each treatment. Data were subjected to analysis of variance and Duncan's multiple range test was used to separate means (5% level).

Results and Discussion

For optimum catch, the trap should be baited with the correct amount of pheromone. To determine the best dosage/dispenser (septum), we compared a range of rates. Results of two tests (Table 1) showed 0.06 to 1.7 mg to be best. Based on these results, we selected 0.2 mg as our standard rate.

Since the WAL pheromone consists of two geometric isomers (EE and EZ), we had to determine the ratio that would be most attractive. An isomer ratio of 1.12:1 (EZ/EE) proved to be optimum in our first test (Table 2). However, in the second, ratios ranging from 0.4-2.7:1 were not significantly different (Table 2). The original identification of the WAL pheromone was based on the combined pheromone gland extracts collected from several females. Therefore, the amount of isomeric variability among individual females was not determined. To answer this question, we analyzed the pheromone extracts from 18 native virgin females and found their average EZ/EE ratio to be 1.31:1 (range 0.5 to 2.29:1). These findings supported the field tests with the synthetic pheromone discussed above.

The type of dispenser used to release the pheromone from the trap is important. The ideal dispenser should release the pheromone at a uniform rate and for as long as possible. We compared rubber septa dispensers with polyethylene caps over an 18 day period. The rubber septa captured an average of 160.8 male moths/trap as opposed to 75/trap for the polyethylene caps.

During the artificial synthesis of pheromones, contaminants are occasionally produced along with the true pheromone. Such was the case during synthesis of the WAL pheromone. Small quantities of a contaminant E10-12:Ac were generated. Since removal of this contaminant is laborious, we decided to determine its effect on the attractiveness of the pheromone before undertaking removal. In a test conducted January 14 to February 10, 1981, it was determined that removal of E10-12:Ac improved trap catches by 30%.

We reported earlier (1) that small quantities of E10-14: Ac were detected in female pheromone gland extracts along with the true pheromone. To determine if E10-14:Ac was an important component and required for maximum trap catch, we compared our standard septa with septa containing 200 ug pheromone plus 2, 6, 20, or 60 ug E10-14:Ac. Results showed that the E10-14: Ac had no effect on trap catch.

Table 1. Capture of Male *A. cuneana* with Various Rates of Sex Pheromone. Test I, June 28-July 4, 1980; Test II, September 5-19, 1980.

Rate/Septum (mg)	$\overline{X}$ No. Moths/Trap Test I	Test II
0.06	9.8a	59.0a*
0.10	7.0a	56.8ab
0.56	8.2a	57.2ab
1.7	6.0a	35.8ab
5.0	2.6 b	29.6 bc
15.0	1.2 bc	16.8 c
Blank	0.2 c	1.0 d

* Means followed by the same letter are not significantly different at the 5% level.

Table 2. Capture of Male *A. cuneana* with Various Isomer Ratios of Sex Attractant. Test I, September 27-October 4, 1980. Test II, February 14-28, 1981.

EZ/EE Ratio	$\overline{X}$ No. Moths/Trap	
	Test I	Test II
0.05:1	0.6 c*	—
0.4:1	—	77.0ab
0.69:1	60.2 b	77.8ab
1.12:1	98.0a	96.2a
2.7:1	61.6 b	92.6ab
4.6:1	—	67.2 b
11.4:1	2.8 c	—
16.5:1	5.2 c	—
Blank	0	0

* Means followed by the same letter are not significantly different at the 5% level.

Table 3. Comparison of Virgin Female *A. cuneana* with Synthetic Pheromone. Feb. 27-March 23, 1981 (Daily Observations).

Treatment	$\overline{X}$ No. Male Moths/Trap/Day
Synthetic Pheromone (pinned)	5.92a*
Virgin Female	3.26 b
Synthetic Pheromone (caged)	2.63 b

* Means followed by the same letter are not significantly different at the 5% level.

Table 4. Longevity of *A. cuneana* Pheromone under Field Conditions. January 15-May 15, 1981. Orange and Riverside Counties, California.

Age of Pheromone (Days)	$\overline{X}$ No. Male Moths/Trap [1]	
	Aging	Fresh [2]
30	43.2	45.0
45	112.2	134.6
60	46.0	50.8
75	18.8	19.6
90	18.4	27.0
105	8.4	18.0
120	2.6	2.6

1 Means are not significantly different at the 5% level.

2 Replaced every 15 days.

A test which is normally included when developing a new pheromone is a comparison of synthetic pheromone with the pheromone emitted by live virgin females. If results of such a test show the pheromone emitted by females to be much better than the synthetic, this may indicate that the synthetic lacks an important component. We found no significant difference between traps baited with caged virgin WAL females and caged septa. However, both caught significantly fewer males than did the standard pinned septa not enclosed in a cage (Table 3). These results indicate that the synthetic pheromone is apparently equivalent to that released by virgin females. It also suggests that the cage itself has an influence on pheromone dispersal and/or male response behavior. We were especially encouraged by these results, because even though the synthetic pheromone used in this test contained the inhibitory contaminant, E10- 12: Ac, it was still as attractive as the virgin female.

The length of time a pheromone-treated dispenser will remain attractive to male moths in the field is critical. If a pheromone-treated dispenser is too short lived, frequent replacements may be required, making it uneconomical. We exposed WAL pheromone treated septa for a total of 120 days and found no significant loss of attractiveness (Table 4). Chemical analysis of the 120 day old pheromone indicated that a shift from the original EZ/EE ratio of 1.65:1 to 0.5:1 had occurred. The 0.5:1 ratio is at the lower limit, but within the range of isomer ratios found in native females and within the optimum range found in one of the synthetic pheromone isomer ratio tests. Based on these results, we will recommend the septa be replaced every 10 weeks.

Soon after the synthetic pheromone became available, we began monitoring population trends in the field in Orange and Ventura Counties. Fig. 1 depicts the seasonal trends for these locations. Three generations per year are clearly shown at the South Coast Field Station location in Orange County; and we assume the same occurred at the Leavens Ranch in Ventura County, even though the trap was not in place in the field until late March. Information as depicted in Fig. 1 is very valuable in helping pinpoint times during the year when damage may occur.

Pheromone traps were also placed in Tulare, Riverside, Santa Barbara, and San Diego Counties. However, it was discovered that the pheromone, as currently formulated, is a very poor attractant in the latter two counties. Since this discovery, native females have been collected from these areas for pheromone extraction and analysis. Results are not yet available from the Santa Barbara females, but analyses of the San Diego females indicate that their isomer ratio is much higher (range 2.6:1 to 13.3:1, EZ/EE).

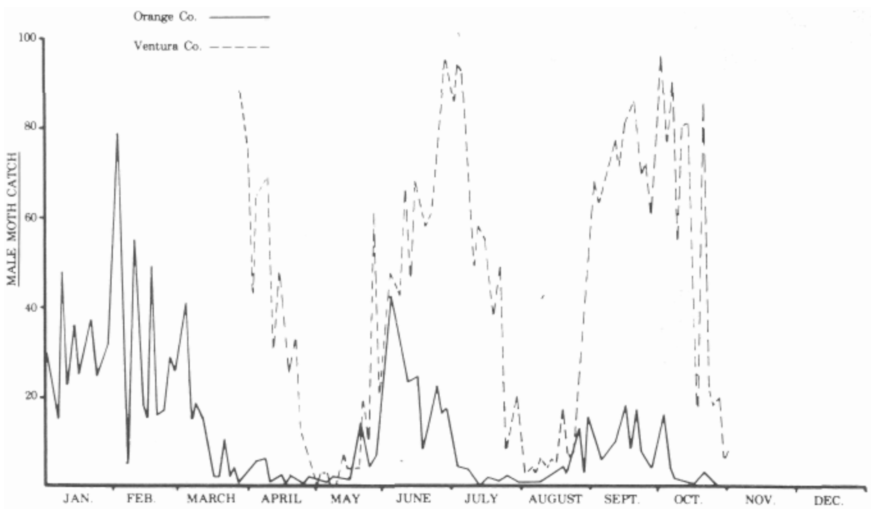

Fig. 1—1981 *Amorbia cuneana* Pheromone Trap Catches

Because this population has a higher isomer ratio, the males are not responding to the 1.6:1 isomer ratio of the synthetic pheromone currently in use, patterned after that extracted from females taken from Orange County. Based on this discovery, we are presently speculating that the WAL moths from the Santa Barbara and San Diego areas may have evolved into biotypes different from those in Orange and Ventura Counties. Field tests with various isomer ratios will be required in the upcoming year to clarify this problem and determine what ratio will be best for possible biotypes in different areas.

This new monitoring tool will eventually be made available to growers when the above difficulties are resolved and as soon as a commercial firm begins pheromone production.

Since the WAL is a sporadic rather than a chronic pest, it is unlikely that our newly developed pheromone could be used successfully for control in either a mass trapping or a male confusion program. Both of these control methods are implemented when population levels are low and in anticipation of an increase. Since the WAL only occasionally reaches economic levels, such control strategies would not be warranted. Neither method is very effective when a heavy infestation is already present.

In addition to the WAL pheromone work, we are currently involved in the early stages of development of a pheromone for the omnivorous looper, the other major worm pest of avocados in California. This pheromone has been identified but not yet synthesized.

Acknowledgements

We thank Steve Griffin, of the Ventura County Cooperative Extension Service, for operating the Leavens Ranch monitor traps, and Kirk N. Olsen for his assistance in making trap counts during the various experiments.

This research was primarily supported by a grant from the California Avocado Commission.

References

1. McDonough, L. M., M. P. Hoffmann, B. A. Bierl-Leonhardt, C. L. Smithhisler, J. B. Bailey, and H. G. Davis. 1981. Sex pheromone of the avocado pest, *Amorbia cuneana* (Walsingham) (Lepidoptera: Tortricidae). J. Chem. Ecol. (In press.)
2. Bailey, J. B., and M. P. Hoffman. 1980. Seasonal population trends of avocado worm pests. California Avocado Society Yearbook. 64: 123 - 130.