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Amorbia cuneana, A Pest of Avocados in California

Discovery of Sibling Species Through the Use of Pheromone

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ABSTRACT

The most effective lure for male *A. cuneana* (Walsingham) in Orange, Ventura, and Riverside counties of California was previously found to be a 1:1 ratio of (E.E)-10, 12- and(E,Z)-10, 12-tetradecadien-1 -ol acetates. In subsequent field tests in San Diego and Santa Barbara counties, this lure was ineffective. Analysis of sex pheromone glands (SPG) of female *A. cuneana* from these two counties showed the EE:EZ ratio to be about 1:9 and synthetic lures of this composition were highly attractive in these areas. Analysis of the SPG of a number of females from both areas showed that there were three population types; two in the low ratio areas possessed 37 and 58% EZ, and the third in the high ratio areas possessed 89% EZ.

INTRODUCTION

A. cuneana is a sporadic pest of avocados and citrus in California. Occasional infestations have caused severe economic losses to some growers. As reported in an earlier article (1), a synthetic sex pheromone for amorbia has been developed. Sex pheromones are chemicals or combinations of chemicals which one organism emits to attract others of the same species for the purpose of mating. Generally, females release pheromones to attract males. These chemicals are very species specific, so that when used in insect traps as lures the great majority of insects caught will be the desired species. Such traps are useful in pest management for detection and population monitoring of pest insects. Under certain conditions, they can even be used for pest control.

Development of the pheromone began with its identification by Dr. Les McDonough, a chemist at the United States Department of Agriculture, Agricultural Research Service (USDA-ARS) lab in Yakima, Washington. He analyzed pheromone extracts from amorbia females collected in Orange County and found the pheromone to be a combination of (E, E}- 10, 12- and (E, Z)- 10, 12 tetradecadien - 1 -ol acetates in a 1:1 ratio (2). He later synthesized the pheromone and sent samples to us for use in field tests in the avocado and citrus growing areas of California.

First, we demonstrated its effectiveness as a lure when used in both sticky and oil traps (2). Subsequently, we conducted several tests (1) which provided information as to how the efficacy of the pheromone could be optimized. These included tests for the optimal pheromone dosage, most effective dispenser, and the best trap design among others.

Subsequently, we conducted studies which indicated to us that two or more populations of *A. cuneana* might exist in California which utilize different pheromones. We tested traps baited with pheromone lures containing the 1:1 ratios of EE:EZ as monitoring tools in 6 counties of southern California and found them to be effective in all areas except in San Diego and Santa Barbara counties, even though blacklight traps in these areas indicated high populations were present.

Chemists at the Yakima lab later analyzed pheromone extracts from *A. cuneana* female adults we had collected as pupae from some of these counties (2). We wanted to determine the range of component ratios among individual females (3). They found percentages of EZ in the pheromone of the individuals from San Diego and Santa Barbara counties to be about 89%, while percentages in the individuals from Ventura County were either 37 or 58%. Here we report these studies.

METHODS AND MATERIALS

Insects

A. cuneana females analyzed in this study were collected as larvae or pupae from commercial avocado groves in San Diego, Santa Barbara, and Ventura counties during the summer and autumn of 1981.

Pupae were sexed and the females were sent to Yakima, Washington, where they were placed on moist vermiculite and kept at 17°C, 63% relative humidity in a photoperiod of 14:10 light to dark. Emerged females were collected and caged daily, where they were offered a diet of beer, sucrose, and ascorbic acid.

Larvae were reared on artificial diet at the U.C. South Coast Field Station in Irvine, California. Upon pupation, they were sent to Yakima where they were treated in the manner previously described.

Females analyzed in the mating studies were the progeny of single adult pairs collected as pupae from a commercial avocado grove in Ventura County. Rearing and handling procedures were the same as previously described except the progeny of mated pairs were kept separate, allowing for determination of the range in component ratios of the sex pheromone in single pair matings. Pheromone extracts were analyzed by gas chromatography.

Field Tests

Tests were conducted in commercial avocado groves in Santa Barbara and San Diego Counties. Pherocon® 1C traps were used with an extra coating of Stickem Special ® applied to the trap bases to improve trap efficiency. Rubber septa were used as the pheromone dispensers. These septa were impregnated with 0.2mg of 98.5%, 90%,

80%, and 50% respectively of the EZ component of the pheromone. The septa were impaled on No. 17 straight pins hung from the top inside center of the traps. All pheromone used in this work was synthesized and formulated at the USDA-ARS lab in Yakima.

Traps were hung on peripheral branches of the trees 5 to 7 feet off the ground and spaced no closer than 100 feet within and between trap rows. A randomized complete block design was used. Four treatments replicated five times were used in the Santa Barbara study. Traps were checked every two days and rotated one position within the block each time they were checked to minimize bias in trap catch due to location. Traps were checked eight times so that each treatment was at each location twice during the study.

The test in San Diego County was conducted in a commercial avocado grove near San Luis Rey. It was conducted in the manner described above except that the distance between traps was about 140 feet and four replicates were used.

Trap catch data were analyzed using ANOVA and Duncan's Multiple Range Test (p=0.05} to separate means.

RESULTS AND DISCUSSION

For our first study, we collected *A. cuneana* pupae from San Diego and Santa Barbara Counties and sent the females to the Yakima lab. Upon adult emergence, pheromone extracts from individual females were analyzed to determine any differences between the pheromone emitted by these females and our synthetic pheromone which was patterned after extracts from Orange County females.

The most obvious difference was in the ratio of the (E, E)-10, 12- and (E, Z)-10, 12-tetradecadien -1 -ol acetates. The 16 females from San Diego County gave an average of 86.6 - 5.1 SD% of the EZ component (1). The 11 females from Santa Barbara County gave an average of 89.7 - 2.4 SD% EZ. Thus, the ratio of the two components was close to 1:9 instead of the 1:1 composition of our synthetic pheromone.

Our second study compared several component ratios of the synthesized pheromone as lures in San Diego and Santa Barbara counties. At both locations, the highest ratio of EZ was found to be the most attractive (Tables 1 and 2). Thus, males were preferentially attracted to the same ratios as found in females from these areas. Results from earlier trapping studies indicated a similar situation exists in the low ratio areas {1). Since the high ratio pheromone will not attract males from a low ratio area and vice versa, the probability of individuals from opposite areas finding one another to mate is slight. In addition, data collected from blacklight traps stationed in Ventura and Santa Barbara Counties indicated the high and low ratio species fly at different times of the year, which would further limit cross mating. These results indicate that the high and low ratio *A. cuneana* populations are probably distinct species because they are not likely to interbreed in the wild. The ability of individuals to interbreed and produce fertile offspring is the most commonly used criterion to distinguish between species.

Table 1. Effect of Pheromone Component Ratios on Trap Catch of Male *A. cuneana* in Santa Barbara County, California*.

Percent EZ	No. of males caught**	
	Total	Per trap-day
98.5	1403	14.8 a
90.0	926	9.7 a
80.0	39	0.4 b
50.0	24	0.3 b

* Test was conducted from 8/24/82 to 9/11/82. There were five replicates of each ratio.

** Means followed by the same letter are not significantly different; ANOVA and DMRT (P=0.05).

Table 2. Effect of Pheromone Component Ratios on Trap Catch of Male *A. cuneana* in San Diego County, California*.

Percent EZ	No. of males caught**	
	Total	Per trap-day
98.5	332	10.4 a
90.0	152	4.8 b
80.0	83	2.6 bc
50.0	14	0.4 c

* The test was conducted from 10/14/82 to 10/22/82.

** Means followed by the same letter are not significantly different; ANOVA and DMRT (P=0.05).

At present, we believe high and low ratio *A. cuneana* to be sibling species. A sibling species is one of two or more species that are nearly indistinguishable morphologically. Except for noticeably darker markings on the forewings of many high ratio males, we have found no major morphological differences between individuals of either population. Our pheromone work provides the only compelling evidence that both populations are separate species.

To verify an earlier observation that females in low ratio areas emit pheromone comprised of EZ percentages grouped around two values, EZ percentages of female offspring from singly mated pairs were determined. The ratios of the pheromone components in females from singly mated pairs from a low ratio area are summarized in Table 3. The EZ percentages fall into two well defined groups with small standard deviations: one at about 37% EZ and the other at about 58% EZ. These results suggest

the possibility of two sibling species or races coexisting in the low ratio areas, We have not determined however, if these are two separate species or if they freely interbreed.

If two or more sibling species of *A. cuneana* exist, each species may have its own distinct habits. For instance, the low ratio species appears to cause more crop damage than the high ratio species. All noticeable economic damage of which we are aware has occurred in the low ratio areas. Thus, different pest management strategies may be necessary to control each species.

Table 3. Percent of Pheromone Component Ratios Found in Offspring from Single Mated Pairs.

Single mated pair	No. of female progeny	No. of females having given values of EZ% ± SD
A	12	12 (36.8 ± 1.7%)
B	8	2 (35.6 ± 2.2)
		6 (59.5 ± 3.2)
C	13	13 (36.7 ± 1.5)
D	8	8 (36.4 ± 0.9)
E	14	8 (37.0 ± 1.9)
		6 (57.9 ± 1.9)
F	9	6 (35.4 ± 1.6)
		3 (55.1 ± 0.7)
G	16	16 (37.1 ± 1.3)

Several field studies involving both *A. cuneana* pheromones remain to be conducted. One study will compare the two pheromones at selected sites near the Riverside-San Diego and Santa Barbara-Ventura County borders. The purpose of this study will be to more precisely define the areas where each pheromone is most effective. Additional tests must be conducted to determine which pheromone is most effective in citrus. The information provided by these tests will allow growers to more easily select the most appropriate pheromone to their specific locations.

Another study will test the longevity of the high ratio pheromone in a new grey rubber septum. Our standard red rubber septum contains sulfur as a by-product of its manufacture. Sulfur catalyzes the transformation of the EZ component into the EE component until the ratio reaches an equilibrium value of close to 1:1, thus rendering it ineffective in the high ratio areas. We speculate the high ratio pheromone will remain effective possibly 10 times longer in the grey septum than in the red septum. This will reduce the frequency of necessary changes which will result in a cost-savings to growers and PC As and in economy to the producer.

Development of the pheromone for the omnivorous looper (4), the other major moth pest of avocados in California is nearing completion. Only a few field tests remain. We are currently finding sites around southern California to station omnivorous looper and *A. cuneana* traps as the basis of a project we call the "Monitoring-Early Warning

System." Cooperators in nine counties at 31 specific locations will check these traps weekly and report moth counts to their local U.C. Farm Advisors, who in turn will make the information available to anyone who phones requesting it. When we have finished field development of the three pheromones, a commercial firm will begin producing them so they will become readily available to growers for individual use.

References

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