Project 79-G3

Volatile Constituents of Almonds and Navel Orange Worm Control

W. G. Jennings, Fong-Yi Lieu and J. A. Settlage

Department of Food Science & Technology, University

of California, Davis, CA 95616 (916-752-2163)

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FINAL REPORT

Previous work on this project has resulted in the isolation and identification of a range of volatile constituents, some of which probably function as long-range attractants and guide the female NOW to a food source. A second short-range stimulus is then required to induce egg laying at that food source. Because infested almonds serve as a powerful stimulus for both attraction and egg laying, it is apparent that they--and ether extracts of infested almonds--must contain both long- and short-range attractants. Previous work has indicated that the active principle is associated with the frass from infested almonds.

A quantity of frass, recovered from large batches of infested almonds, was subjected to ether extraction, and the excess ether removed under a stream of dry nitrogen. The material was subjected to bioassay by Dr. Rice, and found to be highly attractive.

To determine what type of functional groups were important to the biological activity, small lots of frass extract were subjected to a variety of chemical treatments, processed, and re-assayed. It was determined that the activity was destroyed by treatment with strong alkalis, bromine, or hydrogenation. These facts suggested the active principle was acidic, lactonic, or esterified, and that unsaturation in the molecule(s) was vital to attractiveness.

The crude extract was subjected to a degree of purification via gel permeation chromatography; eight fractions, differing in molecular size range, were isolated and subjected to bioassay. Activity was centered in fraction 4, with a lesser amount in fraction 3; these were combined and subjected to preparative thin-layer chromatography. This resulted in five fractions, three of which were discarded when they showed no activity by bioassay. The active fractions were combined and divided into seven sub-fractions by high pressure liquid chromatography. The active fraction from this technique--as indicated by bioassay--was subjected to infrared spectroscopy, which indicated it contained a mixture of long-chain fatty acids, at least some of which possessed olefinic unsaturation.

The mixture was esterified to convert the fatty acid moieties into their volatile methyl esters, and high resolution glass capillary GC and GC/MS revealed the presence of palmitic, stearic, palmitoleic, oleic and linoleic acids. Because the first two are saturated and our initial survey indicated unsaturation was necessary to bioactivity, attention centered on the latter three compounds.

It is interesting to note that these materials also occur in wheat bran baits and in wheat bran frass; Because twig borer infestation encourages subsequent NOW attack, it would be interesting to ascertain if twig borer frass is also high in these materials. The free fatty acid content of sound almonds is ca. 0.1%, while the free fatty acids of NOW frass are ca. 32%. Results of bioassay on synthetic oleic, linoleic and palmitoleic acids have been inconsistent. This may relate to the fact that our bioassays are based on egg count, and if those are ovipositional stimulants, unless they are presented in concert with a long-range attractant, results will usually be negative.

For the coming year, we hope, by using night-viewing binnoculars and electroantennographic equipment, to establish which of the volatiles previously isolated serve as long range attractants. These will then be presented, along

with various combinations of the fatty acids this year, to establish what combinations serve to attract and initiate egg laying.