# Gas Chromatographic Determination of the Components of the Synthetic Boll Weevil Sex Pheromone (Grandlure)

A gas chromatographic procedure was developed by which the four monoterpene components in a synthetic preparation of the sex pheromone of the boll weevil (*Anthonomus grandis* Boheman) could be analyzed with a single injection. The compounds were detected by hydrogen flame ionization after chromatography on a column loaded with 10% fluorosilicone (QF-1).

Previous research demonstrated that male boll weevils, *Anthonomus grandis* Boheman, produce a volatile material (Keller *et al.*, 1964) that is highly attractive to females in the laboratory and to both sexes in the field (Cross and Mitchell, 1966; Hardee *et al.*, 1967a,b). Moreover, traps baited with live males or with extracts of the natural attractant were highly effective in surveying, and might possibly be effective in controlling populations of this species at certain times of the year (Cross *et al.*, 1969; Hardee *et al.*, 1969). The attractive material was isolated and found to consist of two terpenoid alcohols and two terpenoid aldehydes. These compounds were identified, their structures were confirmed by synthesis, and an appropriate mixture of the synthetic compounds (grandlure) was shown to be as attractive as extracts containing the natural pheromone (Tumlinson, 1969; Tumlinson *et al.*, 1969).

Gas chromatography was the major analytical tool used in the identification of the components of the pheromone; however, no single system was developed that would resolve all four compounds. Since the synthetic pheromone probably will be widely used in future research and control programs, a convenient and efficient analytical method was needed for quality control analyses during the preparation of bait formulations and also for studies of the fate of the pheromone. The present paper reports the development of a sensitive gas chromatographic procedure that permits analyzing the four components with a single injection.

## EXPERIMENTAL

**Chemicals.** The four components of the pheromone (I, *cis*-2-isopropenyl-1-methylcyclobutaneethanol; II, *cis*-3,3-dimethyl-$\Delta^{1,8}$-cyclohexaneethanol; III, *cis*-3,3-dimethyl-$\Delta$-$^{1,\alpha}$-cyclohexaneacetaldehyde; and IV, *trans*-3,3-dimethyl-$\Delta^{1,\alpha}$-cyclohexaneacetaldehyde) were synthesized and purified with preparative gas chromatography (by R. C. Gueldner) by the procedures of Tumlinson (1969). Individual samples of I, II, and IV and a solution (8.1 mg per ml) of the grandlure mixture (I, 23.4%; II, 17.3%; III, 29.65%; and IV, 29.65%) were made available by the USDA Boll Weevil Research Laboratory, Starkville, Miss.

**Gas Chromatography.** A F&M 402 instrument equipped with a hydrogen flame ionization detector was used with a 1-mv strip chart recorder (Hewlett-Packard, Pasadena, Calif.).

**Column.** Glass, 183 cm × 4 mm i.d.

**Packing.** Fluorosilicone (QF-1, 10% (w/w) on 80- to 100-mesh Chromosorb W (Analabs, Inc., Hamden, Conn.).

**Carrier Gas.** Nitrogen at 85 ml per min.

**Other Gases.** Hydrogen at 65 ml per min; air at 500 ml per min.

**Temperatures.** Column 110° C; injection port 135° C; detector 190° C.

**Sample.** 0.010 to 2.0 $\mu$g of each compound in 5 $\mu$l of hexane.

The column was conditioned for several days with the indicated conditions and repeated injections of grandlure until a constant response was obtained over the concentration range used. Then a standard calibration curve was established. Peak areas were measured with a planimeter (Gelman Instruments, Co., Ann Arbor, Mich.).

## RESULTS AND DISCUSSION

A representative chromatographic profile of the grandlure mixture is shown in Figure 1; the retention times of I, II, III, and IV were 6.90, 7.85, 22.60, and 23.95 min, respectively.

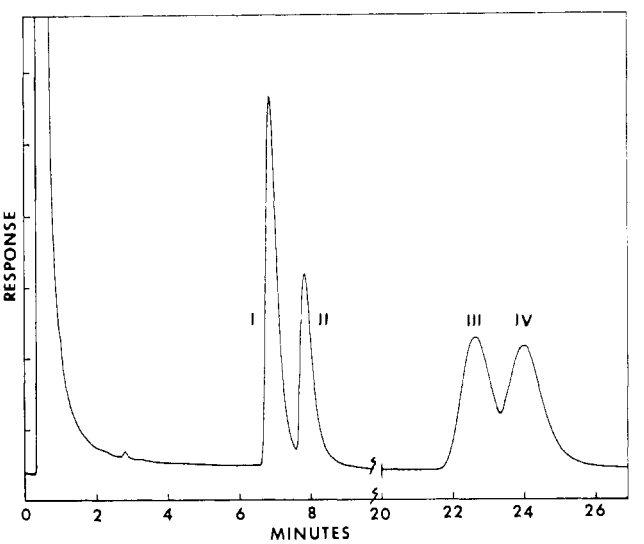


**Figure 1. Chromatogram of the four monoterpene components of grandlure**

Table I. Gas Chromatographic Analyses of Different Concentrations of Grandlure[a]

| I | | II | | III | | IV | |
|---|---|---|---|---|---|---|---|
| Conc. | Response | Conc. | Response | Conc. | Response | Conc. | Response |
| 0.950 | 162 ± 4 | 0.700 | 93 ± 1 | 1.201 | 163 ± 2 | 1.201 | 184 ± 5 |
| 0.475 | 80 ± 1 | 0.350 | 44 ± 2 | 0.600 | 79 ± 2 | 0.600 | 86 ± 1 |
| 0.238 | 36 ± 1 | 0.175 | 24 ± 1 | 0.300 | 40 ± 1 | 0.300 | 45 ± 1 |
| 0.119 | 20 ± <1 | 0.088 | 10 ± <1 | 0.150 | 18 ± 1 | 0.150 | 21 ± 1 |
| 0.060 | 8 ± <1 | 0.044 | 6 ± <1 | 0.075 | 9 ± <1 | 0.075 | 10 ± <1 |
| 0.030 | 4 ± <1 | 0.022 | 1 ± <1 | 0.038 | 5 ± <1 | 0.038 | 5 ± <1 |

[a] Concentrations are μg of component in the injected mixture; response is average (four replicates) peak area in in.$^2$ times the sensitivity factor ± standard deviation.

The elution order of the components of grandlure was confirmed by cochromatography of the mixture with each of the available individual compounds.

The detector response (peak area in in.$^2$ × sensitivity factor) to different concentrations of each of the four components after injection as the grandlure mixture proved to be essentially linear over the range tested (Table I), and the results were in good agreement with standard curves determined with the individual materials. Qualitatively, the lower limits of detection for the four compounds were about 5 ng for I, III, and IV, and 10 ng for II. For accurate quantitation, at least 20 ng of each should be injected.

With pure materials of known concentration, the described method appears accurate and reproducible. Future work will include studies of the relative fate of the components of grandlure in different formulations and at various environmental conditions. The ultimate goal is to provide information that can be used to develop effective, economical baits for use in a variety of trapping systems.

LITERATURE CITED


Cross, W. H., Hardee, D. D., Nichols, F., Mitchell, H. C., Mitchell, E. B., Huddleston, P. M., Tumlinson, J. H., *J. Econ. Entomol.* **62,** 154 (1969).

Cross, W. H., Mitchell, H. C., *J. Econ. Entomol.* **59,** 1503 (1966).

Hardee, D. D., Mitchell, E. B., Huddleston, P. M., *J. Econ. Entomol.* **60,** 169 (1967a).

Hardee, D. D., Mitchell, E. B., Huddleston, P. M., *J. Econ. Entomol.* **60,** 1221 (1967b).

Hardee, D. D., Cross, W. H., Mitchell, E. B., Huddleston, P. M., Mitchell, H. C., Merkl, M. E., Davich, T. B., *J. Econ Entomol.* **62,** 161 (1969).

Keller, J. C., Mitchell, E. B., McKibben, G., Davich, T. B., *J. Econ. Entomol.* **57,** 609 (1964).

Tumlinson, J. H., "Isolation, Identification, and Partial Synthesis of the Boll Weevil Sex Attractant," 76 pp., Ph.D. Dissertation, Miss. State Univ. (1969).

Tumlinson, J. H., Hardee, D. D., Gueldner, R. C., Thompson, A. C., Hedin, P. A., Minyard, J. P., *Science* **166,** 1010 (1969).



D. L. Bull*
Richard A. Stokes
D. D. Hardee[1]
R. C. Gueldner[1]

Entomology Research Division
Agr. Res. Service, USDA
College Station, Texas 77840



*Received for review September 25, 1970. Accepted October 21, 1970. This research was done in cooperation with the Texas Agricultural Experiment Station, Texas A&M University, College Station. Mention of a proprietary product in this paper does not constitute an endorsement of this product by the USDA.*


*[1] Present address: Entomology Research Division, ARS, USDA, State College, Miss. 39762*