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JOHN A. MOORE

National Institute of Environmental Health Sciences, National Institutes of Health, PO Box 12233, *Research Triangle Park, North Carolina* 27709

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Multiple Sex Pheromones of the Codling Moth, *Laspeyresia pomonella* (L.)

NUMEROUS sex pheromones of Lepidoptera have now been identified and synthesized. In general, one or two sex pheromones per species have been reported except that three were found in the silkworm moth, *Bombyx mori* (L.)[1]. If an insect contains more than one sex pheromone, it is important that they all be detected before undertaking structure determination and synthesis, since a single sex pheromone may be unattractive by itself[2,3]. Also, even if it were attractive, the presence of undetected sex pheromones would make it possible for the insect to develop resistance to being controlled by trapping.

Our experience indicates that the standard procedures used for isolation of sex pheromones from extracts of abdominal tips or of whole insects, will not reveal the presence of all sex pheromones. Previously, in two separate studies only one sex pheromone of the codling moth, *Laspeyresia pomonella* (L.), was found in extracts of whole females[4] or abdominal tips of females[5]. Nevertheless, we now report that female codling moths emit several sex pheromones.

In a study to determine the attractiveness of a sex pheromone isolated from female codling moths, tests in an apple orchard showed that partially purified pheromone attracted more, while pure pheromone attracted fewer, males than females[6]. The tests with the purified pheromone, however, were too limited to be free of the possibility of statistical error. Accordingly, more extensive tests were then conducted in a field cage (7.9 m wide by 3.6 m high by 22 m long) maintained by heaters at 24° C. Here traps baited with ten live, virgin females caught 500 males while an equal number of traps baited with an optimum amount (50 μg) of purified natural sex pheromone caught ninety-two. Thus, another chemical, important for attractancy in the field, had been removed during purification: either a synergist or another attractant.

An attempt to find evidence of another sex pheromone in extracts of whole female bodies by gas chromatography was unsuccessful. Because masking[7] might have been a factor, and it is unlikely that unmated females would release a masking agent to their own pheromones, condensed vapours from virgin females were studied. Air was passed over 500 females 2-4 days old in a 15-l. jar at the rate of 20 ml./min into 150 ml. of dichloromethane at 24° C. The air leaving the jar was filtered with a plug of glass wool to remove scales. Any evaporated dichloromethane was replenished each day and 200 ml. of 8% sucrose solution was provided for food. After 4 days, the dichloromethane solution was concentrated to 1 ml. Portions of this solution were then injected into a gas chromatograph equipped with an 'Apiezon-L' column. The gas chromatograph was operated at several temperatures in order to cover a volatility range represented by that from hexanol to octadecanol. Eluted samples were collected each minute and in repeat runs each 0.5 minute in the region of interest and quantitatively bioassayed[4]. The bioassay is based on mating stimulation and consists of increased movement, spinning, and attempted mating. Quantitation is achieved by determining the lowest concentration which will elicit response.

Table 1 Retention Indexes of Sex Pheromones of Codling Moth at the Indicated Temperatures on an 'Apiezon-L' Column and Minimum Concentration (Female Equivalents/ml.) of each Pheromone required to elicit Positive Bioassay

No.	Retention index	Temp. (°C)	Minimum concentration
1	1069	90	10^{-5}
2	1141	90	10^{-5}
3	1262	130	10^{-5}
4	1342	130	10^{-9}
5	1510	160	10^{-5}
6	1633	180	10^{-5}
7	1795	180	10^{-7}

Seven sex pheromones were detected in the collected fractions. Fractions collected between those containing the pheromones elicited no response in the bioassay. Table 1 gives the minimum concentration at which each sex pheromone elicits a response and the retention times expressed as retention indexes[8] at the given temperatures. During the bioassay, we observed no difference in the response of males to any of the seven pheromones. Pheromone No. 5 is the one isolated previously[4]. Subsequently, each of the pheromones has been isolated in μg quantities from extracts of whole female bodies by procedures to be described elsewhere. At present the role of all these sex pheromones is unknown. They may not all be required for attractancy.

Other work at this laboratory with this technique has revealed a similar number of sex pheromones in the zebra caterpillar moth, *Ceramica picta* (Harris). Thus, this large number of sex pheromones could be common for Lepidoptera. Multiple sex pheromones may explain the weak attractancy of some of the synthesized sex pheromones and the ability of two Lepidoptera species having one or more sex pheromones in common to maintain isolation of species, as suggested by Ganyard and Brady[9].

D. A. GEORGE
L. M. MCDONOUGH

Entomology Research Division, Agricultural Research Service, US Department of Agriculture, Yakima, Washington 98902

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Transferable Chloramphenicol Resistance in *Salmonella typhi*

WE report an outbreak of typhoid from which *Salmonella typhi* strains carrying R factors to multiple antibiotics including chloramphenicol have been isolated. Transferable drug resistance is common in *Salmonella* species which cause food poison-