QUEENSLAND DEPARTMENT OF PRIMARY INDUSTRIES
DIVISION OF PLANT INDUSTRY BULLETIN No. 397

P^{32} LABELLING OF CODLING MOTH (CYDIA POMONELLA L.)

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SUMMARY

Newly emerged male codling moths were labelled by P^{32} as orthophosphate in a feeding period of 24 hr. Transference of isotope material to females was achieved by mating. Radioactive spermatophores were detected by autoradiography in 95% of females caged with treated males. A concentration of 0·1 mc per ml appeared to give optimum results. No detrimental effect on reproductive potential was evident at concentrations as high as 0·44 mc per ml.

Introduction

Preliminary work in Queensland with chemosterilants against codling moth (*Cydia pomonella* L.) has given promising results and methods of assessment of their effect on field populations are required.

An important consideration in any work with chemosterilants is the ability of sterile individuals to compete in mating with non-sterile individuals. Labelling of spermatophores with radiosotopes would provide a direct method of assessing competitive ability of treated males.

Several authors have reported the labelling of the sperms of male insects and their subsequent detection in untreated females mated with them. Oftedal and Mostige (1957) labelled sperm of *Drosophila melanogaster* Meigen. Dame and Schmidt (1964) labelled semen of *Anopheles quadrimaculatus* Say and *Aedes aegypti* (L.). Mitlin, Bartlett, and Keller (1965) traced labelled sperm or seminal fluid from treated males to the spermatheca and ovaries of mated females of *Anthonomus grandis* Bohenaan.

The current work was undertaken to determine whether the technique could be applied to codling moth.

Materials and Methods

Moth material.—Larvae of a laboratory colony of codling moth were reared, one per fruit, in small Granny Smith apples, maintained under continuous illumination at approximately 80°F. Fifth instar larvae were collected and sexed daily as they left the fruit. Moths on emergence each day were placed in oviposition tubes maintained at 80°F and receiving natural light from a window with a southerly aspect. The oviposition tube comprised a 4 in. x 1 in. polyethylene cylinder lined with waxed paper and having a 16-mesh wire gauze fused into one end. Moths were provided with water through a cotton-wool plug in a small vial projecting through a cork at the other end. The waxed paper was changed as required and resulting emerging larvae were counted and removed daily. Inactive small larvae proved incapable of penetrating apples and therefore only those designated as active were recorded.

Radioisotope material.—Phosphorus 32 was obtained as a solution of orthophosphate in dilute hydrochloric acid (Australian Atomic Energy Commission P2B1.). The desired radioactivity was obtained by diluting the stock solution with distilled water.

Labelling method.—P^{32} solutions of the desired activity were fed to newly emerged male moths for 24 hr. During this time moths were held under the standard conditions except that the water vial was replaced by one containing P^{32} solution. The male moths were then transferred to oviposition tubes containing the unmated females. Four treated males were placed with four females in each tube.

Autoradiography.—Moths were left in the oviposition tubes for 14 days, which is the average life span under local conditions. At the end of this time the moth sexes were fixed to a thin card with a cellulose tape. The cards were then placed in close contact with Kodak Crystallex X-ray film. After 4 weeks' exposure in the freezing compartment of a refrigerator the film was developed and compared with the card bearing the moths.

Trials.—Trial 1 with a 6 x 5 layout included four isotope concentrations, namely 0·055, 0·11, 0·22 and 0·44 mc per ml. Each unit tube contained four pairs of moths. In the second trial a treatment of 0·1 mc per ml was compared with untreated, using 50 treated tubes and 10 untreated tubes, again with a unit of four pairs of moths.

Results and Discussion

Results showing percentage labelled males, percentage labelled females and number of eggs and active larvae per oviposition tube for both trials are given in Table 1.

In the first trial proximity of the moths under autoradiography precluded clear definition of all of the 24 individuals in each treatment. The numbers which were clearly defined in each trial are those given in brackets after the percentages in Table 1.

TABLE 1

PERCENTAGE LABELLING AND REPRODUCTIVE POTENTIAL OF CODLING MOTH FROM P^{32} FEEDING OF NEWLY EMERGED MALE MOTHS

Concentration of P^{32}	Labelled Males (%)	Labelled Females (%)	Mean No. of Eggs per Tube	Mean No. of Active Larvae per Tuoe
Trial 1				
nil	nil (24)	nil (24)	399·2	137·5
0·055 mc/ml	58·3 (24)	47·4 (19)	298·3	103·8
0·11 mc/ml	100 (19)	78·3 (23)	404·0	151·7
0·22 mc/ml	100 (21)	55·6 (18)	245·3	107·8
0·44 mc/ml	100 (19)	56·6 (18)	319·8	133·8
Trial 2				
nil	nil (40)	nil (40)	306·1	89·0
0·1 mc/ml	98·4 (191)	95·3 (184)	290·4	89·4

Brackets represent numbers of moths on which the percentages are based.

Trial 1 showed that labelling of codling moth was possible over the range 0·055 to 0·44 mc per ml of P^{32}. The dark areas on the autoradiograph were too faint to be entirely reliable with the concentration 0·055 mc per ml and the concentration 0·1 mc per ml was therefore indicated as a suitable minimum. Concentrations as high as 0·44 mc per ml did not affect egg-laying or larval emergence.

In Trial 2, using 0·1 mc per ml, a greater number of moths and improved separation of moths on the autoradiograph proved that a high proportion (95%) of codling moth females caged with treated males had mated and were successfully identified by isotope transference.

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(Received for publication July 22, 1966)

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