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【Research report】

放射性磷標記玉米螟赤眼卵蜂（Trichogramma ostriniae Pang and Chen）【研究報告】

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Abstract

摘要

本試驗以浸漬法及噴佈法，用放射性磷標示玉米螟赤眼卵蜂（Trichogramma ostriniae Pang and Chen）。放射性磷的濃度在0.02、0.23、0.91、4.55、9.00，和74.10μCi/ml均不影響玉米螟赤眼卵蜂的雌蜂壽命，每雌寄生卵數，子代數，和子代羽化率。觀察得的寄生蜂活動性亦未受影響。以濃度4.55μCi/ml的放射性磷噴佈在寄生蜂上，可以有效地標記玉米螟赤眼卵蜂。

Key words:

關鍵詞: 永射性磷、玉米螟赤眼卵蜂、標記。

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Marking *Trichogramma ostriniae* Pang and Chen (Hymenoptera: Trichogrammatidae) with Radioactive Phosphorus

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ABSTRACT

Methods of dipping and spraying with radioactive phosphorus (^{32}P) were used to mark *Trichogramma ostriniae* Pang and Chen. There is no significant effect of ^{32}P at concentrations of 0.02, 0.23, 0.91, 4.55, 9.00, and 74.10 μCi/ml on female longevity, number of parasitized host egg per female, number of progeny per female, or percent emergence. The wasps seemed behaviorally unaffected by treatment with ^{32}P. Spraying with ^{32}P at concentration of 4.55 μCi/ml can be effective for mass marking of adult *T. ostriniae*.

Key words: Radioactive phosphorus, *Trichogramma ostriniae*, marking

放射性磷標記玉米螟赤眼卵蜂 (*Trichogramma ostriniae* Pang and Chen)

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摘 要

本試驗以浸漬法及噴佈法，用放射性磷標示玉米螟赤眼卵蜂（*Trichogramma ostriniae* Pang and Chen）。放射性磷的濃度在 0.02、0.23、0.91、4.55、9.00，和 74.10 μ Ci/ml 均不影響玉米螟赤眼卵蜂的雌蜂壽命，每雌寄生卵數，子代數，和子代羽化率。觀察得的寄生蜂活動性亦未受影響。以濃度 4.55 μ Ci/ml 的放射性磷噴佈在寄生蜂上，可以有效地標記玉米螟赤眼卵蜂。

關鍵字：放射性磷，玉米螟赤眼卵蜂，標記。

Introduction

In Taiwan, maize suffers severe damage by several pests, foremost of which is the Asian corn borer (*Ostrinia furnacalis*). *Trichogramma ostriniae*, a predominant egg parasitoid in Taiwan (Chiu and Chen, 1986) has been extensively used for control of the Asion corn borer throughout the island since 1984. Parasitism of host eggs was generally increased on both field corn and sweet corn after inundative releases of the wasps (Chen and Yu, unpublished data; Tseng, 1972; Tseng and Wu, 1990). Further improved control efficacy could be obtained through an understanding of their population densities, dispersal, and distributions in the field. Mass rearing of *Trichogramma* on alternative hosts is relatively inexpensive, rendering it especially suitable for the inundative release method of biological control (Stinner, 1977). Integration of the parasitoids into pest management systems for maize thus has excellent potential.

For purposes of ecological studies, marking of parasitoids before release would allow us to distinguish them from wild individuals in the field. However, existing methods are usually time-consuming and handling-intensive. These include the use of dyes, paints, fluorescent substances, rare elements, and radioisotopes (Luck *et al.*, 1988; Southwood, 1978). An effective marking method must leave the insects physiologically and behaviorally unaffected and should present no environmental or health hazards. When *T. semifumatum* has been fed honey water marked with radioactive phosphorus (^{32}P), many of the parasitoids gave detectable readings on a G-M scaler (Stern *et al.*, 1965). Based on available literatures and on preliminary tests, ^{32}P was selected as an external marker for *T. ostriniae* with a view to developing a consistently effective marking technique for *Trichogramma*.

Materials and Methods

Trichogramma ostriniae emerging from field collected Asian corn borer egg were used in this study. The culture was maintained on eggs of *Corcyra cephalonica*, an alternative host. For each treatment, wasps emerged within 4 hours were held in a glass tube, 20 cm in length and 8 cm in diameter, with one end sealed with nylon screen and the other end open. The sealed end faced a light so that the wasps remained in the tube on the nylon screen, as *T. ostriniae* is positively phototropic. ^{32}P in the form of H_3PO_4 was diluted in distilled water with 0.1% Tween 80. Solutions of 74.1, 9.0, 4.55, 0.91, 0.23, and 0.02 μCi/ml were prepared. Adding Tween 80 in the solution improved ^{32}P dispersion on the insect surface. The first two solutions with higher radioactivity were used in dipping treatments and the rest in spraying treatments.

Before dipping, wasps were hled in the freezer (−10°C) for ten minutes to make them temporarily inactive. About 30 wasps were used for each treatment. The wasps were wrapped with a piece of nylon screen and then dipped into the prepared radioactive solution for a few seconds. Soon after treatment the nylon screen with treated wasps was placed on filter paper to remove superfluous solution. If this is not done the wasps may be fatally held in the surface tension of the fluid.

For spraying treatments, a hand sprayer was used to apply radioactive solution onto the wasps from the open end of the glass tube. A sheet of filter paper covered the sealed end of the glass tube to prevent the environmental contamination from drifting radioactive solution and to avoid drowning the wasps. About 100 wasps were used for each treatment. For all control treatments of this experiment, the procedure was replicated with distilled water only. Wasps of each treatment were roughly divided into three groups : 1) wasps were paired and placed in small glass vials along with alternative host eggs, then held in an incubator at 27°C, without food or water; activity, longevity, and fecundity were recorded, 2) wasps were prepared for radioactivity counting on the day following treatment, and 3) both ends of the glass tubes were sealed and then placed in a maize field for five days, with honey provided; after five days, they were brought back to the laboratory and survivors were prepared for radioactivity counting.

For radioactivity measurement, individual wasps were sexed and transferred with a small writing brush to a scintillation vial, one wasp per vial. After this 0.5 ml tissue solubilizer (BTS-450, Beckman) was added to kill the wasp and digest tissues. Samples were incubated overnight at room temperature. The digested wasps were added to 10 ml of a liquid scintillation cocktail (Ready organic™, Beckman) and thoroughly mixed with a test tube mixer for 10 seconds before counting. The samples were counted in a Tri-Carb™ Model 1600CA liquid scintillation analyzer (Packard Instrument Company) at ambient temperature. Counting efficiency of this analyzer for detection of beta radiation from ^{32}P is 98%. Radioactivity was measured in counts per minute (CPM).

Results and Discussion

Wasps treated either by the dipping or spraying method regained activity in all treatments. ^{32}P at the concentrations used seems not to cause acute damage to the wasps. Analysis of variance showed no significant effects of ^{32}P on female longevity ($F = 2.21$; df = 6, 65; $P = 0.0527$), number of parasitized host egg per female ($F = 1.54$; df = 6, 65; $P = 0.1795$), number of progeny per female ($F = 0.82$; df = 6, 65; $P = 0.5607$), or percent emergence ($F = 1.63$; df = 6, 57; $P = 0.1555$). However, wasps treated with ^{32}P tended to parasitize fewer

Table 1. Effects of various concentrations of ^{32}P on longevity and fecundity (mean ± SD) of *Trichogramma ostriniae*

^{32}P Concn (μCi/ml)	n	Method of treatment	Female longevity (hr)	No. host eggs parasitized	No. progeny	Percent emergence
74.10	10	dipping	58.3 ± 31.7	22.5 ± 15.9	18.1 ± 14.4	72.5 ± 22.8
9.00	10	dipping	72.9 ± 11.1	31.4 ± 11.7	23.5 ± 9.9	75.5 ± 11.6
4.55	10	spraying	68.3 ± 7.3	25.1 ± 10.3	18.7 ± 9.8	72.6 ± 15.1
0.91	10	spraying	56.0 ± 30.0	26.7 ± 19.2	21.8 ± 14.8	82.7 ± 15.0
0.23	10	spraying	36.8 ± 24.5	15.9 ± 20.0	12.8 ± 16.5	77.6 ± 13.4
0.02	10	spraying	58.3 ± 35.4	20.8 ± 17.6	16.9 ± 14.7	82.7 ± 14.4
0.00	12	spraying	60.0 ± 16.2	35.4 ± 22.1	23.8 ± 16.3	64.6 ± 15.0

host eggs and to produce fewer progeny than those wasps in the control group, while percent emergence was lowest in the control group (Table 1). A relatively wide range of CPM occurred over dipping treatments although wasps appeared to contact well with the radioisotope. Variation in body size may be one of the causes. CPM for each wasp was generally higher at higher ^{32}P concentrations (Table 2). Based on the range of CPM for each treatment, 4.55 μCi/ml is recommended as the minimum acceptable concentration for marking *T. ostriniae*. At this concentration, it is possible to distinguish between marked and unmarked wasps by measuring for radioactivity since the counts for the control group (i.e. background radioactivity) of 23 – 43 CPM were substantially lower than those for other treatments. Differences between females and males were not consistent.

Radioactive *Trichogramma* wasps had been earlier obtained by feeding ^{32}P at a concentration of 5 mCi/ml (Stern *et al.* 1965). However, my preliminary experiments showed that less than 25 % of the wasps marked when allowed to feed on radioactive honey-water (7.4 μCi/ml or 1.48 μCi/ml of ^{32}P water solution + 20 % honey) for 30 minutes. Mass marking of *Trichogramma* by feeding of ^{32}P seems not to be consistent enough to permit the use in some ecological studies especially for estimating absolute population sizes. Loss of the ^{32}P label occurred when the dipping-treated wasps were held in the maize field for five days. This may due to self-grooming and other movements of the wasps, rather radioactive decay. Percent loss over five days respectively were 63% and 79% for males and females in the 74.1 μCi/ml treatment and 80% and 83% for males and females

Table 2. Radioactivity (counts per minute) detected from individual wasps of *Trichogramma ostriniae* treated with ^{32}P

^{32}P Concn (μCi/ml)	Method of treatment	mean ± SD (range) (n)			
		1 day after treatment		5 days after treatment	
		Female	Male	Female	Male
74.10	dipping	795.5 ± 676.0 (101-2169) (12)	1012.0 ± 981.2 (208-2441) (4)	167.6 ± 49.6 (106-239) (10)	376.1 ± 326.1 (134-1166) (10)
9.00	dipping	615.6 ± 263.2 (231-935) (5)	346.3 ± 149.3 (180-599) (6)	103.0 ± 74.6 (43-257) (9)	68.2 ± 32.0 (34-123) (9)
4.55	spraying	55.9 ± 5.2 (48-67) (10)	53.8 ± 9.0 (40-74) (10)	83.1 ± 43.5 (30-187) (10)	80.7 ± 14.9 (53-103) (10)
0.91	spraying	40.1 ± 5.4 (31-50) (10)	43.1 ± 6.5 (35-49) (10)	48.1 ± 6.5 (39-55) (10)	55.7 ± 13.5 (41-75) (10)
0.23	spraying	35.7 ± 4.6 (29-45) (9)	36.0 ± 10.0 (26-58) (9)	48.6 ± 14.8 (31-72) (10)	42.0 ± 7.1 (30-51) (10)
0.02	spraying	31.8 ± 3.8 (26-35) (5)	32.0 ± 3.0 (29-35) (5)	39.5 ± 5.8 (30-51) (10)	43.5 ± 10.2 (31-66) (10)
0.00	spraying	33.8 ± 3.3 (30-37) (5)	30.5 ± 5.8 (23-36) (5)	38.5 ± 2.6 (35-41) (5)	38.0 ± 5.0 (30-43) (5)

in the 9.00 μCi/ml treatment. Without food, adult *T. ostriniae* lived only a few days under laboratory conditions (Chiu and Chen, 1986). Food sources may be available in the field. In this experiment, the radioactive marking persisted for five days in the field, indicating its potential utility in ecological studies. The dipping method seems more efficient in treating wasps for higher CPM, although increased radioactive marking may as well be achieved by an increase in ^{32}P concentration with the method of spraying.

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