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**FIELD TESTS OF KAIROMONES TO INCREASE PARASITISM OF  
SPRUCE BUDWORM (LEPIDOPTERA: TORTRICIDAE) EGGS BY  
*TRICHOGRAMMA* SPP. (HYMENOPTERA:  
TRICHOGRAMMATIDAE)**

Daniel T. Jennings<sup>1</sup> and Richard L. Jones<sup>2</sup>

ABSTRACT

Hexane extracts of spruce budworm, *Choristoneura fumiferana*, moth scales, applied at 0.04 moth-gram equivalents/branch and at 0.06 moth-gram equivalents/tree, failed to increase parasitism rates of *Trichogramma* spp. in two cutover spruce-fir stands in Maine. Releasing "Maine-strain" *T. minutum* apparently increased parasitism rates about 20-fold. However, application of kairomone extracts to whole branches and to upper crowns of small trees may have interfered with host-searching behaviors of *Trichogramma* parasitoids.

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Numerous studies have demonstrated the importance of kairomones for stimulating host-searching behavior of entomophagous parasitoids (Jones et al. 1973; Lewis et al. 1971; Lewis et al. 1975a, 1975b). Kairomones not only stimulate host-seeking behavior but also may help retain released parasitoids within a targeted area (Gross et al. 1975). Most kairomone studies have dealt with field crop pests. However, kairomones also have potential for increasing parasitism of forest insect pests including eggs of the spruce budworm, *Choristoneura fumiferana* (Clemens).

The polyphagous egg parasitoid *Trichogramma minutum* Riley parasitizes eggs of the spruce budworm in northeastern spruce-fir forests. Parasitism of spruce budworm eggs by *T. minutum* may be as high as 77% (Anderson 1976), but is usually less than 15%, and varies by locality and year (Miller 1953, 1963; Neilson 1963; Thomas 1966). Efforts to increase parasitism rates by aerial-broadcast releases of *T. minutum* have been moderately successful (Houseweart et al. 1984), but not sufficient to suppress epidemic spruce budworm populations.

In a preliminary laboratory bioassay (W. J. Lewis, USDA ARS, Tifton, GA) of "Maine-strain" *T. minutum*, hexane extracts of spruce budworm moth scales applied to *Heliothis* sp. egg substratum, increased parasitism from 34% (controls) to 54% (treated). This paper reports results of field tests to determine (a) if kairomones sprayed on host-tree foliage increase parasitism of spruce budworm eggs by native, wild *Trichogramma* spp., and (b) if kairomones sprayed on host-tree foliage in the presence of released "Maine-strain" *T. minutum*, plus native *Trichogramma* spp., increase parasitism of spruce budworm eggs.

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## METHODS

**Kairomone Extracts.** More than 8000 spruce budworm moths were killed by cooling and then shipped in dry ice from the Great Lakes Forest Research Centre, Canadian Forestry Service, Sault Ste. Marie, Ontario, to the Department of Entomology, University of Minnesota, St. Paul, Minnesota. Hexane extracts of budworm moth scales were formulated according to the procedure described by Jones et al. (1973). Extracts were concentrated to 10 moth-grams (1 moth equivalent) per milliliter and refrigerated (5°C) until used in the field.

**Study Areas.** Two study areas were located in a spruce-fir forest heavily infested with spruce budworm; both areas were in Number 14 Plantation, Washington County, Maine, about 16.0 km west of Dennysville and 17.6 km north of East Machias. Area A was 2.4 km northwest of Patrick Lake; area B was 1 km south of area A. Both study areas were in cutover spruce-fir stands with abundant small-tree regeneration of balsam fir, *Abies balsamea* (L.) Mill., and spruces, *Picea* spp.

In area A, 100 balsam fir trees from 1.6 to 4.8 m in height were chosen for study. These small trees were clumped in two openings with a total area of about 0.20 ha. The openings were surrounded by spruce-fir overstory about 15 m in height. Overstory trees were heavily defoliated by the spruce budworm.

In area B, 50 balsam fir trees were chosen and paired in shape and height with 50 nearby trees of the same species. Selected trees were small, ranging from 1.1 to 3.7 m in height, and were densely clumped in a clearing about 0.04 ha. Surrounding overstory spruce, fir, and pine, *Pinus strobus* L., were large; estimated overstory tree heights were 15–20 m for both spruce and fir and 30 m for pines.

**Paired Branches.** We chose two branches, one treated branch and one control branch, on each selected tree in area A, yielding a total of 200 branches. Chosen branches were in the upper and middle crowns of selected trees with each pair member at the same crown level and usually the same whorl. A 1:1000 ml dilution of moth-scale extract: hexane was prepared and applied by pressurized-can sprayer. About 400 ml of hexane, containing 4.0 moth-gram equivalents, were sprayed on the 100 treated branches, yielding 0.04 moth-gram equivalents/branch. During spray application, each control branch was shielded with a large paper bag. Treated branches were sprayed between 1330 and 1500 h Eastern Daylight Time, (EDT), 11 July 1979. This date corresponded with peak egg-laying by spruce budworm moths (Houseweart et al. 1982); fresh, green egg masses were present on some balsam-fir trees in area A.

**Paired Trees.** In area B, 50 trees were treated and paired with 50 control trees. For treated trees, the upper 1/3 (30–60 cm) of each tree was sprayed with 6 ml of the moth-scale extract:hexane dilution, yielding about 0.06 moth-gram equivalents/tree. Nearby paired-control trees were shielded during spray application. The moth-scale extract: hexane dilution was applied by pressurized-can sprayer between 1600 and 1800 h EDT, 11 July 1979; fresh, green egg masses were present on some small balsam-fir trees in area B.

**Parasite Releases.** To supplement native *Trichogramma*, we released lab-reared parasitoids in area B shortly (< 30 min) after applying kairomone to treated trees. Native "Maine-strain" *T. minutum* were sent earlier to Rincon Vitova Insectaries Inc., Oak View, California, for mass production in *Sitotroga cerealella* (Olivier) host eggs. For field releases, host-egg cards with unemerged parasitoids were placed in two carton sizes: 0.47 l ice-cream carton with 16 squares/carton and 0.18 l hot cold drinking cup with 10 squares/cup. Each container was sprayed with 1 ml (0.01 moth-gram equivalent) of scale extract prior to loading with host-egg cards. Four cartons and two cups were hung from upper-crown branches of small trees; deployment sites were distributed uniformly throughout the 0.04-ha area.

**Egg Densities.** Foliage samples were collected from both study areas 7 days after treatment. Paired treated and control branches were pruned from selected trees in area A. Foliated branch lengths and widths were measured (cm) and branch surface areas

calculated by the formula  $L \times W/2$  (Sanders 1980). Branches were then bagged individually and transported to the laboratory for foliage examination.

For treated and control trees in area B, the entire upper 1/3 of each tree was removed, bagged, and transported to the laboratory. This destructive sampling helped to thin some of the dense, overstocked regeneration in area B.

Following the procedures outlined by Dixon et al. (1978), trained examiners inspected the foliage in the laboratory and removed all needles bearing egg masses. Egg masses were then examined microscopically and categorized as new or old, based on the descriptions of egg-mass age given by Morris (1955). The length of each new egg mass was measured (0.01 mm) with an ocular micrometer, and the number of egg rows counted. Regression equations of Leonard et al. (1973) were used to estimate eggs/mass, except eggs in 1- and 1.5 row masses were counted individually. All parasitized and nonviable eggs were counted individually. Egg densities were expressed as eggs/m<sup>2</sup> of branch foliage area or eggs/1/3-tree crown.

**Data Analyses.** Means and standard errors were calculated for tree heights in both study areas. A paired *t*-test ( $P = 0.01$ ) was used to compare tree heights of treated and control trees in area B.

Egg-density data were subjected to Hartley's test for homogeneity of variances; transformations were not required. However, arcsine transformations were made on all percentages prior to statistical tests. Analysis of variance (ANOVA) was used to evaluate differences between egg densities by treatment (treated, control) and differences among percentages for each egg category (eclosed, parasitized, nonviable). Regression analyses were used to determine relationships between tree heights and combined (treated and control) egg densities for each experiment.

## RESULTS

**Tree Heights.** Trees in area A were distributed about equally among five height classes: 1.5–2.1, 2.2–2.6, 2.7–3.0, 3.1–3.5, and 3.6–4.8 m. Overall mean height was  $2.86 \pm 0.07$  m for the 100 paired branch trees in area A.

For the 50 paired trees in area B, a paired *t*-test indicated treated trees were significantly ( $P < 0.01$ ) taller than control trees; however, the mean difference was  $< 0.5$  m. Mean heights were  $1.97 \pm 0.07$  m for treated trees and  $1.54 \pm 0.09$  m for control trees in area B.

**Paired Branches.** An analysis of variance indicated no significant difference between egg densities for treated ( $\bar{x} = 487.66$  eggs/m<sup>2</sup>) and control ( $\bar{x} = 495.53$  eggs/m<sup>2</sup>) branches ( $P > 0.95$ ). Percentages of eggs in each category (hatched, parasitized, nonviable) were also not significantly different between treated and control branches (Table 1). Parasitism by native *Trichogramma* spp. was extremely low ( $< 0.25\%$ , Table 1) in area A. More eggs were parasitized by *Trichogramma* spp. on control branches than on treated branches, but the mean percentages were not significantly different.

Table 1. Comparison of 100 paired treated and control branches by egg category, area A, Washington County, Maine, with native *Trichogramma* spp. as parasites.

Variable	Percentage of eggs <sup>a</sup>		
	Hatched	Parasitized	Nonviable
Treated	85.91	0.09	14.00
Control	89.07	0.23	10.70
<i>F</i> -value ( <i>P</i> ) <sup>b</sup>	1.90 (0.17)	0.09 (0.76)	1.98 (0.16)

<sup>a</sup>Arcsine transformation of percentages before analysis.

<sup>b</sup>Differences not significant.

Regression analysis indicated virtually no relationship ( $r^2 = 0.05$ ) between tree height and egg density (eggs/m<sup>2</sup>) for the 100 paired branch trees in area A.

**Paired Trees.** Mean egg densities for treated ( $\bar{x} = 52.16$  eggs/1/3-tree crown) and control ( $\bar{x} = 59.80$  eggs/1/3-tree crown) trees in area B were not significantly different (ANOVA,  $P = 0.44$ ). Percentages of eggs in each category (hatched, parasitized, nonviable) were also not significantly different for the paired trees (Table 2). Parasitism rates of 3.66% (treated) and 4.66% (control) were not significantly different; however, again more eggs were parasitized on control branches than on treated branches.

Regression analysis indicated a weak relationship ( $r^2 = 0.24$ ) between tree height and egg density for the 50 paired trees in area B.

**Parasite Releases.** We estimated ca. 287,000 "Maine strain" *T. minutum* emerged and dispersed from the host egg cards deployed in area B. Subsamples of host egg cards indicated release densities of reared parasitoids were 67,497 for small cups and 219,581 for large cartons.

We have no data on the retention of released parasitoids in area B. However, the increased rates of parasitism in area B compared to area A indicate some treatment effect due to parasitoid releases.

Table 2. Comparison of 50 paired treated and control trees by egg category, area B, Washington County, Maine, with native *Trichogramma* spp. and released "Maine strain" *T. minutum* (< 5,000,000/ha) as parasites.

Variable	Percentage of eggs <sup>a</sup>		
	Hatched	Parasitized	Nonviable
Treated	83.53	3.66	12.81
Control	81.23	4.66	14.11
<i>F</i> -value ( <i>P</i> ) <sup>b</sup>	0.67 (0.42)	0.27 (0.60)	0.31 (0.58)

<sup>a</sup>Arcsine transformation of percentages before analysis.

<sup>b</sup>Differences not significant.

## CONCLUSION

We found no evidence that (a) spruce budworm moth-scale extracts sprayed on host-tree foliage increased parasitization of budworm eggs by native, wild *Trichogramma* spp., or (b) spruce budworm moth-scale extracts sprayed on host-tree foliage in the presence of released "Maine-strain" *T. minutum* parasitoids increased parasitization of budworm eggs.

Comparing parasitism rates between experimental areas indicates that releasing "Maine-strain" *T. minutum* apparently increased parasitism of spruce budworm eggs about 20-fold. However, the observed low parasitism rates, combined with the low parasitoid release rate (< 5,000,000 *Trichogramma*/ha), also indicates that most parasitoids either (a) left the area in spite of kairomone present in the release container, (b) died, or (c) failed to search for spruce budworm eggs.

Chiri and Legner (1983) concluded that complete foliar coverage of host food plants with extracted kairomones might lead to a significant loss in parasitoid searching time. Total coverage applications may "mask" naturally occurring cues and cause early dispersal of parasitoids from treated plots. Although our observed parasitism rates were low, the data in both field tests may reflect a lowered searching efficiency of *Trichogramma* on individually treated branches and small tree crowns of balsam fir.

Much more research is needed to elucidate important relationships between *Trichogramma* spp. and the spruce budworm. Particularly important are laboratory

studies to quantify response-area curves of *T. minutum* to varying concentrations of kairomone extracts. The quest to improve parasitoid searching efficiencies via appropriate distribution of moth-scale extracts on host-tree foliage remains unresolved.

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