

EVALUATION OF INUNDATIVE RELEASES OF *TRICHOGRAMMA EXIGUUM*
(HYMENOPTERA: TRICHOGRAMMATIDAE) FOR SUPPRESSION OF NANTUCKET
PINE TIP MOTH (LEPIDOPTERA: TORTRICIDAE) IN PINE (PINACEAE)
PLANTATIONS

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Abstract

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Inundative releases of *Trichogramma exiguum* Pinto and Platner were evaluated for suppression of the Nantucket pine tip moth, *Rhyacionia frustrana* (Comstock), in first-year loblolly pine, *Pinus taeda* L., plantations. Three releases, spaced 7 d apart, were made in three 0.4-ha plots during second-generation *R. frustrana* egg deposition. Each release included three cohorts of *T. exiguum* developmentally separated by 25 degree-days. Mean \pm SD field release rate for each cohort was 328 238 \pm 88 379 females/ha. Mean *T. exiguum* emergence under laboratory conditions for released cohorts was 96 \pm 2%, with 74 \pm 3% females, of which 1 \pm 1% of females displayed brachyptery; female longevity was 18 \pm 3 d. Field emergence averaged 96 \pm 4%. Parasitism of *R. frustrana* eggs was significantly increased, ranging from 40 \pm 19 to 73 \pm 22% in *T. exiguum*-treated plots and 17 \pm 17 to 67 \pm 21% in control plots. Data from all treated plots combined showed *R. frustrana* egg survival (hatching) was significantly reduced by 46%, and larval populations were significantly reduced by 60%. There was no significant difference in the percentage of terminals damaged between *T. exiguum*-treated (31 \pm 16%) and control plots (45 \pm 10%); however, length of terminal damage was significantly lower in treated plots. The percentage of damage to top whorl shoots was significantly lower in *T. exiguum*-treated plots compared with control plots, but there was no significant difference in length of tunneling damage. Damage to remaining shoots was not significantly different between *T. exiguum*-treated and control plots. Microhabitat significantly influenced both mean maximum and minimum temperature and the number of consecutive hours per day that were at or above 35°C (critical temperature for *T. exiguum* survival). Soil surface with no cover had the greatest number of hours at or above 35°C, followed by soil surface with herbaceous cover, and canopies of small trees (0.4 m tall). Canopy habitats in larger trees (0.9–1.8 m tall) had the most moderate temperature conditions. Parasitoid emergence was significantly reduced in response to increasing number of consecutive hours at or above 35°C. Predation of parasitoids prior to emergence was significantly affected by microhabitat and by the length of time capsules were in the field before *T. exiguum* emergence (i.e., cohort number).

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la lutte contre le Perce-rameau du pin (Lepidoptera: Tortricidae) dans les plantations de pins (Pinaceae). *The Canadian Entomologist* 132 : 373-386.

Résumé

La libération massive de *Trichogramma exiguum* Pinto et Platner comme méthode de lutte contre le Perce-rameau du pin, *Rhyacionia frustrana* (Comstock), a été évaluée dans des plantations de pins taeda, *Pinus taeda* L., d'un an. Trois traitements ont été administrés à 7 jours d'intervalle dans trois parcelles de terrain de 0,4 ha au cours de la ponte de la deuxième génération de *R. frustrana*. À chaque traitement, des individus de trois cohortes de *T. exiguum* séparées par 25 degrés-jours étaient libérés. Le taux de libération pour chaque cohorte, moyenne \pm écart type, était de $328\ 238 \pm 88\ 379$ femelles/ha. L'émergence moyenne de *T. exiguum* dans des conditions de laboratoire chez les cohortes relâchées a été de $96 \pm 2\%$, dont $74 \pm 3\%$ de femelles, parmi lesquelles $1 \pm 1\%$ étaient brachyptères; la longévité des femelles était de 18 ± 3 jours. En nature, l'émergence moyenne a été de $96 \pm 4\%$. Les parasitisme des oeufs de *R. frustrana* a augmenté de façon significative, allant de 40 ± 19 à $73 \pm 22\%$ dans les parcelles traitées et de 17 ± 17 à $67 \pm 21\%$ dans les parcelles témoins. Les données de toutes les parcelles traitées combinées indiquent que la survie des oeufs de *R. frustrana* (à l'éclosion) est réduite significativement, de 46%, et que les populations de larves sont réduites significativement aussi, de 60%. Il n'y avait pas de différence significative dans le pourcentage de flèches attaquées par la tordeuse entre les parcelles traitées au moyen de *T. exiguum* ($31 \pm 16\%$) et les parcelles témoins ($45 \pm 10\%$), mais la longueur des tunnels creusés dans les flèches était significativement plus courte dans les parcelles traitées. Le pourcentage de pousses attaquées du premier verticille était significativement plus faible dans les parcelles traitées que dans les parcelles témoins, mais il n'y avait pas de différence significative dans la longueur des tunnels. Les dommages aux autres pousses ne différaient pas significativement dans les parcelles traitées et les parcelles témoins. Le microhabitat influençait fortement le maximum et le minimum moyens de température et le nombre d'heures consécutives dans la journée où la température était égale ou supérieure à 35°C (température seuil de la survie de *T. exiguum*). Les surfaces de sol sans couverture restaient exposées au plus grand nombre d'heures à 35°C ou plus, suivies des sols avec couverture d'herbacées, puis des couvertures de petits arbres (0,4 m de hauteur). Le feuillage des grands arbres (0,9 à 1,8 m de hauteur) offrait les conditions de température les plus modérées. L'émergence du parasitoïde était fortement réduite en réaction au nombre progressivement plus grand d'heures à 35°C ou plus. La prédation des parasitoïdes avant l'émergence était significativement affectée par le microhabitat et par la durée de séjour des capsules en nature avant l'émergence de *T. exiguum* (i.e., le rang de la cohorte).

[Traduit par la Rédaction]

Introduction

The Nantucket pine tip moth, *Rhyacionia frustrana* (Comstock), is one of the most abundant insect pests of southeastern pine forests, primarily attacking seedlings and saplings of loblolly (*Pinus taeda* L.), shortleaf (*Pinus echinata* Mill.), and Virginia (*Pinus virginiana* Mill.) pine (Pinaceae) (Yates *et al.* 1981; Berisford 1988). Eggs are laid on needles and shoots. Early-instar larvae of this multivoltine pest mine needles and fascicle sheaths, whereas later instars feed directly on the meristematic tissue of shoots and buds, causing loss of growth, form, and wood quality (Berisford 1988).

Over the past two decades the timber industry in the southern United States has shortened rotations in loblolly pine plantations through intense management (Schultz 1997). Herbicide applications to reduce competition and increase tree growth limit vegetational diversity in these plantations and may impact natural enemies of

R. frustrana (McCravy 1998). Fertilization to enhance tree growth does not increase the percentage of shoots damaged by *R. frustrana* (Pritchett and Smith 1972; Berisford *et al.* 1989), but in a greenhouse study pupal densities were significantly higher in treatments with high nutrient levels (Ross and Berisford 1990). Timing of insecticide applications is critical, and in some locations populations are very difficult to predict, making control difficult (Fettig and Berisford 1999).

Public perception of aerial applications of pesticides, whether they are herbicides or insecticides, in the plantations is generally negative. Applications of many pesticides are not permitted near wetlands or urban areas, leaving large acreages untreated. Biological control might be useful as a component of future integrated pest management programs directed toward *R. frustrana* in these environmentally sensitive areas.

One possible approach to biological control of *R. frustrana* in pine plantations is the augmentation of natural enemies. Garguillo and Berisford (1983) found that biotic mortality factors affecting *R. frustrana* are most important in the egg and pupal stages. Because pupal mortality occurs after the trees are already damaged, we focused on the egg stage as a target for natural enemy augmentation.

Natural populations of *Trichogramma* spp. wasps cause most *R. frustrana* egg mortality. Garguillo and Berisford (1983) found parasitism of *R. frustrana* eggs by *Trichogramma* spp. to be as high as 47%. Similarly, Yates (1966) found 64.5% egg mortality due to *Trichogramma minutum* Riley in central Georgia. McCravy and Berisford (1998) reported 37.2 and 43.3% parasitism of spring- and summer-generation eggs, respectively. The species responsible for this parasitism were identified as primarily *Trichogramma pretiosum* Riley and *Trichogramma exiguum* Pinto and Platner (Hymenoptera: Trichogrammatidae), with a small number of eggs attacked by *Trichogramma marthae* Goodpasture.

Wasps of the genus *Trichogramma* are the most widely used arthropod natural enemy in augmentation programs, being mass reared and field released annually on 32 million hectares worldwide against primarily lepidopteran pests of forestry and agriculture (Li 1994). These parasitoids have several advantages as biological control agents, including relative ease of rearing and the fact that they kill their host in the egg stage before it causes feeding injury (Wajnberg and Hassan 1994).

We evaluated the extent to which inundative releases of *T. exiguum* would increase parasitism over natural levels, then assessed the impact these releases had on shoot damage by *R. frustrana*. We selected *T. exiguum* because prior experience had indicated this was a vigorous species that retained high quality under long-term mass rearing (Suh *et al.* 1998). To provide a fair evaluation of the potential for inundative releases, we released a locally adapted strain of *T. exiguum*, for which strict quality controls were implemented and monitored. We also monitored air temperature and *T. exiguum* emergence in six distinct microhabitats found within first- and second-year pine plantations to determine the optimal location to place *T. exiguum* for release.

Materials and Methods

Experimental Design. The experiment was set up using a complete block design, with three plantations acting as three blocks. Within each plantation was a treatment plot and control plot, each approximately 0.4 ha in size. The field sites were located in Bertie County, North Carolina. Two plantations were near the town of Connarista (lat. 36°12'00"N, long. 77°05'00"W), and the third plantation was located near Aulander (lat. 36°15'00"N, long. 77°00'00"W), all in Bertie County, North Carolina. The treatment was *T. exiguum* release; the control plots were not treated with *T. exiguum*, and insecticides were not used on any of the plots. Within each block, the release plot was

located at least 400 m downwind (based on prevailing wind direction for that area) from the control plot to reduce the possibility of parasitoid dispersal into control plots.

Plot Management. *Pinus taeda* seedlings (1–0) were planted in rows spaced 3.5 m apart from furrow to furrow, and 1.7 m apart within rows. Blocks 1 and 2 were planted on 23–27 January 1997, and block 3 on 31 January – 1 February 1997. All three blocks received the following per hectare herbicide treatment: May 1997, 946 mL Velpar L[®] (hexazinone) and 89 mL Oust[®] (sulfoneturon) (banded on rows); June 1997, 118 mL Arsenal[®] (imazapyr) (broadcast), 0.74 kg Velpar DF[®], and 89 mL Oust[®] (broadcast).

Two-year-old trees used for the microclimate–emergence study were planted on 1 March 1996 on the same spacing described above and were located adjacent to block 1. The stand received the following herbicide treatments: April 1996, banded 946 mL Velpar L[®] and 89 mL Oust[®]; June 1996, broadcast 118 mL Arsenal[®]; April 1997, broadcast 946 mL Velpar L[®] and 89 mL Oust[®]; June 1997, broadcast 118 mL Arsenal[®].

Trichogramma Source. *Trichogramma exiguum* were reared from parasitized sentinel corn ear worm, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), eggs placed in woodlots adjacent to agricultural fields near Plymouth (lat. 35°52'00"N, long. 76°45'00"W), North Carolina. Eight isolines (colonies begun from a single mated female) were collected and species identity confirmed by John Pinto, University of California at Riverside. Parasitoids were shipped to BIOTOP (Valbonne, France) where isolines were combined, mass reared, and formulated for field release. The formulation consisted of waxed cardboard capsules (about 5 cm³) each containing an average (\pm SD) of 1319 (\pm 140) *T. exiguum* developing inside *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs. Four small holes made during the encapsulation process were large enough for adults to escape but small enough to prevent most predators from entering the capsules.

Shipments from BIOTOP to North Carolina State University (NCSU) were made weekly, beginning 25 May 1998, via commercial air freight. Each shipment consisted of three cohorts of parasitoids whose development was staggered approximately 25 degree-days apart. For each shipment, a HOBO XT[®] Temperature Logger (Onset Computer Corp., Pocasset, Massachusetts), programmed to record temperature hourly, was placed alongside capsules to monitor temperature fluctuations. These temperature data allowed estimation of degree-day accumulation during shipping and handling so accurate predictions of adult emergence could be made.

After clearing USDA Animal and Plant Health Inspection Service and United States customs inspections, packages were taken to the North Carolina Department of Agriculture and Consumer Services (NCDA and CS) Quarantine Laboratory, Cary, North Carolina. Once cleared by NCDA and CS, parasitoids were immediately taken to NCSU and held at standard rearing conditions (25°C, 80% RH, and a 16L:8D photoperiod) until required for experimental use.

Parasitoid Releases. Our treatment for each plot consisted of three releases (R1–R3), spaced 7 d apart beginning 29 May 1998 (Fig. 1). Each release contained three cohorts of capsules differing in *T. exiguum* development. One cohort of capsules contained *T. exiguum* expected to emerge within 12–24 h, the second within 60–72 h, and the third within 96–108 h after field release. *Trichogramma* capsules were hand-placed at 100 release points evenly spaced throughout each release plot. Each release point had a pair of waxed, cone-shaped paper cups, located 0.3–0.5 m above the soil surface in the plant canopy, into which capsules were placed. Cups were spaced about 5.5 m apart within rows and 7 m apart across rows. The mean (\pm SD) release rate of *T. exiguum* for

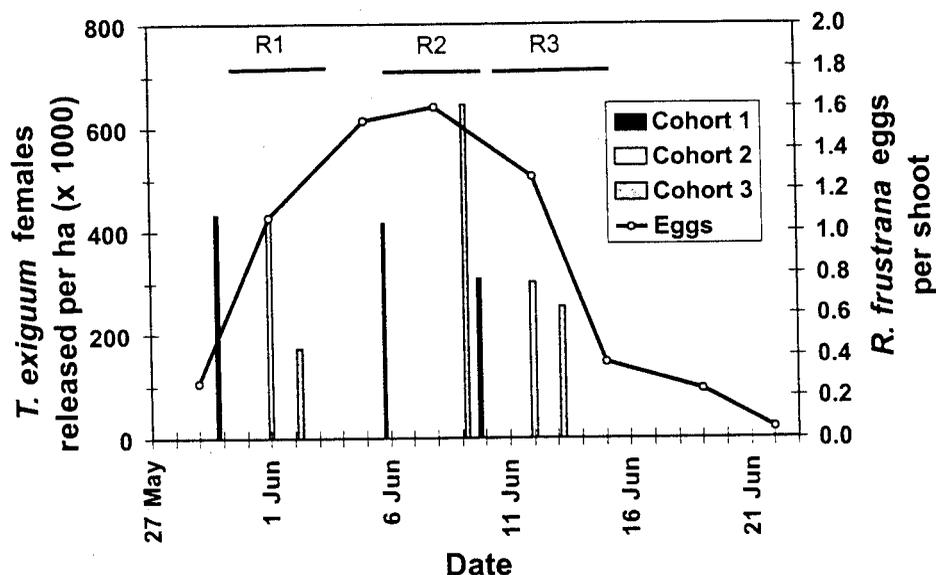


FIGURE 1. Mean number of *Rhyacionia frustrana* eggs per shoot and mean number of *Trichogramma exiguum* released with date of peak emergence for each cohort (three releases R1–R3, three cohorts each), Bertie County, North Carolina, United States, 1998. Data presented are from all release plots combined.

each cohort was 328 238 (\pm 88 379) females/ha (determined from data presented in Results and Fig. 1).

Parasitoid Quality Control. To ensure that high-quality parasitoids were used throughout the experiment, field and laboratory quality-control samples were taken from each released cohort. For each shipment from BIOTOP, five capsules per cohort were frozen immediately upon receipt to determine production emergence (*i.e.*, percentage of parasitized eggs added to fresh eggs during production). Emergence percentages were determined by sampling 50 black eggs per capsule for adult *T. exiguum* emergence holes.

A second sample of 10 capsules per cohort was placed into capped diet cups (30 mL; one capsule per cup) at standard rearing conditions. *Trichogramma exiguum* adults were allowed to emerge over a 7-d period, after which capsules were frozen. From each of the 10 individual capsules, 30 adults were randomly selected to determine sex ratio and 10 females were randomly selected to determine percent brachyptery. Emergence percentages under laboratory conditions were determined for each of the 10 capsules using the same procedure as that stated earlier.

A third sample of five capsules per cohort was used to assess adult longevity according to procedures described by Cerutti and Bigler (1991). A section (containing approximately 100 parasitized eggs) from each capsule was placed into individual vials (12 \times 75 mm) each containing a streak of honey-water (50% solution). Vials were plugged with cotton and held at standard rearing conditions. Capsule sections were removed from vials 24 h after initial emergence so that only 10–30 adults less than 24 h old remained in each vial. Vials were checked daily and the number of dead females and males in each was recorded.

To estimate field release rates, a fourth sample of 10 capsules per cohort was taken just prior to each release, and the total number of parasitized eggs (blackened) per capsule was counted. To assess field emergence, a fifth sample of 25 capsules per cohort was hand-placed in waxed, cone-shaped paper cups located 0.5 m above the soil

surface in the plant canopy, adjacent to a release plot. Five capsules from each cohort were collected on the day of release (day 0) and 3, 5, 7, 10, and 11 d after each release and frozen immediately. Emergence percentages were determined by the same procedure used to calculate emergence under laboratory conditions.

***Rhyacionia frustrana* Egg Density.** *Rhyacionia frustrana* egg density was estimated in each release and control plot by clipping the upper 22–25 cm section of 20 subterminal shoots (one shoot per tree) randomly selected from the central 50% of the plot. Shoot samples were placed in Zip-loc® bags and immediately taken to a laboratory where they were examined for *R. frustrana* eggs. The total number of parasitized eggs (indicated by black shiny appearance of egg chorion) and viable eggs were recorded for each plot. Shoots were collected and examined every 3–4 d beginning 29 May 1998.

Parasitism Levels. The level of egg parasitism in *T. exiguum* release and check plots was measured on 1, 5, 8, 12, and 15 June 1998. For each date, *R. frustrana* eggs were collected from shoots used to estimate egg densities. To standardize parasitism data, only light orange colored eggs (2–3 d old) were clipped from shoots and used to determine percent parasitism. Clipped eggs (10–35 eggs per plot) were placed on pre-moistened filter paper (Qualitative P5, Fisher Scientific, Pittsburg, Pennsylvania; 5.5 cm diam.) within Petri dishes (6 cm diam.) and held at standard rearing conditions for 7–8 d, at which time the eggs were classified as hatched, black-head stage, nonviable, or parasitized. A percentage was calculated for each category.

Larval Infestation and Tree Damage. During shoot examinations for eggs, numbers of instar 1–2, 3–4, 5 larvae (determined by head capsule size; see Fox *et al.* 1972), and pupae found in shoots and needles were recorded for each release and control plot. Additionally, the number of mined needles and the percentage of shoots damaged were recorded.

A final shoot damage assessment was made on 20 July in each release and control plot. For each plot, 50 trees were randomly selected from the central 50% of the plot. Each tree was divided into three sections (terminal shoot, top whorl shoots, and remaining tree shoots), all shoots were sampled, and the length of damage in shoots within the first two sections was measured with a ruler.

Pine Microclimate and Emergence from Capsules. To determine which habitats in pine plantations were suitable (in terms of temperature) for release of encapsulated *Trichogramma*, a datalogger (model CR10, Campbell Scientific, Inc., Logan, Utah) with 24 temperature probes (model 107 on 32 m wire leads) was set up in a field between a stand of 1- and 2-year-old *P. taeda*. Each temperature probe was completely enclosed within a sheath, constructed from the cardboard used to encapsulate *T. exiguum*, to simulate conditions inside capsules. Probes were placed in six types of habitats, replicated four times (total of 24). The habitats were (1) soil surface with no cover in first-year plantation, (2) soil surface with herbaceous cover in first-year plantation, (3) canopy (mid-height) of small (0.46 m) first-year plantation trees, (4) canopy (mid-height) of tall (0.92 m) first-year plantation trees, (5) canopy (mid-height) of second-year plantation trees, and (6) soil surface with herbaceous cover in second-year plantation. The datalogger was programmed to take one measurement every minute and record the average on an hourly basis for each temperature probe. Measurements were begun 29 May 1998 and continued through 19 June. The critical temperature at which *T. exiguum* survival is reduced is 35°C (Harrison *et al.* 1985). We therefore calculated the number of consecutive hours each day that were at or above this temperature.

To assess the effect of microclimate on *T. exiguum* emergence in different microhabitats, two capsules from each cohort for two releases (R1 and R3) were placed in white nylon mesh bags and placed alongside probes. Capsules located in the canopy were placed in waxed, cone-shaped paper cups used for the release study. One cohort was programmed to emerge within 12–24 h, the second within 60–72 h, and the third within 96–108 h after field release. Capsules from cohorts 1–3 were collected 7, 10, and 14 d, respectively, following field release and then were immediately frozen. The emergence percentage for each capsule was determined using procedures described in the section Parasitoid Quality Control. The first set of capsules was placed in the field on 29 May, and the second set on 12 June.

Predation of parasitized *E. kuehniella* eggs within capsules was also noted while emergence data were being recorded. Predation was either heavy (<100 eggs remaining in a capsule) or not present. When both capsules of a pair had heavy predation, we recorded 100% predation for the pair; if only one capsule had heavy predation, 50% was recorded; if neither capsule had obvious predation, 0% was recorded. Ants collected from inside capsules were identified to genus by David Stephan, Department of Entomology, North Carolina State University.

Statistical Analysis. Egg, larval, and pupal counts were transformed by taking the square root prior to analysis. These data and damage data were analyzed using analysis of variance (PROC GLM, SAS Institute Inc. 1996). Hatch and parasitism data were subjected to logistic regression analysis (PROC GENMOD with PSCALE option and likelihood ratio tests, SAS Institute Inc. 1996). Temperature, parasitoid emergence, and predation data were analyzed using analysis of variance (PROC GLM, SAS Institute Inc. 1996). Parasitoid emergence data were subjected to arcsine square root transformation prior to analysis. The influence of temperature on *T. exiguum* emergence was examined with a correlation analysis (PROC CORR, SAS Institute Inc. 1996). All means are provided with standard deviations.

Results

The beginning and peak of second-generation *R. frustrana* oviposition were relatively well synchronized throughout all blocks and plots. Eggs were first detected 29 May and peak oviposition was around 8 June (Fig. 1). Overall, there was no difference in egg numbers ($F_{1,14} = 0.64$, $P > 0.05$) between *T. exiguum*-treated plots and control plots, thus there was no need to use a formula to compare treated and control plots.

Mean *T. exiguum* emergence under laboratory conditions for released cohorts was $96 \pm 2\%$, consisting of $74 \pm 3\%$ females, of which $1 \pm 1\%$ displayed brachyptery. Female longevity for released cohorts averaged 18 ± 3 d. Mean emergence of parasitoids under field conditions was $96 \pm 4\%$. A release rate for each cohort of 328 238 (± 88 379) females/ha was estimated after taking into account the number of parasitized *E. kuehniella* eggs per capsule, percentage of females emerging from capsules, removal of brachypterous females, and emergence of parasitoids under field conditions (Fig. 1). Peak emergence of parasitoid cohorts from the three releases occurred every 1–4 d throughout the peak oviposition period of *R. frustrana* (Fig. 1).

Because date of egg collection affected parasitism ($F_{4,10} = 8.64$, $P = 0.003$), and the treatment \times date interaction was marginal ($F_{4,10} = 2.65$, $P = 0.096$), the egg parasitism data are presented by date of collection (Table 1). Overall, parasitism of *R. frustrana* eggs collected throughout the study period was increased by 29% ($F_{1,10} = 8.30$, $P = 0.016$) and egg hatch was reduced by 46% ($F_{1,8} = 62.0$, $P < 0.001$) in *T. exiguum*-treated plots compared with control plots (Table 1). The mean parasitism

TABLE 1. Mean \pm SD percent parasitism, egg hatch, and viability of *Rhyacionia frustrana* eggs collected from *Trichogramma exiguum*-treated and control plots in *Pinus taeda* plantations, Bertie County, North Carolina, United States, 1998.

Date	n	Treatment	% parasitized	% hatched	% nonviable
1 June	32	<i>T. exiguum</i>	49.9 \pm 23.4	24.2 \pm 9.5	23.7 \pm 23.1
	18	Control	16.7 \pm 16.7	52.8 \pm 31.5	30.7 \pm 17.2
5 June	78	<i>T. exiguum</i>	55.9 \pm 13.2	12.2 \pm 10.7	34.0 \pm 21.7
	56	Control	36.4 \pm 10.0	32.3 \pm 17.2	31.0 \pm 27.9
8 June	80	<i>T. exiguum</i>	73.0 \pm 21.7	11.4 \pm 10.0	15.3 \pm 12.3
	60	Control	66.6 \pm 20.9	25.3 \pm 13.9	8.3 \pm 7.2
12 June	74	<i>T. exiguum</i>	49.9 \pm 7.2	36.8 \pm 14.5	16.7 \pm 10.0
	65	Control	29.3 \pm 16.8	58.0 \pm 14.3	12.7 \pm 12.4
15 June	23	<i>T. exiguum</i>	40.0 \pm 19.1	24.6 \pm 9.0	36.0 \pm 12.8
	38	Control	59.2 \pm 13.1	38.0 \pm 13.6	12.0 \pm 13.7
Over all dates	287	<i>T. exiguum</i>	53.7 \pm 12.2a	22 \pm 4b	24 \pm 10a
	241	Control	41.6 \pm 20.8b	41 \pm 6a	17 \pm 13a

NOTE: Means within a column followed by a different letter are significantly different (logistic regression, $P \leq 0.05$).

ranged from 40 ± 19 to $73 \pm 22\%$ in *T. exiguum*-treated plots and 17 ± 17 to $67 \pm 21\%$ in control plots. The species responsible for parasitism in *T. exiguum*-release plots was *T. exiguum* (100% of parasitized eggs collected); *T. exiguum* (92.4%), *T. marthae* (3.8%), *T. pretiosum* (1.9%), and *T. minutum* (1.9%) were responsible for parasitism in the control plots.

The number of larvae per shoot in instar categories 1–2, 3–4, and 5 was lower in *T. exiguum*-treated plots compared with control plots (instar 1–2, $F_{1,14} = 51.41$, $P = 0.0001$; instar 3–4, $F_{1,14} = 16.79$, $P = 0.0004$; instar 5, $F_{1,14} = 26.02$, $P = 0.0001$), with a mean reduction in *T. exiguum*-treated plots of 65 ± 8 , 63 ± 10 , and $57 \pm 4\%$, respectively (Fig. 2). The number of pupae per shoot was also reduced ($F_{1,14} = 6.91$, $P = 0.02$) by $62 \pm 30\%$ in treated plots.

Despite significant reductions in larval numbers, no difference in percentages of terminal damage ($F_{1,2} = 1.28$, $P = 0.376$) was found (Table 2). There was a difference in the length of damage on terminals ($F_{1,2} = 5.69$, $P = 0.019$). No difference was found between release and control plots in the number of top whorl shoots per tree ($F_{1,2} = 1.04$, $P = 0.416$), but a greater number of top whorl shoots were attacked in control plots ($F_{1,2} = 28.36$, $P = 0.034$). However, there were no differences in the length of tunneling within top whorl shoots ($F_{1,2} = 1.70$, $P = 0.322$). There were no differences between release and control plots in the number of shoots below the top whorl ($F_{1,2} = 0.28$, $P = 0.651$), but the number of these shoots that were attacked was lower in *T. exiguum* plots ($F_{1,2} = 5.48$, $P = 0.144$) (Table 2).

The mean maximum and minimum temperatures differed among microhabitats ($F_{5,18} = 830.5$, $P < 0.001$; $F_{5,18} = 143.1$, $P < 0.001$), and so did the number of consecutive hours per day that were at or above 35°C ($F_{5,18} = 3.34$, $P = 0.025$) (Table 3). Mean minimum temperatures and the number of consecutive hours per day that were at or above 35°C in the six microhabitats varied between the two releases for which microclimate data were recorded ($F_{1,18} = 1846.0$, $P < 0.001$; $F_{1,18} = 9.56$, $P = 0.006$), therefore subsequent analyses were conducted on the two releases separately (Table 3). During the first release period (29 May – 5 June) the bare soil surface had the greatest number of hours at or above 35°C , followed by the two soil surface microhabitats with herbaceous cover and the 0.4-m tree canopies. The two canopy habitats in the largest trees

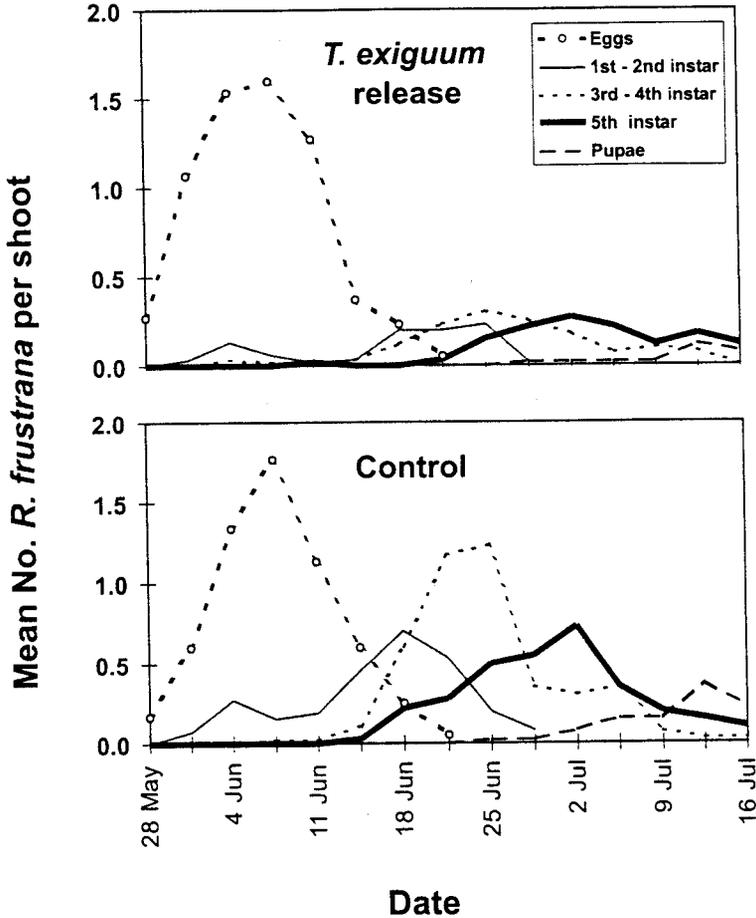


FIGURE 2. Mean number of *Rhyacionia frustrana* eggs, larvae, and pupae per shoot in *Trichogramma exiguum* release plots and control plots, Bertie County, North Carolina, United States, 1998.

TABLE 2. Mean \pm SD percentage of *Pinus taeda* shoots attacked by *Rhyacionia frustrana* and length of tunneling in shoots occurring on different parts of trees in each block, Bertie County, North Carolina, United States, 1998.

Treatment	% of shoots attacked			Length of tunneling (cm)	
	Terminal	Top whorl	Remainder	Terminal	Top whorl
<i>Trichogramma exiguum</i>	31 \pm 16a	30 \pm 4a	33 \pm 4a	2.3 \pm 1.3a	1.8 \pm 0.3a
Control	45 \pm 10a	49 \pm 7b	53 \pm 3b	3.5 \pm 2.5b	2.1 \pm 0.2a

NOTE: Fifty trees were sampled in each plot; data presented are averaged over blocks. Means within a column followed by a different letter are significantly different (ANOVA, $P < 0.05$).

had the most moderate temperature conditions. During the second release period (12–19 June) temperature conditions were more moderate due to cloudy weather and the soil surface with no cover had a significantly greater number of hours at or above 35°C or greater when compared with all other microhabitats.

Parasitoid emergence (all microhabitats combined) was reduced in response to increasing number of consecutive hours at or above 35°C ($r_s = -0.673$, $P = 0.0001$) in the first release period, but was not reduced in the second period ($r_s = -0.191$, $P = 0.23$)

TABLE 3. Mean \pm SD microhabitat temperatures, percent emergence, and percent predation of encapsulated *Trichogramma exiguum* exposed to various microhabitats within *Pinus taeda* plantations, Bertie County, North Carolina, United States, 1998.

Microhabitat description	Mean temperature (\pm SD)		Successive No. of hours per day > 35°C	% emergence of <i>T. exiguum</i>	% predation of <i>T. exiguum</i>
	Maximum	Minimum			
	Exposure period 29 May - 5 June				
First-year trees, soil surface, no cover	39.6 \pm 4.2	17.8 \pm 0.9	5.2 \pm 3.0c	47.8 \pm 37.3a	0.0 \pm 0.0a
First-year trees, soil surface, herbaceous cover	34.5 \pm 3.3	18.6 \pm 0.8	1.5 \pm 2.4b	68.3 \pm 31.5ab	16.7 \pm 32.6a
First-year 0.4-m trees, canopy	34.1 \pm 1.8	18.0 \pm 0.9	0.9 \pm 1.0b	90.5 \pm 11.3b	25.0 \pm 33.7ab
First-year 0.9-m trees, canopy	33.4 \pm 1.4	18.3 \pm 0.1	0.3 \pm 0.3a	95.0 \pm 6.9b	8.3 \pm 19.5a
Second-year 1.8-m trees, soil surface, herbaceous cover	35.0 \pm 4.9	18.5 \pm 1.1	1.6 \pm 2.5b	98.2 \pm 2.5*	55.0 \pm 49.7b
Second-year 1.8-m trees, canopy	33.0 \pm 1.0	18.1 \pm 1.0	0.4 \pm 0.6a	94.2 \pm 6.7b	4.2 \pm 14.4a
	Exposure period 12-19 June				
First-year trees, soil surface, no cover	38.6 \pm 3.7	20.4 \pm 0.8	3.9 \pm 2.5b	82.6 \pm 31.5a	8.3 \pm 19.5a
First-year trees, soil surface, herbaceous cover	34.5 \pm 3.1	20.7 \pm 0.8	1.0 \pm 1.9a	91.8 \pm 9.1a	16.7 \pm 24.6a
First-year 0.4-m trees, canopy	33.7 \pm 1.3	20.3 \pm 0.9	0.4 \pm 0.6a	90.7 \pm 12.8a	16.7 \pm 32.6a
First-year 0.9-m trees, canopy	32.9 \pm 1.1	20.5 \pm 0.8	0.1 \pm 0.2a	89.9 \pm 11.5a	4.2 \pm 14.4a
Second-year 1.8-m trees, soil surface, herbaceous cover	33.8 \pm 3.1	20.8 \pm 0.8	0.8 \pm 1.0a	78.6 \pm 28.9*	58.3 \pm 46.9b
Second-year 1.8-m trees, canopy	33.2 \pm 1.0	20.4 \pm 0.9	0.1 \pm 0.2a	97.2 \pm 4.5a	22.7 \pm 34.4ab

NOTE: Within a given date, means within a column followed by a different letter are significantly different (LS means, $P \leq 0.05$).

* Heavy predation resulted in too many missing data points for PROC GLM to estimate a comparative value.

TABLE 4. Mean \pm SD emergence and predation of *Trichogramma exiguum* following varying periods of exposure to field conditions within *Pinus taeda* plantations, Bertie County, North Carolina, United States, 1998.

No. of days <i>T. exiguum</i> exposed to field conditions prior to emergence	% emergence of <i>T. exiguum</i>	% predation of <i>T. exiguum</i>
Exposure period 29 May – 5 June		
2	86.4 \pm 24.5a	10.4 \pm 25.4a
4	79.1 \pm 26.8a	4.4 \pm 20.9a
6	75.0 \pm 34.2a	37.0 \pm 40.5b
Exposure period 12–19 June		
2	89.6 \pm 21.4a	13.0 \pm 31.0a
4	92.5 \pm 15.7a	22.9 \pm 36.1ab
6	85.1 \pm 18.1a	27.1 \pm 36.1b

NOTE: Within a given date, means within a column followed by a different letter are significantly different (LS means, $P \leq 0.05$).

(Table 3). Predation of parasitized *E. kuehniella* eggs within capsules was noted during the course of this study and recorded. Ants of the genus *Crematogaster* Lund (Hymenoptera: Formicidae) appeared to be responsible for this predation, since they were the only potential predators found inside capsules from which predation was noted. The ants apparently gained entry to the capsules by chewing the perimeter of and widening the holes that are punched in capsules during production to allow *Trichogramma* spp. escape in the field. Predation was affected by microhabitat ($F_{5,18} = 3.30$, $P = 0.027$); the soil surface in second-year plantations with herbaceous ground cover had significantly more predation than the other microhabitats [least squared (LS) means, $P \leq 0.05$] (Table 3). The more time capsules were in the field before *T. exiguum* emergence (i.e., cohort number), the higher predation was ($F_{2,69} = 16.3$, $P < 0.001$), but not emergence ($F_{2,58} = 2.71$, $P = 0.075$). Capsules in the third cohort which had been in the field 6 d prior to parasitoid emergence had higher predation than those exposed for 2 or 4 d (LS means, $P \leq 0.05$) (Table 4).

Discussion

Parasitism of *R. frustrana* eggs in release plots was increased 29% compared with control plots in this study. Although several *Trichogramma* species (*T. exiguum*, *T. marthae*, *T. pretiosum*, and *T. minutum*) were collected from parasitized eggs in control plots, only *T. exiguum* was collected from release plots, substantiating that the increase in parasitism was the result of our releases. Parasitism in our control plots was 42% (Table 2) and in line with the 43.3% parasitism of summer-generation *R. frustrana* eggs by resident populations of *T. exiguum*, *T. pretiosum*, and *T. marthae* in Georgia pine plantations reported by McCravy and Berisford (1998).

Population levels of *R. frustrana* were high in all our blocks (see Fig. 2), presenting a challenging environment in which to test augmentation of natural enemies for suppression of this pest. If conducted properly, the degree of success of *Trichogramma* spp. releases depends on the degree to which egg parasitism and larval mortality are density dependent (Smith 1996). We did not examine whether egg parasitism was density dependent in this study, but in apple orchards parasitism by resident populations of *T. exiguum* and *T. minutum* was not density dependent (Shetty 2000). In *T. exiguum*-treated plots in our study the percent decrease in egg survival and the percent reduction

in neonate and fifth-instar larval numbers all had approximately the same absolute value (46, 65, and 57%, respectively). This suggests there was no compensatory mortality in the larval stage during this study. This is an important consideration because compensatory mortality in larval stages followed *Trichogramma* spp. releases in rice (van Hamburg and Hassell 1984) and *T. exiguum* releases in cotton (Suh *et al.* 1998). Andow *et al.* (1995) found a direct positive relationship between egg mortality caused by *Trichogramma nubilale* Ertle and Davis and larval mortality of European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera Pyralidae), in corn. Although there are few field studies addressing this concern, the variability in results suggests that the issue of compensatory mortality should be addressed in each system in which populations of *Trichogramma* spp. are to be augmented.

Considerable reductions in larval *R. frustrana* populations were noted in this study, although the percent infestation of top whorl shoots was not as greatly reduced. This reflects the fact that there were multiple larvae in infested shoots in control plots, and therefore a reduction in larval numbers did not translate into a proportional reduction in the number of infested shoots. The significant reduction in larval densities and length of tunneling within shoots, plus the apparent lack of compensatory mortality, suggests that *T. exiguum* releases may have potential for suppression of *R. frustrana*.

There are a variety of questions that need to be addressed before an operational system could be implemented. For example, release plots in this study were each 0.4 ha and were surrounded by large untreated portions of the study plantations. Releasing *Trichogramma* on a much larger scale, for example on an entire plantation, would allow an evaluation of whether *R. frustrana* suppression can be improved by area-wide treatment effects. Such area-wide effects have been demonstrated for other biologically based pest management tactics such as mating disruption with semiochemicals (Niwa *et al.* 1988; Cardé and Minks 1995).

An examination of temperatures and *T. exiguum* emergence within various microhabitats of pine plantations in this study revealed significant differences in survival of *T. exiguum* in the different microhabitats. The canopy of both first- and second-year trees was the most suitable microclimate for *T. exiguum* emergence. Distributing capsules into tree canopies would not be a practical way to release *Trichogramma* spp. in large pine plantations. The most acceptable means of distribution would likely be broadcast application by helicopter. Capsules distributed in this manner would probably end up on the soil surface. Where the soil surface was bare in our study, *T. exiguum* capsules distributed on the soil surface faced considerable reductions in emergence.

Combining several cohorts of *Trichogramma* spp. into a single release, as in this study, extends the emergence period and the length of time a single application can potentially suppress *R. frustrana* populations. We found that the longer capsules remained in the field prior to adult emergence, the lower the emergence. Future considerations for optimizing the success of *Trichogramma* spp. release methodology in pine plantations should include a consideration of the possible selection of high temperature tolerant strains, and an examination of various types of vegetational management on microhabitat and *Trichogramma* spp. emergence.

Apparent ant predation in this study reduced the number of *T. exiguum* available for emergence in field plots below originally planned rates. Although the potential impediment of predation has been noted in other *Trichogramma* spp. release projects (Smith 1996), none utilized encapsulated material as in this study. Significant predation was not observed in cotton or apples following release of encapsulated *T. exiguum* (Suh *et al.* 1998; Shetty 2000), nor in corn when encapsulated *Trichogramma brassicae* Bezd. (Hymenoptera: Trichogrammatidae) were released (Kabiri *et al.* 1990; Orr 1993). Predation in our study may have been exacerbated by the clustering of capsules in cups or on the ground as part of our experimental design. Encapsulated *Trichogramma* are

usually broadcast, resulting in a uniform rather than clumped distribution. It would be of value to assess broadcast application of capsules on the ground versus clumped distribution (as in this study) as a method of reducing predation.

To date, a relationship between vegetational diversity in southeastern pine plantations and natural enemy activity has not been demonstrated (McCrahy 1998). Egg parasitoid longevity and parasitism of target pests can be substantially increased by the presence of nectar-bearing plants and (or) honeydew in some agroecosystems (Orr 1988). In our study we found that ground cover and its effect on microclimate significantly influenced emergence of released parasitoids. Consideration of pine plantation management practices, their effect on ground cover, and the subsequent impact on *Trichogramma* releases may lead to improved *Trichogramma* spp. performance, and overall control of *R. frustrana*.

Our study is the first to consider the augmentation of *Trichogramma* spp. populations for *R. frustrana* suppression. We demonstrated that reduction of *R. frustrana* populations by mass releases of *T. exiguum* was feasible under our experimental conditions. Additional studies will be required to evaluate the potential of this technology from an operational perspective.

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