THE POTENTIAL FOR *TRICHOGRAMMA* RELEASES TO SUPPRESS TIP MOTH POPULATIONS IN PINE PLANTATIONS

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Abstract—Because the Nantucket pine tip moth is a native pest, augmentation (mass-release) of native natural enemies may be the most promising method of tip moth biocontrol. The tip moth has several important egg, larval, and pupal parasitoids. Egg parasitoids are most effective as biocontrol agents because they eliminate the host before it reaches a damaging stage. *Trichogramma* egg parasitoids are the most important of these, and are the most commonly augmented arthropod in biocontrol programs worldwide. Inundative releases of encapsulated *Trichogramma exiguum* Pinto & Platner were evaluated for suppression of *R. frustrana* in first year loblolly pine, *Pinus taeda* L., plantations. Three releases of 328,238 ± 88,379 ♀/ha, spaced 7 d apart, were made in three 0.4 ha plots during second generation *R. frustrana* egg deposition. The quality of each release was very high in comparison with published values of biological characteristics of *T. exiguum*. Parasitism of *R. frustrana* eggs was significantly increased by 29 percent when compared to check plots, egg survival (hatching) was significantly reduced by 47 percent, and larval populations were significantly reduced 60 percent. There was no significant difference in the percentage of terminals damaged between *T. exiguum*-treated (31 ± 16 percent) and control plots (45 ± 10 percent), 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 damage. Microhabitat significantly affected the number of consecutive hours per day that were 35°C or above (critical temperature for preimaginal *T. exiguum* survival). Soil surface with no cover had the greatest number of hours 35°C or greater, 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 35°C or greater. Predation of *T. exiguum* prior to emergence, though relatively minor, was significantly affected by microhabitat and by the length of time capsules were in the field before emergence (i.e. cohort number). The reduction of *R. frustrana* populations by mass-release of *Trichogramma* is technically feasible. In order to make these releases practical, however, we suggest several considerations for future research. Large scale *Trichogramma* releases could be studied to evaluate possible area-wide effects and residual effects over multiple tip moth generations. Combining several releases of *Trichogramma* into a single application may be accomplished by a single release of multiple cohorts timed to emerge over a 7–8 day period. The efficacy of such an approach should be studied. Consideration might be made of plantation management practices that result in adequate vegetational cover for survival of encapsulated *Trichogramma* broadcast on the soil surface, but do not interfere with tree growth. Though unlikely with a native parasitoid, the potential for non-target impacts should be considered in any future evaluations.

INTRODUCTION

The purpose of this paper is to examine the potential for using biological control as a practical pest management tool for *R. frustrana* in southeastern pine plantations. We briefly review the literature on naturally occurring parasitoids and predators of *R. frustrana*, and the use of one parasitoid group, *Trichogramma*, in pest management. We also summarize data from a study demonstrating the efficacy of *Trichogramma* releases and the suitability of microclimate in pine plantations for *Trichogramma* survival. Finally, we include a discussion of the research questions that should be addressed in determining the potential for *Trichogramma* releases to suppress *R. frustrana* in commercial pine plantations.

Biological Control and *R. frustrana*

There are three general approaches to implementing biological control in pest management systems. The first, importation (or classical) biological control involves importing effective natural enemies of exotic pests from the pests' homeland. However, *R. frustrana* is a native pest, so the classical approach does not apply to it. The so-called "neo-classical" approach, in which natural enemies that attack related pests in other geographic locations are sought (Hokkanen and Pimentel 1989), might be considered. However, the neoclassical approach is considered ecologically risky and is frowned upon by some in the conservation community (Simberloff and Stiling 1996).

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Citation for proceedings: Berisford, C. Wayne; Grosman, Donald M., eds. 2002. The Nantucket pine tip moth: old problems, new research. Proceedings of an informal conference, the Entomological Society of America, annual meeting. 1999 December 12–16. Gen. Tech. Rep. SRS–51. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station. 68 p.

Conservation biological control involves remediation of factors in systems that limit the effectiveness of natural enemies. This can involve activities as diverse as modifying pesticide use practices or the creation of additional habitat for natural enemies. The potential for conservation biological control of *R. frustrana* through pesticide use modification appears low because of the limited range of insecticide products available and the narrow and sometimes unpredictable timing window for their application (Fettig and Berisford 1999, McCravy 1998). In addition, resident populations of natural enemies do not adequately suppress *R. frustrana* populations on their own (McCravy 1998). The use of habitat manipulation to increase natural enemy effectiveness may also be difficult. The intensive site preparation and early chemical weed control employed to increase survival and growth rate of seedling pines has apparently resulted in higher tip moth densities. The levels of associated vegetation in pine plantations has been shown to be a factor that predisposes stands to tip moth damage (Warren 1963, Berisford and Kulman 1967, Ross and others 1990). Berisford (1988) suggested that this relationship might be due to variation in the availability of alternate hosts or pollen and nectar sources for natural enemies. However, McCravy (1998) demonstrated no relationship between parasitism levels of *R. frustrana* and vegetational diversity.

Augmentation biological control involves augmenting populations of natural enemies not present in high enough numbers to be effective, by means of releasing lab reared enemies. This approach has been evaluated by Orr and others (2000), and has been shown to be technically feasible for *R. frustrana* suppression in a study presented below.

Natural Enemies of *R. frustrana* in Pine Plantations

The concealed nature of *R. frustrana* (i.e. small larvae mine in needles and larger larvae tunnel into shoots) means that larvae may not be as susceptible to predation, possibly reducing the role predators play in natural regulation of tip moth populations. However, there is a rich complex of over 60 parasitoid species that attack *R. frustrana* eggs, larvae, and pupae (Frank and Foltz 1997). Some of the parasitoids attacking *R. frustrana* may be important in regulating populations of this pest (Berisford 1988).

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

McCravy (1998) found overall pine tip moth larval and pupal parasitism to be 44.8 percent on the Georgia coastal plain, and Freeman and Berisford (1979) found 42 percent parasitism on the Georgia piedmont. Eikenbary and Fox (1965) reported tip moth parasitism to 41 percent and 26 percent on the South Carolina coastal plain and piedmont, respectively. The species primarily responsible for tip moth parasitism are the tachinid fly *Lixophaga mediocris* Aldrich, the ichneumonid wasp *Campoplex frustranae* Cushman, the eurytomid wasp *Eurytome pini* Bugbee, and the eulophid wasp *Hyssopus rhyacioniae* Gahan (McCravy 1998).

As indicated above, 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. Since pupal mortality occurs after the trees are already damaged, it seems logical to focus on the egg stage as a target and *Trichogramma* wasps as a natural enemy for augmentation.

Use of *Trichogramma* in Augmentation Biological Control

Parasitic wasps in the genus *Trichogramma* are the most commonly employed arthropod in augmentation biological control programs. 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). *Trichogramma* releases are currently used to treat an estimated 32 million hectares annually for control of agricultural and forest pests (Li 1994), and are being studied in over 50 countries worldwide (Smith 1996). Of the approximately 180 species of *Trichogramma* known, over 70 have been employed as biocontrol agents, and 20 are commonly mass reared and released in augmentation programs (Li 1994, Pinto 1998). Individuals to be mass reared are usually collected near the planned release site to ensure that the *Trichogramma* are adapted to that area (Smith 1996).

Trichogramma are exclusively egg parasitoids seldom exceeding 0.7 mm in length (Pinto 1998). They have been recorded from hosts in seven insect orders, but are predominantly parasitoids of lepidoptera (Pinto and Stouthamer 1994). *Trichogramma* have a worldwide distribution and comprise the largest genus within the family Trichogrammatidae (Pinto 1998).

Augmentation biocontrol with *Trichogramma* can take the form of inundative or inoculative releases (Li 1994). Inundative releases require the mass rearing and release of many individuals to combat an existing pest problem. Inoculative releases involve fewer individuals released early in the cropping season to prevent pests from reaching damaging levels. Many *Trichogramma* release programs are actually a hybrid of these two release approaches. Parasitoids are released inundatively only at the beginning of the oviposition period of the targeted pest with the expectation that their progeny will continue to provide pest suppression through the remainder of the oviposition period. Reducing the number of releases required for a single generation of a pest makes this hybrid approach more economical.

FEASIBILITY OF *TRICHOGRAMMA* RELEASES FOR *R. FRUSTRANA* SUPPRESSION.

A study conducted in North Carolina in 1998 evaluated the feasibility of using inundative releases of *T. exiguum* for suppression of second generation *R. frustrana* in loblolly pine, *P. taeda* L., plantations. This was the first study to consider the augmentation of natural enemy populations for *R. frustrana* control.

Study Design and Plot Management

The experiment was set up using a complete block design, with three field locations (each with a treatment and a control plot) acting as three blocks. Two field locations were near Connarista, North Carolina, and a third was near Aulander, in Bertie County, North Carolina. The treatment was *T. exiguum* release; controls were not treated with *T. exiguum*, and insecticides were not used on any of the plots. Within each location, the *Trichogramma* release plot was at least 400 m downwind (based on prevailing wind direction for that area) from the control plot to reduce possibility of parasitoid dispersal into control plots. Plots were approximately 0.4 ha each.

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. Field locations 1 and 2 were planted on 23–27 January, 1997, and field location 3 on 31 Jan – 1 Feb, 1997. All three locations received the following herbicide treatment per ha: May, 97, 946 ml Velpar L® (hexazinone) and 89 ml Oust® (sulfoneturon) (banded on rows); June, 97, 118 ml Arsenal® (imazapyr) (broadcast), 0.74 kg Velpar DF® and 89 ml Oust® (broadcast).

Data Analysis

Tip moth 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, Cary, NC, USA, 1996). Hatch and parasitism data were subjected to logistic regression analysis (PROC GENMOD with PSCALE option and likelihood ratio tests, SAS Institute, Cary, NC, USA, 1996). Temperature, parasitoid emergence, and predation data were analyzed using analysis of variance (PROC GLM, SAS Institute, Cary, NC, USA, 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, Cary, NC, USA, 1996).

R. frustrana Population Sampling

At each of the three field locations, *R. frustrana* egg density was estimated in release and control plots by clipping the upper 22–25 cm section of 20 sub-terminal shoots (1 shoot per tree) randomly selected from the central 50 percent 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. Total number of parasitized and viable eggs were recorded for each plot. Shoots were collected and examined every 3–4 d beginning 29 May.

Second generation *R. frustrana* eggs were first detected between 29 May and 1 June at all three field locations (data for all release plots combined presented in fig. 1). At field site 1, at peak oviposition *R. frustrana* egg density in both *T. exiguum*-treated and control plots was 2.2 eggs per shoot, with both peaks occurring on 8 June. At field site 2, peak oviposition in the *T. exiguum*-treated plot was 1.7 eggs per shoot (5 June) and 2.3 eggs per shoot (8 June) in the control plot. At field site 3, peak oviposition in the *T. exiguum*-treated plot was 1.0 eggs per shoot (8 June) and 0.8 eggs per shoot (8 June) in the control plot. Overall, there was no significant difference in egg numbers (df=1,14, F=0.64, P>0.05) between *T. exiguum*-treated and control plots, therefore data from the three fields sites were combined.

Source, Release Rates, and Quality of *Trichogramma*

Proper selection of species, biotypes or ecotypes has been shown to be crucial for successful *Trichogramma* augmentation (Kot 1979; Voronin and Grinberg 1981; Pak 1988). McCravy and Berisford (1998) reported that two species responsible for *R. frustrana* parasitism were *T. pretiosum* and *T. exiguum*. We selected *T. exiguum* because prior experience had indicated this was a vigorous species that retained very high quality under long-term mass rearing (Suh and others 1998). To ensure we had a strain that was well adapted to the area of intended release, we collected material for our culture from a site near Plymouth, North Carolina, within 50 km of release sites.

Trichogramma exiguum were reared from parasitized sentinel corn earworm, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) eggs placed in woodlots adjacent to agricultural fields near Plymouth, 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 (approx. 5 cm^3) each containing an average of 1319 ± 140 *T. exiguum* developing inside Mediterranean flour moth, *Ephestia kuehniella* Zeller, eggs. Four small holes made during the encapsulation process were just large enough for adults to escape but small enough to prevent most predators from entering the capsules.

Shipments from BIOTOP to NCSU were made weekly, via commercial air freight. Each of three weekly shipments (beginning 25 May) consisted of three cohorts of parasitoids whose development was staggered approximately 25 Celsius degree days apart. For each shipment, a HOBO XT® Temperature Logger (Onset Computer Corp., 536 MacArthur Blvd., Box 3450, Pocasset, MA 02559-3450), 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.

Three releases (R1 - R3), spaced 7 d apart, were made in each release plot beginning 29 May (fig. 1). Each release contained three cohorts of capsules differing in *T. exiguum* development. One set of capsules contained *T. exiguum* expected to emerge within 12–24 h, the second within 60–

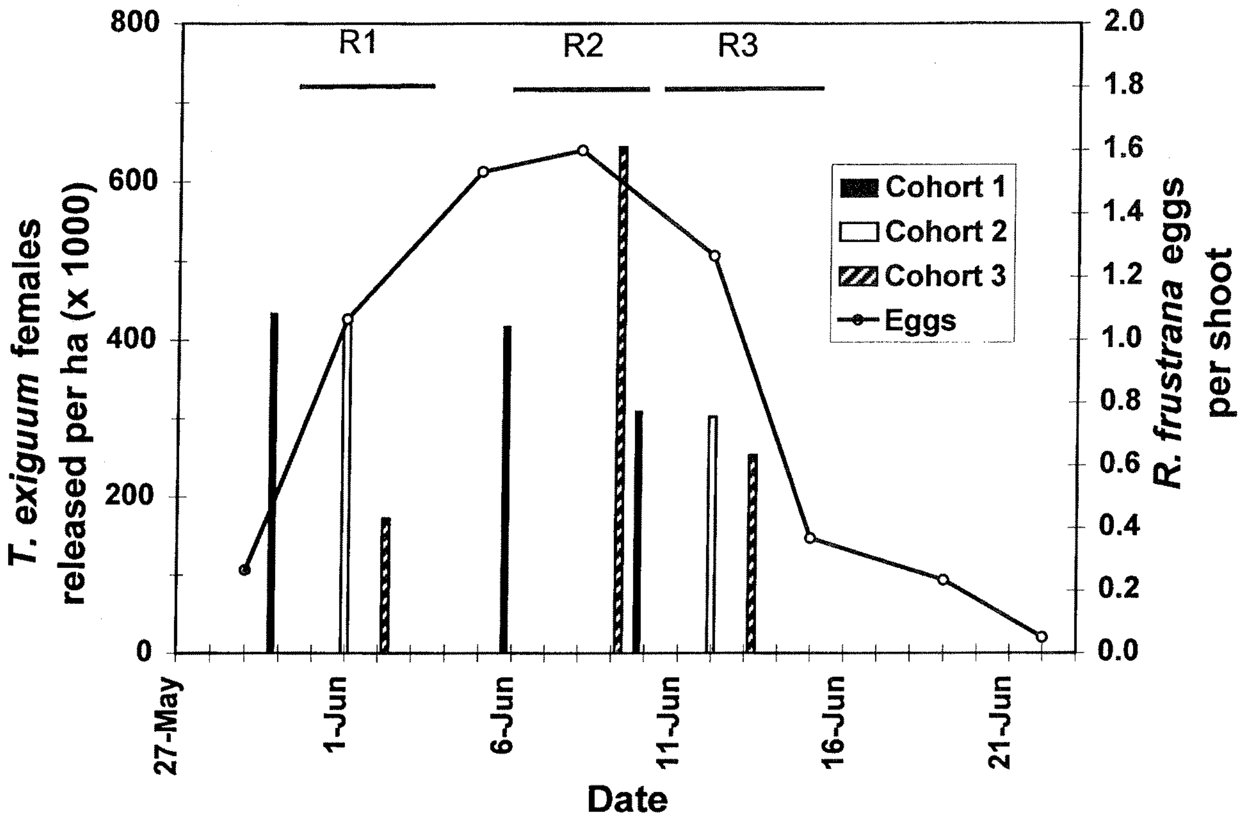

Figure 1—Mean *Rhyacionia frustrana* eggs per shoot and mean number of *Trichogramma exiguum* released with date of peak emergence for each cohort (three releases R1 – R3, three cohorts each). Data presented are from all release plots combined. Bertie County, NC, USA, 1998.

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 to 0.5 m above the soil surface in the tree canopy, into which capsules were placed. Cups were spaced approx. 5.5 m apart within rows and 7 m apart across rows. *T. exiguum* were released at a rate of 328,238 ± 88,379 females/ha/ cohort/release (determined from data presented below and in fig. 1).

Stringent quality control protocols were followed once material arrived in North Carolina to ensure that only high quality *T. exiguum* were utilized in this study. Poor quality of released natural enemies can lead to unsatisfactory pest suppression and unpredictable results (Hoy and others 1991).

Samples of five capsules per *T. exiguum* cohort were frozen immediately upon receipt to determine production emergence (i.e. percentage of parasitized eggs added to fresh eggs during production). Emergence percentages from all subsequent samples were estimated by counting emergence holes in 50 black (parasitized) eggs/capsule, and subtracting production emergence. *Trichogramma exiguum* adults from a second sample of 10 capsules per cohort were allowed to emerge over a seven day period at 25° C, 80 percent RH, and a 16L:8D photoperiod after which capsules were frozen. For each of the 10 individual capsules, emergence was estimated, 30 adults were randomly selected to estimate sex ratio, and 10 females were randomly selected to estimate percent brachyptery. A third sample of 5 capsules per cohort was used to estimate female longevity according to procedures described by Cerutti and Bigler (1991).

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 (blackened) eggs per capsule was counted. To assess field emergence (i.e. timing of releases), 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 tree canopy, adjacent to a release plot. Five capsules from each cohort were collected on the day of release (day 0), 3, 5, 7, 10, and 11 d after each release, and frozen until they could be sampled.

Mean (± SD) *T. exiguum* emergence under laboratory conditions for released cohorts was 96 ± 2 percent, consisting of 74 ± 3 percent females, of which 1 ± 1 percent displayed brachyptery. Female longevity for released cohorts averaged 18 ± 3 d. In comparison, the mean life span of adult female *T. exiguum* reported in other laboratory studies ranged from 4.1 to 8.6 d (Harrison and others 1985, Ram and Irulandi 1989). Mean emergence of parasitoids under field conditions was 96 ± 4 percent. A release rate of 328,238 ± 88,379 females/ha/cohort/release was estimated after taking into account the number of parasitized *E. kuehniella* eggs per capsule, percent 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 to 4 days throughout the peak oviposition period of *R. frustrana* (fig. 1).

Parasitism of *R. frustrana* Eggs

The level of egg parasitism in *T. exiguum* release and check plots was measured on 1, 5, 8, 12, and 15 June. 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 premoistened filter paper (Qualitative P5, 5.5 cm diam.) within petri dishes (6 cm diam.) and held at 25°C, 80 percent humidity, and a 16L:8D photoperiod for 7–8 d at which time eggs were classified as either hatched, black-head stage, non-viable, or parasitized. A percentage was calculated for each category.

Parasitism of *R. frustrana* eggs collected throughout the study period was significantly increased by 29 percent ($F_{1,10}$=8.30, P=0.016) and egg hatch was significantly reduced by 47 percent ($F_{1,8}$=62.0, P<0.001) in *T. exiguum*-treated plots (combined) compared with control plots (combined) (table 1). The species responsible for parasitism in *T. exiguum*-release plots were *T. exiguum* (100 percent of parasitized eggs collected), and in control plots *T. exiguum* (92.4 percent), *T. marthae* (3.8 percent), *T. pretiosum* (1.9 percent), and *T. minutum* (1.9 percent). Parasitism in control plots was approximately 42 percent (table 1) which seems relatively high, but is in line with the 43.3 percent 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).

Larval infestation

During shoot examinations for eggs, numbers of instar 1–2, 3–4, 5 larvae (determined by head capsule size; see Fox and others 1972), and pupae found in shoots and needles were recorded for each release and control plot. The number of larvae in instar categories 1–2, 3–4, and 5 per shoot was significantly lower in *T. exiguum*-treated plots compared to control plots (df=1,14, F=51.41, P=0.0001; df=1,14, F=16.79, P=0.0004; df=1,14, F=26.02, P=0.0001), with a mean (± SD) reduction in *T. exiguum*-treated plots of 65 ± 8 percent, 63 ± 10 percent, and 57 ± 4 percent, respectively (fig. 2). The number of pupae per shoot was also significantly (df=1,14, F=6.91, P=0.02) reduced by 62 ± 30 percent in treated plots.

Population levels of *R. frustrana* were high in all plots used in this study (see fig. 2), presenting a challenging environment in which to test augmentation of natural enemies for suppression of this pest. The percentage decrease in egg survival, and the percentage reduction in neonate and fifth instar larval numbers all had approximately the same absolute value (47 percent, 65 percent and 57 percent, respectively). This suggests there was no compensatory mortality in the larval stage during this study. This is an important consideration since compensatory mortality in larval stages followed *Trichogramma* releases in rice (Van Hamburg and Hassell 1984) and cotton (Suh and others 1998). Andow and others (1995) found a direct relationship between egg mortality and larval mortality in corn. Although there are few studies addressing this concern, the variability in results suggests that the issue of compensatory mortality should be addressed in each system in which *Trichogramma* populations are to be augmented.

Damage to trees

The number of mined needles and percentage of shoots damaged was also recorded while shoots were examined for eggs. 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 percent 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 measured with a ruler.

Despite significant reductions in larval numbers, no significant difference in percentages of terminal damage

Table 1—Mean ± SD percent parasitism and egg hatch of second generation *Rhyacionia frustrana* eggs collected from *T. exiguum*-treated and control plots in *Pinus taeda* plantations, June 1–15, 1998 in Bertie County, NC

		Percentage of *R. frustrana* eggs which were:		
Plot type	n	Parasitized	Hatched	Non-viable
T. exiguum release	287	53.7 ± 12.2 a	22 ± 4 b	24 ± 10 a
Control	241	41.6 ± 20.8 b	41 ± 6 a	17 ± 13 a

Values within a column followed by different letters are significantly different (logistic regression, P≤ 0.05).

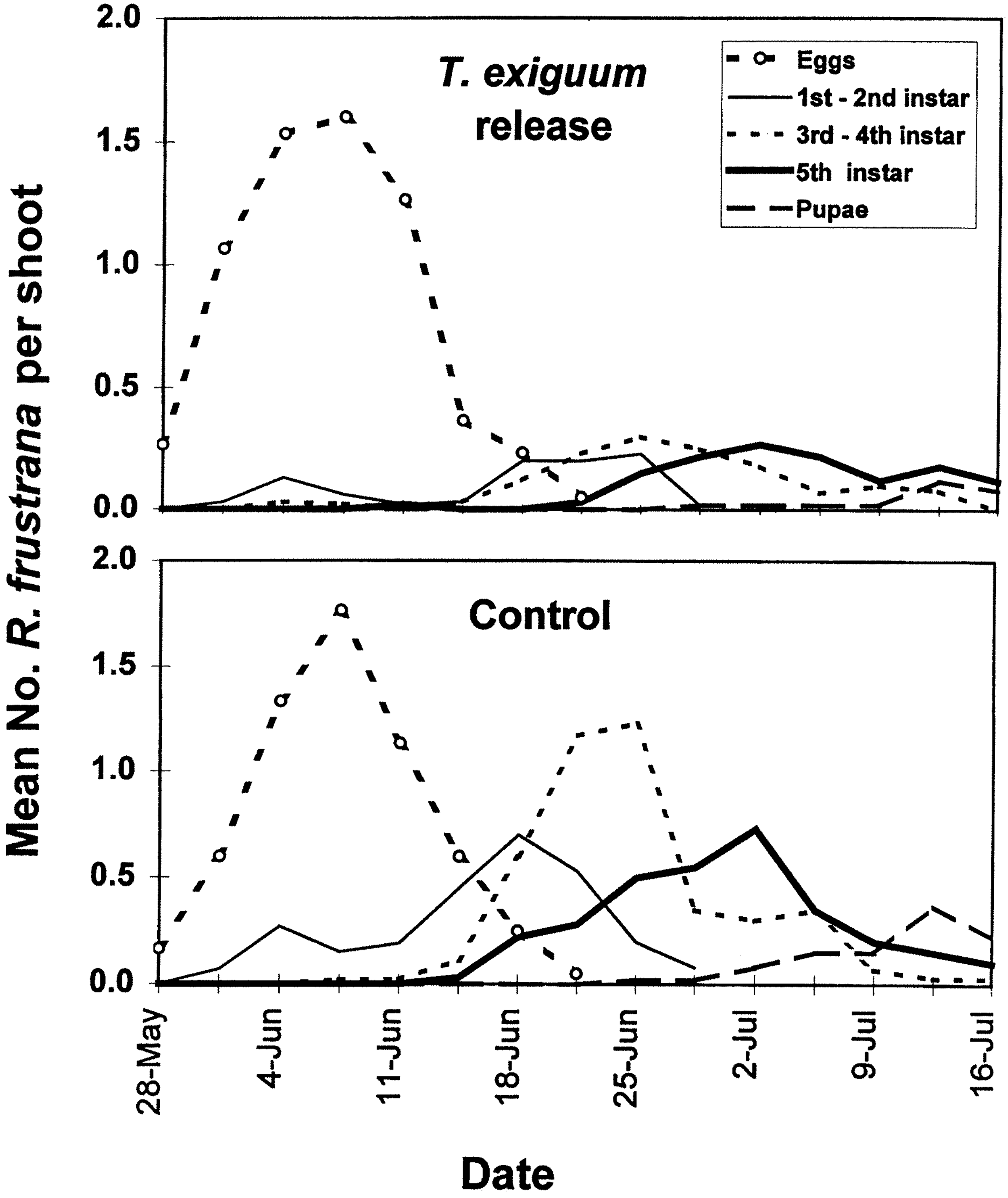

Figure 2—Mean number of *Rhyacionia frustrana* eggs, larvae, and pupae per shoot in *Trichogramma exiguum* release plots and control plots. Bertie County, NC, USA, 1998.

(df=1,2, F=1.28, P=0.376) was found (table 2). However, there was a significant difference in the length of tunneling damage within terminals (df=1,2, F=5.69, P=0.019). No significant difference was found between release and control plots in the number of top whorl shoots (df=1,2, F=1.04, P=0.416), but there was a significant difference in number of top whorl shoots that were attacked (df=1,2, F=28.36, P=0.034). However, there were no significant differences in the length of tunneling within top whorl shoots (df=1,2, F=1.70, P=0.322). There were no significant differences between release and control plots in the number of shoots below the top whorl (df=1,2, F=0.28, P=0.651), but the

Table 2—Mean ± SD percent of *Pinus taeda* shoots attacked by second generation *Rhyacionia frustrana* and length of tunneling in shoots occurring on different parts of trees[a] at three field sites. Bertie Co., NC, USA, 1998

	Shoots attacked on			Length of tunneling	
Treatment	Terminal	Top whorl	Remainder of tree	Terminal	Top whorl
	- - - - - - - - - - - - - *Percent* - - - - - - - - - - - - -			- - - - - - - - - - *Cm* - - - - - - - - - -	
T. exiguum release	31 ± 16	30 ± 4 a	33 ± 4 a	2.3 ± 1.3 a	1.8 ± 0.3 a
Control	45 ± 10 a	49 ± 7 b	53 ± 3 b	3.5 ± 2.5 b	2.1 ± 0.2 a

Values within a column followed by different letters are significantly different (ANOVA, $P < 0.05$)

[a] Fifty trees were sampled in each plot; data presented are averaged from 150 trees each for the *T. exiguum* release and control plots.

number of these shoots that were attacked was significantly lower in *T. exiguum* plots (df=1,2, F=5.48, P=0.144) (table 2).

Microclimatic effects on *T. exiguum*

In addition to assessing efficacy of *T. exiguum* releases, we also assessed *T. exiguum* application methodology by monitoring temperature, *T. exiguum* emergence, and predation of *T. exiguum* in six distinct microhabitats found within first and second year pine plantations. One-year-old trees used for this study were in field location 1 used in the release study. The two-year-old trees were located adjacent to field location 1, and were planted 1 March, 1996 on the same spacing described in the release study. The stand received the following herbicide treatments: April 96, banded 946 ml Velpar L® and 89 ml Oust®; June 96, broadcast 118 ml Arsenal®; April 97, broadcast 946 ml Velpar L® and 89 ml Oust®; June 97, broadcast 118 ml Arsenal®.

The microhabitats studied were: 1) soil surface with no cover in 1st year plantation; 2); soil surface with herbaceous cover in 1st year plantation 3) canopy (mid-height) of small (0.46 m) 1st year plantation trees; 4) canopy (mid-height) of tall (0.92 m) 1st year plantation trees; 5) canopy (mid-height) of 2nd year plantation trees; 6) soil surface with herbaceous cover in 2nd year plantation. Temperature was monitored in each habitat with a datalogger (Model CR10, Campbell Scientific, Inc., 815 W. 1800N, Logan, Utah 84321-1784) with 24 temperature probes (Model 107 on 100 ft. wire leads) set up in between the stands of 1- and 2-yr-old *P. taeda*. Each temperature probe was completely enclosed within a sheath, constructed from the cardboard used to encapsulate *Trichogramma,* in order to simulate conditions inside capsules. Probes were placed in the six microhabitats, replicated four times (total of 24 sites). 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, and continued through 19 June. The critical temperature at which *T. exiguum* survival is reduced is 35°C (Harrison and others 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 the different microhabitats, two capsules from each cohort for two releases (1st and 3rd) were placed in white nylon mesh bags alongside probes. In the canopy, capsules were placed in cone-shaped waxed 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 quality control section. The first set of capsules was placed in the field on 29 May, and the second set on 12 June.

Microhabitat had a significant influence on the number of consecutive hours per day that were 35°C or above ($F_{5,18}$= 3.34, P = 0.025) (table 3). This value varied significantly between the two releases for which microclimate data were collected ($F_{1,18}$= 9.56, P = 0.006), therefore subsequent analyses were conducted on the two releases separately (table 3). During the first release period (May 29 – June 5) the bare soil surface had the greatest number of hours 35°C or greater, 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 had the most moderate temperature conditions. During the second release period (June 12 – 19) temperature conditions were more moderate due to cloudy weather and the soil surface with no cover had significantly greater number of hours 35°C or greater when compared to all other microhabitats.

Parasitoid emergence (all microhabitats combined) was significantly reduced in response to increasing number of consecutive hours 35°C or above (Pearson's Correlation Coefficient = -0.673, P = 0.0001) in the first release period, but was not significantly reduced in the second period (Pearson's Correlation Coefficient = -0.191, P = 0.23) (table 3).

Table 3—Mean ± SD microhabitat temperatures, and emergence and predation of encapsulated *Trichogramma exiguum* exposed to various microhabitats within *Pinus taeda* plantations. Bertie County, NC, USA, 1998

Exposure period	Microhabitat description	Successive hrs (± SD) above lethal temperature (35° C) per day	T. exiguu emergence	Predation of T. exiguum
			Percent	*Percent*
May 29–June 5	1st yr. trees, soil surface, no cover	5.2 ± 3.0 c	47.8 ± 37.3 a	0.0 ± 0.0 a
	1st yr. trees, soil surface, herbaceous cover	1.5 ± 2.4 b	68.3 ± 31.5 ab	16.7 ± 32.6 a
	1st yr. 0.4 m trees, canopy	0.9 ± 1.0 b	90.5 ± 11.3 b	25.0 ± 33.7 ab
	1st yr. 0.9 m trees, canopy	0.3 ± 0.3 a	95.0 ± 6.9 b	8.3 ± 19.5 a
	2nd yr. 1.8 m trees, soil surface, herbaceous cover	1.6 ± 2.5 b	98.2 ± 2.5[a]	55.0 ± 49.7 b
	2nd yr. 1.8 m trees, canopy	0.4± 0.6 a	94.2 ± 6.7 b	4.2 ± 14.4 a
June 12–19	1st yr. trees, soil surface, no cover	3.9 ± 2.5 b	82.6 ± 31.5 a	8.3 ± 19.5 a
	1st yr. trees, soil surface, herbaceous cover	1.0 ± 1.9 a	91.8 ± 9.1 a	16.7 ± 24.6 a
	1st yr. 0.4 m trees, canopy	0.4 ± 0.6 a	90.7 ± 12.8 a	16.7 ± 32.6 a
	1st yr. 0.9 m trees, canopy	0.1 ± 0.2 a	89.9 ± 11.5 a	4.2 ± 14.4 a
	2nd yr. 1.8 m trees, soil surface, herbaceous cover	0.8 ± 1.0 a	78.6 ± 28.9[a]	58.3 ± 46.9 b
	2nd yr. 1.8 m trees, canopy	0.1 ± 0.2 a	97.2 ± 4.5 a	22.7 ± 34.4 ab

Within a given date, values within a column followed by different letters are significantly different (LS Means, $P \leq 0.05$).

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

Predation of *T. exiguum* capsules

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

Predation by ants in the genus *Crematogaster* was significantly 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 (LS Means, P £ 0.05) (table 3). The length of time capsules were in the field before *T. exiguum* emergence (i.e. cohort number) also significantly affected predation ($F_{2,69}$= 16.3, P < 0.001), but not emergence ($F_{2,58}$= 2.71, P = 0.075). Capsules in the third cohort that had been in the field 6 d prior to parasitoid emergence had significantly higher predation than those exposed for 2 or 4 d (LS Means, P £ 0.05) (table 4).

Predation in this study reduced the number of *Trichogramma* available for emergence in field plots, thereby reducing the actual numbers of wasps/ha considerably below those originally planned. Ants of the genus *Crematogaster* 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* escape in the field. Although the potential impediment of predation has been noted in other *Trichogramma* 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 and others 1998, Shetty 2000), nor in corn when encapsulated *T. brassicae* were released (Kabiri and others 1990, Orr 1993).

SUMMARY OF FEASIBILITY STUDY

Larval *R. frustrana* populations were reduced considerably by *T. exiguum* in this study. However, the percentage 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 necessarily lead to a proportional reduction in number of infested shoots. There was, however, a significant reduction in the length of damage within shoots. This, plus the apparent lack of compensatory mortality, indicates that *Trichogramma* release for suppression of *R. frustrana* populations is technically feasible. Additional studies will be required to evaluate the potential of this technology to be used on an operational basis. Some possible considerations for these studies are presented below.

Table 4—Mean ± SD emergence and predation of *Trichogramma exiguum* following varying periods of exposure to field conditions within *Pinus taeda* plantations. Bertie Co., NC, USA, 1998

Exposure period	No. days *T. exiguum* exposed to field conditions prior to emergence	*T. exiguum* emergence	Predation of *T. exiguum*
		- - - - - - - - - - Percent	*- - - - - - - - - -*
May 29–June 5	2	86.4 ± 24.5 a	10.4 ± 25.4 a
	4	79.1 ± 26.8 a	4.4 ± 20.9 a
	6	75.0 ± 34.2 a	37.0 ± 40.5 b
June 12–19	2	89.6 ± 21.4 a	13.0 ± 31.0 a
	4	92.5 ± 15.7 a	22.9 ± 36.1 ab
	6	85.1 ± 18.1 a	27.1 ± 36.1 b

Within a given date, values within a column followed by different letters are significantly different (LS Means, P ≤0.05).

FUTURE RESEARCH CONSIDERATIONS.

There are a variety of questions that should be addressed before an operational *Trichogramma* release 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 over multiple generations. Such area-wide effects have been demonstrated for other biologically-based pest management tactics such as mating disruption with semiochemicals (Niwa and others 1988, Cardé and Minks 1995).

An examination of temperatures and *T. exiguum* emergence within various microhabitats of pine plantations in the feasibility 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. However, distributing capsules into tree canopies would not be a practical way to release *Trichogramma* in large pine plantations. Likely, the most acceptable means of distribution would 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, *Trichogramma* capsules distributed on the soil surface faced considerable reductions in emergence. We also found that the longer capsules remained in the field prior to adult emergence, the lower the emergence. This could be especially important since in order to make *Trichogramma* releases practical, only one application (containing several cohorts that would emerge over a 7–8 d period) could be made per *R. frustrana* generation. Future considerations for optimizing the success of *Trichogramma* 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* emergence.

To date, a relationship between vegetational diversity in pine plantations and natural enemy activity has not been demonstrated (McCravy 1998). However, 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 the current 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* performance, and overall control of *R. frustrana*.

The predation of *T. exiguum* by ants in our study may have been due to the high numbers of capsules clustered in cups or on the ground as part of our experimental design. Encapsulated *Trichogramma* are usually broadcast, so that they would have 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.

Another consideration that should be made prior to commercial implementation is the potential for *Trichogramma* releases to have non-target impacts. Historically, biological control has enjoyed a reputation as an environmentally safe alternative to chemical. Recently, however, concern has been raised regarding potential non-target impacts of biocontrol practices (Follett and Duan 1999). Although the focus of most attention is on the introduction of non-native organisms in classical biocontrol programs, the augmentation of native natural enemies may also a concern (Orr and others 1999).

Because non-target impacts are of greatest concern outside the release area, dispersal ability of *Trichogramma* is a key component of potential non-target impacts. Studies on dispersal of *Trichogramma* have demonstrated contradicting results. Most studies indicate that *Trichogramma* dispersal is limited (Bigler and others 1989, McDougall and Mills 1997).

However, Stern and others (1965) demonstrated that individuals could travel up to 1100 m from the release site. Bigler and others (1990) found that levels of parasitism by *Trichogramma evanescens* of *Ostrinia nubialis* eggs placed 400m downwind of the point of parasitoid release were equal to parasitism at the release site. These results led to the conclusion that the effective release area for these parasitoids was at least twice the area of the plot releases were made in.

Host specificity is another key component to understanding potential nontarget impacts. Parasitoid species in the genus *Trichogramma* are considered highly polyphagous, together attacking more than 400 species in 203 genera, 44 families, and 7 orders (Pinto 1998, Bao and Chen 1989). However, individual species tend to have narrow host and habitat preferences or climatic tolerances, making them less likely to cause undesired impacts (Orr and others 1999).

Future studies of *Trichogramma* releases in pine plantations should consider the dispersal of parasitoids, and the potential impact of releases on both predatory and herbivorous species within and downwind of release sites. This could be done by estimating parasitism of selected species' eggs when they are placed within plantations and in surrounding habitats into which *Trichogramma* could potentially disperse.

ACKNOWLEDGMENTS

We thank R S Cameron and M Davidson, Union Camp Corporation, for their assistance in locating the sites for this study. This research was funded in part by the Pine Tip Moth Research Consortium: Champion International Corporation; International Paper Corporation; Smurfit-Stone Container Corporation; Union Camp Corporation; USDA Forest Service, Southern Research Station; The Timber Company; and Weyerhaeuser Company. Helpful comments on an earlier version of this manuscript were provided by F. Hain, F. Hastings, and C Nalepa. We are grateful to F Kabiri and J Frandon (BIOTOP, Valbonne, France) for rearing and formulation of *Trichogramma*, and J Shurtleff (NCDA&CS Quarantine Lab) for handling shipments.

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