

EVALUATION OF *LastCall*[™] NPTM FOR THE CONTROL OF THE NANTUCKET
PINE TIP MOTH

by

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(Under the Direction of C. Wayne Berisford)

ABSTRACT

The Nantucket pine tip moth (*Rhyacionia frustrana*) is a significant pest of pine plantations in the southeastern United States. Current industry practice for controlling tip moth includes backpack application of permethrin insecticides. *LastCall*[™] NPTM is an attracticide encapsulating both pheromone and insecticide components. This technology attempts to address the environmental concerns associated with conventional insecticide sprays. Varied rates of *LastCall*[™] were tested in loblolly pine plantations in the Georgia Coastal Plain to determine optimal application procedures. Applications varied in the area treated and the per acre rate of application. Efficacy of treatments was determined by quantifying shoot damage and pre- and post-treatment tree volume measurements. All treated plots had significantly less damage than control plots with the most intensive treatment consistently yielding the greatest control. Alternative treatments resulted in adequate control but posed additional problems in untreated areas.

INDEX WORDS: Nantucket pine tip moth, *Rhyacionia frustrana*, attracticide, loblolly pine

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INTRODUCTION

The Nantucket pine tip moth, *Rhyacionia frustrana* (Comstock) (Lepidoptera: Tortricidae) is a significant pest of pine plantations throughout the eastern United States. The importance of *R. frustrana* as a pest has been dramatically increased by the increased popularity of intensively managed monoculture plantations, sparking a renewed interest in the management of this pest.

Current management practices center around the application of broad spectrum insecticides which are timed using well established degree day models based on historic and contemporary emergence data (Fettig et al. 1999b). However, the loss of registration for forestry uses of many commercial insecticides and concern about potential nontarget effects of broad spectrum insecticides has increased the demand for more environmentally friendly pest control options. One favorable and effective technique is to use “attract and kill” strategies. While these techniques are not necessarily new, they are increasing in popularity and technologies for application.

LITERATURE REVIEW

Attract and kill products (called attracticides) are currently being developed, registered, and marketed for many tortricid moths including *R. frustrana*. This review will encompass literature concerning the biology, life history, and management strategies for *R. frustrana* as well as attract and kill mechanisms, their use and success in related systems.

Biology

Throughout its range, the life cycle of the Nantucket pine tip moth has become roughly synchronized with that of its primary hosts, so that a new generation of egg laying adults is produced with each new growth flush (Berisford 1988). This moth has two to five generations per year (Fettig et al. 2000a) with the number of generations generally increasing with a decrease in latitude and increase in average annual temperature.

Most species of hard pines are susceptible to attack by the Nantucket pine tip moth. In the Southeast, loblolly (*Pinus taeda* L.), shortleaf (*P. echinata* Mill.), and Virginia (*P. virginiana* Mill.) pines are the preferred hosts while Scotch pine (*P. sylvestris* L.), pitch pine (*P. rigida* Mill.), red pine (*P. resinosa* Ait.), and Mugo pine (*P. mugo* Turra.) are attacked in the Northeast (Gibson 1968). Ponderosa pine (*P. ponderosa* Dougl. Ex. Laws.) and Monterey pine (*P. radiata* D. Don) are common hosts in the Southwest (Scriven and Luck 1980). Slash (*P. elliottii* Engelm.), longleaf (*P. palustris* Mill.) and eastern white (*P. strobus* L.) pines are generally resistant to attack, though light infestations can occur in these species (Wakeley 1928, 1935a, b,

Smith et al. 1930, Asaro et al. 2003). Damage to any pine species due to Nantucket pine tip moth is most severe on seedlings and saplings less than five years old (Berisford 1988).

The moths overwinter in host shoots as pupae, emerging as adults as early as December and January in the southernmost portions of its range (Berisford 1988) and as late as April in the northernmost portions (Yates 1960). Male moths typically emerge a few days prior to females (Berisford and Brady 1972, Canalos et al. 1984) which ensures ample potential mates as females emerge. Early emergence of females has been observed but is thought not to contribute substantially to the pest status of the moth (Young 2006). Although adult moths are crepuscular they have been observed in late afternoon flight on days when evening temperatures may fall below the flight threshold temperature of 9.5° C (Webb and Berisford 1978).

Female *R. frustrana* emit sex pheromones to attract males for mating. This pheromone-mediated attraction was first observed using live virgin females (Wray and Farrier 1963). Chemical composition of the pheromone was reported by Hill et al. (1981) as being a blend of the major component, E-9-dodecenyl acetate, and a minor component, E-9, 11-dodecadienyl acetate which occur in a ratio of approximately 20:1. Actual pheromone release rates have not been measured for *R. frustrana* due to the difficulty in inducing calling and mating behaviors in artificial environments (Richmond and Thomas 1977); therefore, the above ratios are based on gland extraction and empirical field tests of differing ratios. However, synthetic baits which use a 20:1 ratio of these components are highly attractive.

Multiple avenues of pheromone dissemination can be used to attract male moths to traps. Crude extract from female abdominal tips can be equally attractive to males as live females (Berisford and Brady 1972). Rubber septa impregnated with pheromone can also be as attractive as live females or crude extracts (Hill et al. 1981). Current monitoring programs use traps baited with pheromone-loaded septa (Asaro et al. 2003). Pheromone trap catch data are used to determine spray dates using a spray-timing model based on degree day accumulations (Gargiullo et al. 1985, Fettig and Berisford 1999b).

Life History

The natural range of the Nantucket pine tip moth extends north to Massachusetts, west into Texas and Oklahoma and south through the northern two-thirds of Florida; it also occurs in parts of Central America and the Caribbean Islands. Introduced populations occur throughout much of New Mexico, southeast Arizona and extreme southwest California (Asaro et al. 2003) though detailed information on its range is lacking for these areas.

The distribution of *R. frustrana* overlaps with three other common *Rhyacionia* species: the pitch pine tip moth, *R. rigidana* (Fernald), the subtropical pine tip moth, *R. subtropica* Miller, and the European pine shoot moth, *R. buoliana* (Denis and Schiffermüller) (Asaro et al. 2003). *R. frustrana* and *R. rigidana* can often be found infesting the same tree and may emerge at the same time in the spring (Berisford 1974b, Canalos and Berisford 1981). The pheromones of *R. frustrana* and *R. rigidana* are mutually inhibitory (Berisford and Brady 1973, Berisford et al 1974, Berisford 1977). *R. frustrana* is only weakly attracted to the sex pheromones of *R.*

subtropica and *R. buoliana* (Berisford et al. 1979). This might be expected since E-9-dodecenyl acetate is shared as the major pheromone component of each (Berisford et al. 1979, Roelofs et al. 1979).

Female *Rhyacionia frustrana* deposit eggs singly on needles and shoots (Gargiullo and Berisford 1983). It appears that females can discriminate between susceptible and resistant host species and will preferentially lay eggs on susceptible hosts (Yates 1966b, Hood et al. 1985). Host preference and location mechanisms for oviposition remain unknown (Ross et al. 1995). The temperature threshold above which development of all life stages occurs is 9.5 C (Haugen and Stephen 1984, Richmond and Becheler 1989). Eggs may take from 5 days (during summer) to 30 days (during spring) to hatch depending on temperatures (Yates et al 1981). Following egg hatch larvae complete five instars (Fox et al. 1971) before pupating. First instar larvae primarily mine needles, but may also feed on shoots or buds (Asaro et al. 2003). Second and third instar larvae feed at needle and bud axils. Resin coated silken tents, which develop at these feeding sites, are the first obvious signs of attack (Asaro et al. 2003). Late instars feed entirely within buds and shoots severing the meristematic tissue, resulting in tissue necrosis and shoot death. Larval feeding on conelets of shortleaf pine can also cause a reduction in seed production (Ebel and Yates 1974). Fully developed larvae pupate within the dead shoots (Berisford 1988, Asaro et al. 2003).

R. frustrana overwinters as pupae in the dead shoots of the host tree (Asaro et al. 2003). When adults are ready to eclose, circular movements of the abdomen, which possesses rows of spines, aid in maneuvering the pupa out of infested shoots (Yates 1960). Wallis and Stephen

(1980) observed diapause patterns in *R. frustrana* in Arkansas for four years. Field collected pupae were held in the laboratory at 25° C until adult emergence occurred. For all collection years a small proportion of moths emerged in the usual generation time (about sixty days) while the majority of moths emerged between January and March, after a significant diapause period which for the 1976 collection was as long as seven months. Based on these observations, Wallis and Stephen (1980) determined that *R. frustrana* has a diapause mechanism which is not affected by temperatures above the development threshold.

Invasion of pine stands by *R. frustrana* can occur shortly after establishment (Asaro et al. 2003). Low rates of parasitism in newly colonized stands may aid in rapid establishment and expansion (Lashomb et al. 1980). Tree resistance to tip moth attack increases with age. Tip moth populations typically level off in three to five years and are significantly diminished or absent by stand maturity (Berisford 1988). There are many explanations as to why older trees are more resistant to *R. frustrana* attack. Yates (1962) showed that resin of loblolly pine is toxic to first instar larvae. High resin flow in older trees may encapsulate larvae or repel them (Asaro et al. 2003). Shaded shoots common in closed canopy stands suffer lower *R. frustrana* attacks than those exposed to sun; removal of shade can cause an almost immediate increase in shoot attacks (Berisford and Kulman 1967). Growth flushes decrease as trees get older and become asynchronous with *R. frustrana*. This may decrease the success of larvae due to a lack of access to soft tissues in new shoots (Berisford 1988, Asaro et al. 2003). As trees become older they become more susceptible to pitch pine tip moth and competition between the two *Rhyacionia* species increases with pitch pine tip moth being the more successful invader at this stage (Asaro et al. 2003).

Management strategies

Silvicultural control

Theories on management of *R. frustrana* are diverse. Historically, management called for the burning of infested twigs during winter (Comstock 1880) or the complete removal of all pines to eliminate host species (Scudder 1883). Integrated strategies which combine silvicultural, chemical and biological controls are favored today (Asaro et al. 2003).

Wakeley (1928) proposed some of the original silvicultural treatments for *R. frustrana* in timber production stands. Tip moth mitigation methods included planting resistant species, only planting on favorable sites, and employing shelterwood techniques for regeneration. Wakeley further recommended any technique that maintained some competing vegetation, increased stand vigor or enhanced biodiversity. Tip moth populations tend to be highest in even-aged stands with minimal competing vegetation and studies have shown that *R. frustrana* densities are inversely correlated to the amount of competing vegetation (Berisford and Kulman 1967, Hertel and Benjamin 1977). Initial research suggested that removing shade adversely affected *R. frustrana* development (Harrington 1955) however it is now thought that it is actually maintaining shade that adversely affects the moth (Yates 1960, Berisford and Kulman 1967)

In the 1930s, Japanese black pine (*Pinus thunbergii* Parlatores) was extensively planted along the Northeast coast due to its high resistance to *R. frustrana* (Jones 1930, Littlefield 1942, Afanasiev 1949). Slash pine is virtually immune to *R. frustrana* attack except as a newly planted

seedling, throughout its range (Williston 1958, Williston and Huckenpanler 1960). However, resistance to tip moth attack is of modest importance to today's growers who typically base planting decisions on other factors (Asaro et al. 2003). Breeding for resistance or tolerance to tip moth has been researched since the late 1950s with mixed results (Holst and Heimmurger 1955, Henry and Hepting 1957, Harris 1960, Yates 1962, Holst 1963, Warren and Young 1972, Hertel and Benjamin 1975, Hood et al. 1985). Cade and Hedden (1989) found significant differences between 12 half-sib families of loblolly pine, each from genetically improved parents and in stands representing operational plantings in eastern North Carolina. However, no family-related differences in attack frequency were found in loblolly plantations in Florida (Lopez-Upton et al. 2000). Similarly, Nowak and Berisford (2000) found no significant differences in damage between four seed sources of genetically improved loblolly pine. As with species selection, selection of loblolly cultivars in current timber stands is made with little consideration of *R. frustrana* resistance or tolerance (Asaro et al. 2003).

Berisford (1988) suggests silvicultural practices which include less intensive site preparation, direct seeding, and reducing the size of regeneration blocks, all of which are preventative in nature but they conflict with the goals of rapid juvenile growth and simplified harvest and regeneration (Asaro et al. 2003). Silvicultural treatment for tip moth control receives little to no attention from managers. Chemical control however, is more widely used and often regarded as more feasible for *R. frustrana* management.

Chemical Control

The feasibility of pre-planting control of tip moth was first shown by Baumhoffer (1936) who demonstrated the importance of seedling dips prior to shipment from nurseries to prevent early *R. frustrana* attacks. Fumigation of seedlings with sodium cyanide was reported as being satisfactory in control by Beal et al. (1952). Seedlings are now commonly treated with insecticides for *R. frustrana* and pine regeneration weevils (*Hylobius pales* and *Pachylobius picivorus* sp.) in the nursery prior to any shipment.

Numerous studies have examined a great variety of insecticides used to control *R. frustrana* populations. Initially it was shown that 2% nicotine dust was highly effective at controlling *R. frustrana* by killing adults (Howard 1925). Dichlorodiphenyltrichloroethane (DDT) first became available in the 1940s and was shown to effectively control *R. frustrana* infestations (Fenton and Afanasiev 1946, Afanasiev and Fenton 1947, Beal 1958) and was used until it was banned by the United States Environmental Protection Agency in 1973. Nicotine sulfate (Blackleaf 40), lead arsenate, benzenehexachloride (BHC), parathion and DDT were all tested by Stearns (1953); only DDT and parathion provided satisfactory control. Pyrethroid class insecticides are commonly used today (Fettig et al. 2000a, Nowak et al. 2000). There is still potential for effective control using other compounds such as more host specific chemicals, botanicals (i.e. neem), or biological insecticides (i.e. *Bacillus thuringiensis* var. *Kurstaki*) but they are often more expensive and in most cases require precise timing (Dalusky and Berisford 2002).

The economics of foliar insecticide sprays, often requiring multiple applications along with the potential nontarget effects, resulted in the investigation of systemic insecticides for *R. frustrana* control. Systemic insecticides typically provide adequate and often prolonged control; however uptake and translocation is highly dependant on soil moisture. Less than optimal conditions can lead to failure of absorption by roots if conditions are too dry or leaching into the ground water if conditions are too wet. Disulfoton was found to be highly effective, though some phytotoxic effects were observed, at controlling *R. frustrana* for the first two years after application (Thor and Beavers 1964). However, Cade and Heikkenen (1965) found disulfoton provided no control when applied as granules at rates of 27.5 and 82.5 kg/ha. Phorate was also applied at these concentrations and found to be efficacious. Application of these systemics to seedling roots resulted in highly phytotoxic effects from phorate, and only adequate control with disulfoton (Cade and Heikkenen 1965, Yates 1970). Carbofuran was the industry standard for tip moth control for many years before that use was lost to regulatory action (Kerr and Owebs 1973, Overgaard et al. 1976, 1978). Carbofuran was also shown to reduce fusiform rust infections (Powers and Stone 1988), though it has not been observed to have fungicidal properties in other uses.

Insecticides are most commonly used in high value stands such as Christmas tree farms, seed orchards, progeny tests, and short rotation stands (Asaro et al. 2003). A list of currently registered insecticides for use in pine plantations can be found in the most recent pest control handbook which can be obtained through the State Cooperative Extension office. The efficacy of foliar insecticide applications is strongly influenced by timing. Applications must be made before larvae bore into shoots and buds where they are protected from sprays. However, if

applied too early insecticides are often ineffective because of weathering and the steady accrual of new, hence unprotected tissue. In the early 1980s, new methods for timing applications of insecticide sprays were proposed. Gargiullo et al. (1983b) devised a degree-day spray model using dimethoate insecticides in the Georgia Piedmont and Richmond et al. (1983) developed a method for predicting spring flight using heat unit accumulation. Berisford et al. (1984) found that insecticide treatments applied once per generation at 30-80% egg hatch provided the best control. Spray models for esfenvalerate were developed in the Georgia Coastal Plain (Gargiullo et al. 1985). Kudon et al. (1988) and Fettig et al. 1998 have refined these models to increase their applicability. Because these studies were done in the Georgia Piedmont and Coastal Plain, spray model values are only available for areas where tip moth has 3 or 4 generations per year (Asaro et al. 2003).

A simplified system for predicting optimal spray intervals for the entire Southeastern U.S. where the moth has three to four generations per year was developed by Fettig et al. (2000a, 2003). This system uses long-term historical temperature data to predict the optimal spray periods for *R. frustrana* control. This system is easier for managers to use than earlier methods because it decreases the cost and labor inputs, and reduces the need for detailed knowledge of pheromone trap deployment, degree day calculation, and general tip moth biology and identification (Asaro et al. 2003). While spray insecticides are effective for control of *R. frustrana*, it is impractical and uneconomical to control all tip moth generations in commercial timber plantations (Asaro et al. 2003). Combinations of spray schedules have been evaluated to determine the most economical schedule. A single spray during the first generation of the first two years following stand establishment was found to be the most effective and economically

feasible in the Georgia Piedmont with 3 annual tip moth generations (Fettig et al. 2000b). A similar study in the Georgia Coastal Plain, where there are 4 annual tip moth generations, produced similar results (Young 2006).

Biological Control

There are numerous natural enemies of the Nantucket pine tip moth, some of which have been studied as biological control agents. Frank and Foltz (1997) provided a list of parasitoids of *R. frustrana* and Nowak et al. (2001a) presents a key to the identification of arthropod natural enemies of *R. frustrana*. Several publications address the entire genus *Rhyacionia*. Harman and Kulman (1973) published a world survey of parasitoids and predators. Yates (1967a) published a key to the Nearctic parasitoids of the genus. Pathogens and parasitoids have been the most widely researched for use in biological control systems. Of these, insecticides containing the bacterium *Bacillus thuringiensis* var *kurstaki* Berliner have been the most successful (Asaro et al. 2003). Scriven and Luck (1978) were able to substantially decrease tip moth damage in an introduced population in California, through the release of a native parasitoid *Campoplex frustranae* Cushman from Georgia and Arkansas (Eikenbary and Fox 1968b). Experimental releases of the egg parasitoid *Trichogramma exiguum* Pinto and Platner showed substantial increases in egg parasitism and decreases in egg hatch in *R. frustrana* at release sites in North Carolina (Orr et al. 2000). These releases also resulted in decreases in both the percentage of infested shoots and the length of damage within the shoot.

There are also numerous predators of the Nantucket pine tip moth (Eikenbary and Fox 1968a); however there is a paucity of information concerning their impact on *R. frustrana* populations. Clerid beetles (*Phyllobaeuus* sp.) and various spiders seem to be the most important predators of *R. frustrana* larvae (Eikenbary and Fox 1968a). The black imported fire ant (*Solenopsis richteri* Forei), though found in association with *R. frustrana*, appears to have little impact on tip moth populations in Southeastern Louisiana (Wilson and Oliver 1970). The predatory wasp *Zethus spinipes* Say has been observed removing tip moth larvae from infested loblolly pine shoots (Lashomb and Steinhauer 1980) but no data on impact on the tip moth are available.

Attract and Kill

Though a variety of control options are currently available for the Nantucket pine tip moth, economic action thresholds are vague and pesticide use restrictions are increasing (Asaro et al. 2005). “Attract and kill” techniques (attracticides) are another potential control option that may reduce pesticide use issues such as nontarget effects while providing adequate control. Attract and kill management strategies combine an attractant with an insecticide to target a specific pest. Such strategies have been successfully developed to control the Mediterranean fruit fly *Ceratitis capitata* Wiedemann (Steiner et al. 1961), the oriental fruit fly *Dacus dorsalis* Hendel (Steiner et al. 1965, 1970), the olive fruit fly *Dacus oleae* (Gmelin) (Haniotakis et al. 1991), the pink bollworm *Pectinophora gossypiella* (Saunders) (Baker 1984), as well as the oriental fruit moth *Grapholita molesta* (Busck) (Evenden and McLaughlin 2004a, b), the codling moth *Cydia pomonella* (L.) (Charmillot et al. 1996, Charmillot and Hofer 1997), and the light

brown apple moth *Epiphyas postvittana* (Walker) (Suckling and Brockerhoff 1999). Point source formulations have been described by McKibben et al. (1990) to control the boll weevil *Anthonomus grandis* Boheman, while Hofer and Brassel (1992) described the use of droplets of an insecticide-pheromone combination on cotton and apple crops for the control of *P. gossypiella* and *C. pomonella* respectively (Lösel et al. 2000). For Lepidopteran pest species, a low concentration of sex pheromones is used as the attractant and coupled with a pyrethroid insecticide as the toxicant (Butler and Las 1983, Haynes et al. 1986, Miller et al. 1990, Downham et al. 1995, Charmillot et al. 1996, Charmillot and Hofer 1997, Brockerhoff and Suckling 1999, Suckling and Brockerhoff 1999, Charmillot et al. 2000, Krupke et al. 2002, Evenden and McLaughlin 2004a,b). This approach is an extension of mating disruption strategies which have had mixed success with Lepidopteran pests. Cardé and Minks (1995) provide an inclusive review of literature concerning the mechanisms for mating disruption and its use as a pest management strategy for many economically important moth species. Mating disruption has yielded little success in tip moth control. Air permeation with the major pheromone component for *R. frustrana* reduced male attraction to female-baited traps; however there was no reduction in tip moth associated damage (Berisford and Hedden 1978). Mating disruption has been shown to be effective against other tortricid pests such as Oriental fruit moth, *Grapholita molesta*, codling moth, *Cydia pomonella*, and the light brown apple moth, *Epiphyas postvittana*.

Attracticides represent a pheromone based technology developed for better control at high population densities and using reduced pheromone concentrations (Conlee and Staten 1981, Evenden and McLaughlin 2004a). The effectiveness of attracticide products is dependant on

insect contact exposure to the insecticide within the formulation (Charmillot et al. 1996, Suckling and Brockerhoff 1999). This requires that formulations are highly attractive and that males can follow synthetic pheromone trails directly to the source. Charmillot et al. (1996) and Suckling and Brockerhoff (1999) assessed control and orientation of male moths of codling moth and light brown apple moth with attracticide formulations both with and without insecticide. Approximately 50% of orientation disruption was the result of pheromone alone with the additional 50% being the result of male removal through insecticide exposure.

Recently attracticide formulations have been developed for use against the Oriental fruit moth and the codling moth and the light brown apple moth. Field and laboratory success of these products (Brockerhoff and Suckling 1999, Suckling and Brockerhoff 1999, Charmillot et al. 2000, Krupke et al. 2002) suggested that this technology may have potential against other tortricid pests. Consequently, an attracticide formulation for the Nantucket pine tip moth has been developed under the trade name *LastCall*[™] NPTM (IPM Development Co. Marylhurst, OR). This formulation consists of a viscous matrix and incorporates a permethrin insecticide with the *R. frustrana* sex pheromone in a UV-stable gel-carrier material. Asaro et al. (2005) conducted preliminary tests on the efficacy of *LastCall*[™] NPTM formulation. *LastCall*[™] NPTM was applied at a rate of two 50 µl droplets per tree, or approximately 3,000 droplets per ha (1,215 droplets per ac); treatment blocks were treated to control the first, second, third, or fourth generation of *R. frustrana*. Attracticide treatments were most effective at controlling damage during the first generation compared to subsequent generations; treatments were moderately successful in the second generation but damage was not suppressed in the third and fourth generations (Asaro et al. 2005). These preliminary tests of *LastCall*[™] NPTM were used

as a basis for more extensive testing of the product. While there is a paucity of information related to attract and kill in Nantucket pine tip moth, more information is available on the use of attract and kill methods for related tortricid pests using similar attracticide formulations.

LastCall™ commercial formulations contain 0.16% pheromone. When tested at varying rates of 0.016, 0.16, and 1.6%, formulations of *LastCall™* for Oriental fruit moth control were all attractive to male moths; however more moths were attracted to traps baited with 0.016 and 0.16% pheromone (Evenden and McLaughlin 2004a).

Attract and kill management strategies can be very effective in controlling pest populations. The combined effects of mating disruption and lethal or sublethal insecticide poisoning offer a great advantage over the use of either technique alone. Continued evaluation of attract and kill products should further refine the most effective and economical application rates and techniques. My study was designed to extend the preliminary research of Asaro et al. (2005) in evaluating the efficacy of *LastCall™* NPTM. I evaluated varied rates and application techniques for *LastCall™* NPTM application to determine their control efficacy and to identify the most reliable and cost effective application methods. This study was conducted in the coastal plain of Georgia for two years beginning in January 2005.

MATERIALS AND METHODS

Year 1

Three loblolly pine plantations were selected in the Georgia Coastal Plain on land provided by International Paper Company. These sites were located in Jefferson and Emanuel Counties and met the following requirements: 1) the stand was at least 70 acres, 2) the trees were entering their second growing season, and 3) trees had sufficient pre-existing tip moth populations.

At each site, eight five-acre plots were established in December 2004 to be treated with *LastCall* NPTM. Plots were square and measured 467 feet per side. Plots were randomly assigned one of four treatments yielding two replications of each treatment per site.

Treatments

This study tested three variations of attracticide treatments plus an untreated control (C). Based on preliminary work by Asaro et al. (2005), I established a standard treatment, which treated all trees in the plot with two attracticide drops per tree (AT). As variations, one treatment reduced only the number of trees treated while maintaining the per acre application rate (AR) by treating trees in alternate rows with four drops per tree, and another treatment that reduced both the number of trees treated and the rate applied per acre (RR) by treating trees in alternate rows

with two drops per tree. Treatment of alternating rows was tested in an effort to make the application of LastCall more cost effective by reducing labor costs.

Attracticide applications were made for the first, second and third generations in January, May and June 2005, respectively. Applications were made prior to the main emergence for each generation as determined by the trap catch data and historical spray dates (Fettig et al. 2000a) in order to maximize the attraction of males to the droplets. Droplets were applied in the top whorl of the tree using a pump supplied by IPM Development Co., which dispenses 50 µl pre-measured droplets. For the “standard” and AR treatments 3.64 g/ac of permethrin and 0.097 g/ac of pheromone were utilized, while only 1.82 g/ac of permethrin and 0.0485 g/ac of pheromone were dispensed for the RR treatment.

Measurements of Efficacy

Three wing traps (Trecé Inc., Salinas, CA) were placed approximately equidistant from each other along a diagonal transect within each plot. Traps were used to monitor the progression of generations and to detect any reduction in male flight due to treatments. A synthetic *R. frustrana* bait (Trecé Inc., Salinas, CA) was placed in each trap. Baits were replaced every three to four weeks to ensure adequate release and attractancy of pheromone. Traps were checked at least once per week and twice per week during peak moth emergence. Numbers of male moths caught were recorded for each trap.

Damage estimates were made prior to any treatments and at the end of the first, second and third generations as well as at the end of the growing season. Damage was expressed as a ratio of damaged shoots to the total number of shoots in the top whorl (Fettig and Berisford 1999a). Data were arcsine transformed and analyzed using one-way analysis of variance (ANOVA) and a Tukey HSD test ($\alpha=.05$) for homogenous groups.

Measurements were taken from 50 randomly selected trees in each plot along the trap line in January 2005 prior to any treatment and in December 2005 at the end of the growing season. In the alternate row plots, the data requirements were revised to include an additional 50 trees per plot so that damage assessments and volume measurements from 50 trees in both treated and untreated rows could be collected. Height and ground level diameter were measured for each tree and used to calculate initial and final stem volumes. We used the volume equation for a cone ($V=1/3\pi r^2h$) and converted diameter to radius for each measurement. Volume was used as an indicator of treatment efficacy when treated stands exhibited higher post-treatment volumes than control stands. Volume data were analyzed using the same methods as the analysis of damage. The data were initially tested for normality and equal variance and they met both assumptions. They were then tested for homogenous groups using a Tukey HSD test ($\alpha=.05$).

Year 2

A similar process was followed for the second year of treatment with minor modifications. Research sites were located on land owned by Rayonier Inc. These sites were located in Burke and Emanuel Counties. One site in Burke County and one in Emanuel County

were entering their second growing season; a second Burke County site was entering its third growing season. The site area and tip moth population requirements remained the same.

Plot design and setup was the same as the 2005 study plots.

Treatments

2006 treatments retained our “standard” treatment of every tree (AT) and the untreated control plots (C); however we altered two experimental treatments in an effort to improve efficiency and cost of application while preserving the efficacy of the product. Our alternative treatments in 2006 were to treat every tree with one drop of the attracticide (HD) or to treat alternating trees within each row with two drops per tree (SD).

Attracticide treatments were made for the first and second generations only in February and May respectively. All other treatment procedures were as in 2005.

Measurements

Measurements were made as in 2005 with the addition of growth measurements taken at the end of the first and second generations. Final growth measurements will be made in November 2006.

Data for 2006 were not analyzed due to the failure to meet normality and equal variance assumptions within the untransformed and transformed data and because tip moth populations were extremely low.

RESULTS AND DISCUSSION

Year 1

Trap Catch

Trap catches were dramatically reduced in all treated plots compared to untreated controls and complete trap shutdown was often observed. **Figure 1** shows the average trap catch for each treatment throughout the trapping period. Trap shutdown is commonly observed in mating disruption efforts (Asaro et al. 2005) yet it does not necessarily suggest treatment efficacy, as trap shutdown can sometimes occur in stands which still have high levels of damage (Berisford and Hedden 1978). Trap shutdown occurred during all three treated generations and lasted ca. eight weeks between February and April and four weeks each in May and July. This suggests that the attracticide droplets were actively releasing pheromone during this time. Trap catch during the fourth generation application period, for which no attracticide applications were made, was similar across treatment blocks.

Damage

Damage data were averaged for each plot and each generation. Plot averages were grouped by treatment and each generation was tested for normality and equal variance using SigmaStat 3.1 software (SYSTAT Software, Inc. 2004); however, in all cases the raw data values failed to meet these assumptions. The data were arcsine transformed and retested for normality

and equal variance. All generations met these assumptions with the exception of the 1st generation which still failed normality. Since only one generation failed to meet the assumption of normality, the data from this generation were analyzed similar to the other generations. A one-way analysis of variance (ANOVA) was used to test for homogenous groups at the $\alpha = .05$ significance level (Statistica, StatSoft 2003). **Table 1** shows the results of these analyses for each generation, as well as the percent damage for each treatment. Pretreatment damage levels were not significantly different among sites or among plots within sites. The control plots had significantly higher infestations than those that received any of the three treatments. Of the three treatments, the all treated blocks (AT, our predetermined standard and “positive control”), incurred the least damage and was significantly different from the reduced area and reduced rate (RR) treatments. The reduced area (AR) treatment was not significantly different from either the AT or the RR treatments in the first generation.

The second generation results are slightly more ambiguous compared to the first generation. These findings are also consistent with those of Asaro et al. (2005). Damage in all treated blocks decreased substantially. While the AT blocks were lower and statistically different from the RR and C blocks, damage was only lower by approximately 8 and 12% respectively. The RR and the C treatments were not significantly different from each other. Damage in the AR treatment blocks was not significantly different from the AT blocks and the RR blocks. However the actual percent damage is so low, they may not be practically significant, with or without being statistically significant.

There were significant differences between the positive and negative controls and the treatment blocks in the third generation. During this generation there was a substantial increase in damage in all plots compared to previous generations, indicating a population rebound. The AT treatment was still most effective at controlling damage, though it was statistically similar to the AR treatment, there is approximately a 16% numerical difference in the damage values with the AR treatments being somewhat less effective than the AT treatment but still more effective than the RR treatment. Predictably, the control blocks suffered the most damage.

Damage estimates taken at the end of the growing season were more variable. The average damage for each treatment increased by the end of the growing season. Damage in the control plots reached levels equivalent to those prior to any treatments. The RR treatment blocks also significantly increased in damage and were statistically similar to the control plots and the AR plots. Damage in plots receiving the AT treatment numerically maintained the lowest damage of all plots during the final measurement period and was statistically similar to both the AR and RR treatment block damage. This suggests that there may be a residual effect of continuous treatment on future populations of tip moth.

I noted early in these evaluations that there was a difference between the treated and untreated rows in the AR and RR treatment blocks. This was first observed during weekly trap checks when trap shutdown was occurring in all the traps in the treated sites; however, a few traps in the AR and RR plots continuously had high trap catches compared to the rest of the traps in a particular plot. These traps had been placed on trees in untreated rows in the random assignment of traps. **Figure 2a** shows the 1st generation percent damage of trees in treated and

untreated rows along with damage from the AT and C plots. Damage in the AT plots is not significantly different from damage in the treated rows of the AR and RR plots, while damage in the C plots is similar to damage in the untreated rows of the AR and RR plots. Possible explanations for the failure of these treatments to control damage across an untreated row include: pheromone release from the droplets may not be sufficient to attract males from distances of more than 8 to 10 feet; however, it is attractive enough when each tree is individually treated, skipping entire rows alters the “even distribution” of the product and makes it less effective due to the creation of long extended corridors where calling females might be more attractive than the adjacent synthetic pheromone. There could also be visual or behavioral aspects of mating that are unknown at this time that may be effecting the ability to control tip moth populations across rows.

Damage in treated and untreated rows for the 2nd generation was similar to that of the 1st generation. **Figure 2b** shows the 2nd generation data for treated versus untreated rows. The AT plots were not significantly different to the treated rows of the AR and RR plots while the amount of damage in the C blocks was similar to that in the untreated rows of the AR and RR block.

Tree Volume

Tree volume measurements were recorded at the beginning of the growing season and again at the end of the growing season. Initial and final volume results are presented in **Figure 3**. Initial volumes ranged between ca. 44 and 57 cm³/tree. The initial volume data showed there

were no significant differences among the trees prior to treatments. Final tree volumes were determined at the end of the growing season. Differences in plot volumes are rather large from a purely numerical standpoint. Final volumes ranged between 623 and 893 cm³/tree. Statistically there were no differences in volumes. However, based solely on numbers, there is a 170 cm³ difference between the volume of trees in control plots and trees in AT plots. Even though final volume averages were not statistically different there was considerable difference in volume between the some of the treatments. Change in volume between each treatment and the control was calculated and is presented in **Table 2**. Overall, there was 30.26% more volume at plots receiving the AT treatment and 13.22% more volume in the AR plots when compared to the control. The RR plot exhibited the least volume gain of the three treatments with only 0.73% more volume in treated plots compared to control plots.

It is unclear why volume differences were not statistically significant. It may be due to the small *n* size. Since treatment blocks are the unit of measure and not individual trees sample size decreases from *n*=300 to *n*=6. Difference in growth (or lack thereof) may be due to treating only one year. Perhaps treating the same blocks for two consecutive years would yield more amplified differences in volume (Young 2006).

Year 2

Overall, data collected during the 2006 field season were ambiguous and indeterminate. **Table 3** shows the compiled average damage and average volume data by treatment for the three sites. At all sites I observed extremely low damage after the second generation of treatment even

in the untreated control plots. A tip moth population crash which began with the overwintering generation and peaked in the second generation of 2006 made it difficult to find differences among treatments. The initial damage levels at these sites in 2006 were extremely low compared to the initial and even the final damage levels at some of the 2005 sites. Damage data failed to meet normality and equal variance assumptions even when arcsine transformed for all generations.

Tree volume measurements were made prior to treatment, after the first generation and after the second generation in 2006. Data were collected using the same methods as in 2005. As with the damage data for year 2, the volume data are highly variable and offer little insight into the efficacy of the attracticide. Final volume measurements (to be collected in November 2006) may express clearer differences among treatments. Data collected from all sites during all generations failed to meet normality and equal variance assumptions.

CONCLUSIONS

After the first year of treatment, I found that trees treated with *LastCall*TM NPTM exhibited consistently less damage and higher average stem volumes than the untreated controls. Overall, the standard treatment (AT) and the reduced area treatment (AR) were most effective at reducing tip moth damage and increasing average stem volume. However, the AR treatment incurred a high level of damage in the untreated rows while the AT treatment is the most labor intensive and therefore the most costly of the treatments making it less attractive for field implementation. Costs and labor limitations can be a significant impediment to pest control implementation in large scale plantations. Treating alternate rows proved effective in controlling damage in the treated rows and therefore lowered average damage across the plots; however untreated rows experienced levels of damage similar to that in control plots. While these treatments reduce the cost and labor inputs of treatment they are only moderately successful at controlling damage and increasing volume on the stand level because of the disparity between treated and untreated rows. These results may be due to a lack of consistent and even coverage throughout the stand as a result of the creation of long linear gaps between treated areas.

Limited success of the 2006 field trials was due to a dramatic decline of tip moth populations independent of the attracticide treatments. Damage estimates were initially low at two of the sites. Although first generation estimates show infestation differences between the treated plots and the control plots, there were no significant differences among the treatment groups. Second generation damage levels were consistent across all plots and all sites and represented a restricted range of values due to the population crash. Differences in damage for

this generation are nonexistent except for the control plots at site 1051. All other blocks were statistically similar.

Volume data provided little evidence of treatment effects. However there is some evidence that the HD treatment (one drop per tree, every tree) treatment was most effective at increasing volume. The change in volume between the initial and first generation as well as between the first and second generations was consistently high at all of the sites. It should also be noted that at two of the three sites (1051 and 1075) the second generation volume was the highest in the HD blocks. Without final volume and damage measurements for this season (to be taken in November 2006), conclusions about the efficacy of *LastCall*TM NPTM treatments cannot be made for the second year.

Overall, this study supports preliminary data on *LastCall*TM NPTM as an effective method for controlling tip moth in pine plantations. From the 2005 data we know it has clear and defined effects on tip moth populations, shoot damage, and tree volume. Obstacles to implementing this technology in an operational setting are the cost and labor inputs involved. These issues might be addressed through development of automated application techniques or reduced application rates. As with any pheromone mediated control technique there is a host of behavioral questions that arise. Since there is little currently known about tip moth mating behaviors such as calling and mate location, it would be beneficial to understand this aspect of the biology for the creation and improvement of future pheromone mediated control products.

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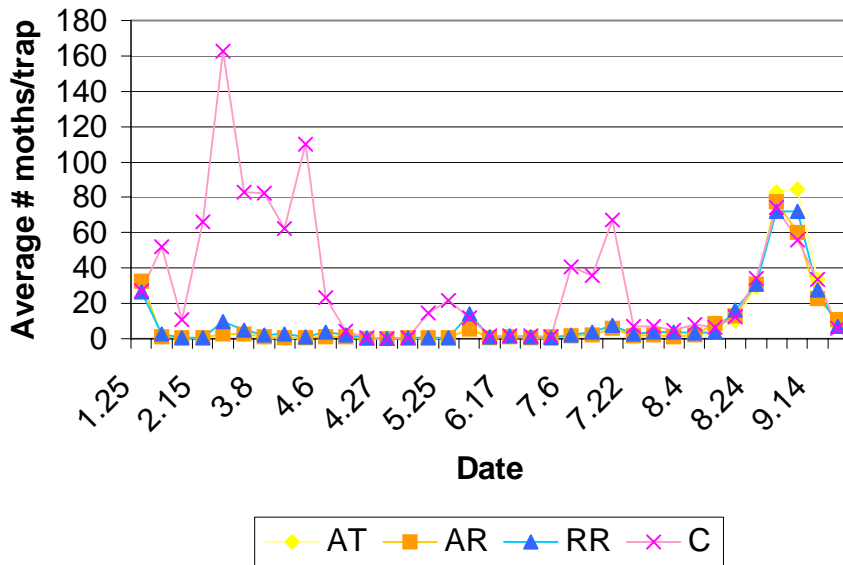


Figure 1: Mean male NPTM caught in 2005 in pheromone traps in plots treated with different rates of *LastCall*TM NPTM.

Table 1: 2005 percent damage by treatment for all generations. Superscript letters represent significant differences in damage among treatments within a generation.

	Average Percent Damage (untransformed)				
	Initial	1st Gen	2nd Gen	3rd Gen	Final
AT	72 ^a	9 ^a	4 ^a	15 ^a	27 ^a
AR	72 ^a	18 ^{ab}	10 ^{ab}	31 ^{ab}	29 ^a
RR	74 ^a	21 ^b	11 ^b	37 ^{bc}	45 ^{ab}
C	65 ^a	37 ^c	17 ^b	51 ^c	67 ^b

Raw data was arcsine transformed and analyzed using a one-way ANOVA; means separated by Tukey's HSD $\alpha=0.05$

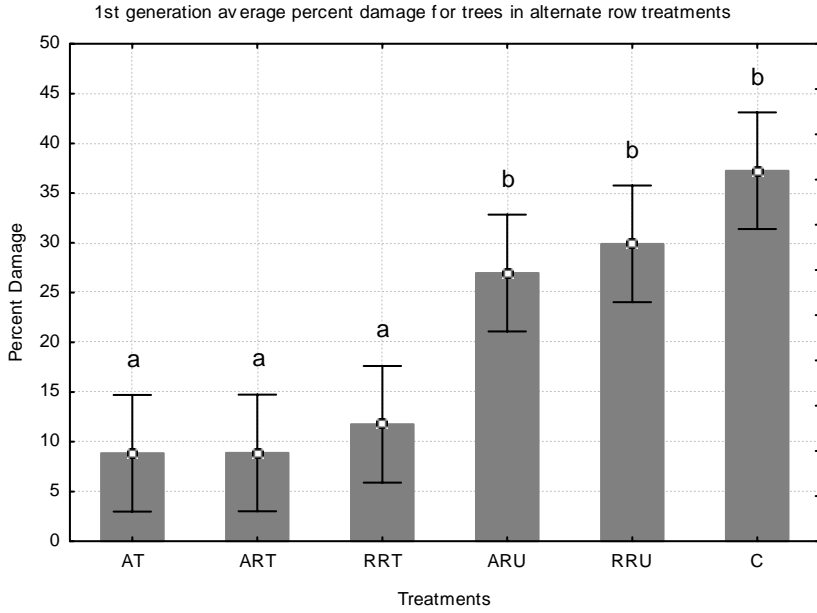


Figure 2a: Percent damage comparison between treated and untreated rows in alternate row treatments after the first generation in 2005. (ART= AR treated rows; RRT= RR treated rows; ARU= AR untreated rows; RRU= RR untreated rows).

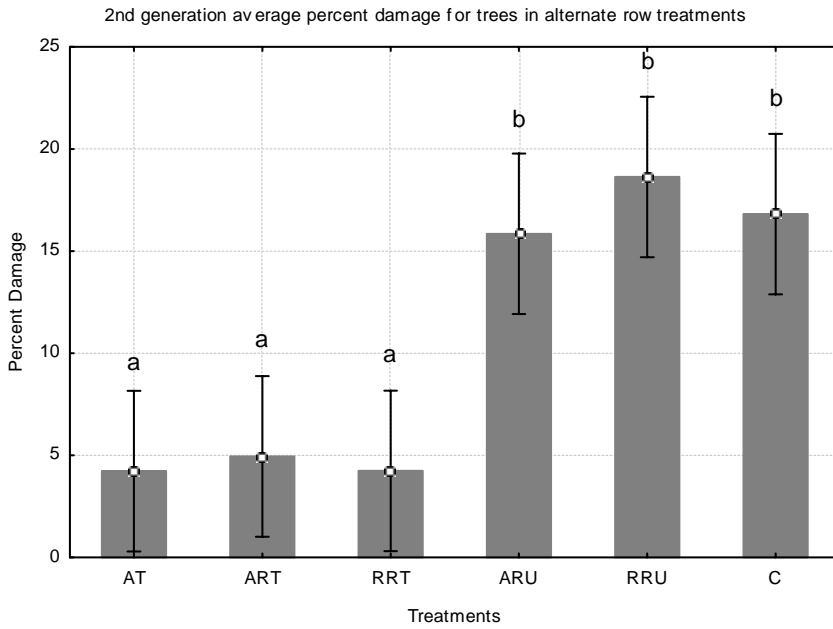


Figure 2b: Percent damage comparison between treated and untreated rows after the second generation in 2005.

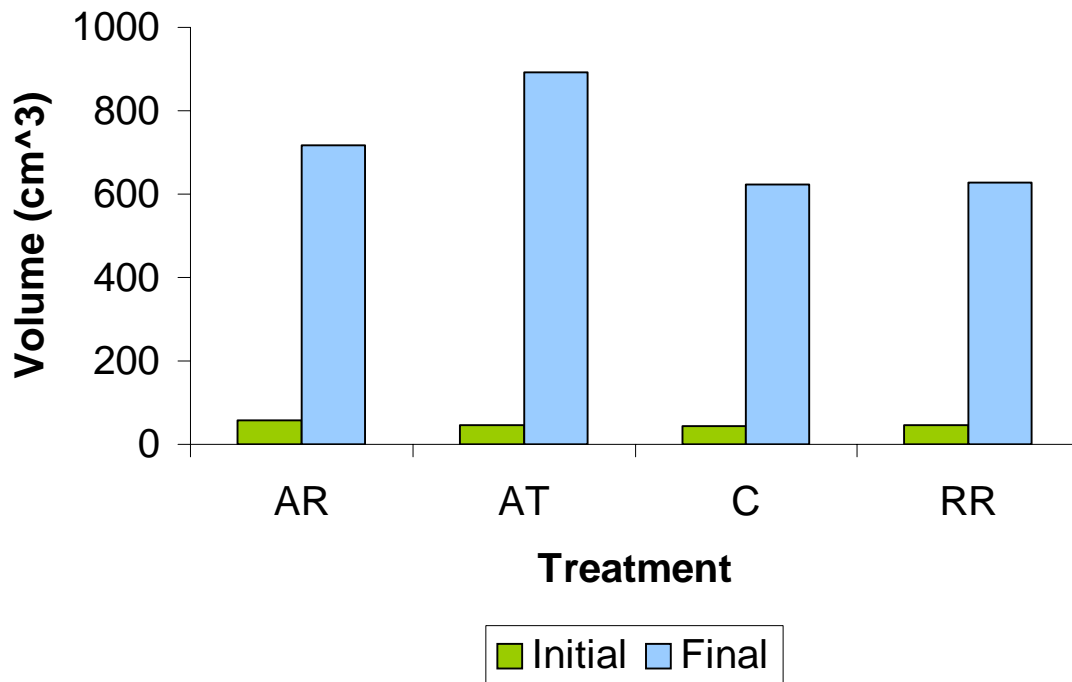


Figure 3: 2005 Initial and final volume averages by treatment.

Table 2: 2005 Final volume by treatment and percent change in volume between the treatment and control.

	Final Volume	% Change from Control
AT	893.1	30.26
AR	717.8	13.22
RR	627.4	0.73
C	622.9	--

Table 3: 2006 average damage and volume for all treatments and sites

	Average Percent Damage			Average Volume (cm ³)		
	Site 1047			Site 1047		
	Initial	1st Generation	2nd Generation	Initial	1st Generation	2nd Generation
AT	52	9.6	4.2	1057.8	1663	1799.9
SD	45.5	12.5	3.8	794.9	1272.8	1402.4
HD	46.2	11	3.8	681.1	1082.9	1204.4
C	46.9	22.8	6.2	536.3	942.4	978.7
	Site 1051			Site 1051		
AT	25	5.8	2.6	19.5	70.6	241.8
SD	24.9	6.9	4.7	50	84.9	164.6
HD	24.1	6.3	4.5	41.7	100.3	392.3
C	27.5	12.8	11.4	25	81.5	141.7
	Site 1075			Site 1075		
AT	19.6	2.2	2.7	45.2	123.2	180.7
SD	18.8	8.9	6.5	15.7	60.5	126.8
HD	21.5	9.9	2.4	24.9	122.2	263.2
C	23.7	20.5	8.2	32.1	90.9	196.1