FHP SPECIAL TECHNOLOGY DEVELOPMENT PROGRESS REPORT FOR FY 1999

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PROJECT STATUS: Continuing _Progress Report

PROJECT NUMBER: NA-99-04

PROJECT TITLE: **Development of an Improved Lure for Detection, Survey, and Management of the Pine Shoot Beetle**

SUBJECT: Bark beetles

PROJECT OBJECTIVE: The objectives of this project were to evaluate responses by the pine shoot beetle, *Tomicus piniperda* (L.), to attractive semiochemicals (pheromones and host compounds) and determine the optimal mixture for an improved lure to be used in detection, survey, mass-trapping, and reducing the spread of *T. piniperda*. The ultimate goal was to develop and implement a cost-effective pheromone-trapping technique as a means for detecting new infestations, preventing further spread, and reducing currently established populations of *T. piniperda.*

BRIEF DESCRIPTION OF PROJECT:

Tomicus piniperda is among the most damaging insect pests of pine trees in Europe and Asia (Långström and Hellqvist 1991; Ye 1994). In July 1992, *T. piniperda* was found near Cleveland, Ohio, and as of October 1999, it was established in 270 counties in 11 US states (Illinois, Indiana, Maryland, Michigan, New Hampshire, New York, Ohio, Pennsylvania, Vermont, West Virginia, and Wisconsin; NAPIS 1998), 24 counties in Ontario, and 8 counties in Quebec. Because this insect poses a potentially significant threat to major pine-producing areas of the US, a federal quarantine was implemented in November 1992 that regulates movement of pine logs with attached bark, pine Christmas trees, and pine nursery stock from infested to uninfested counties in the US. The enactment of the quarantine has had a significant economic impact on Christmas tree growers, lumber companies, and nursery producers in the infested counties, as well as on regulatory agencies in both infested and uninfested states.

Accurate delimitation of the area infested with *T. piniperda* is necessary to ensure that regulations and management tactics are implemented to reduce the risk of accidentally introducing *T. piniperda* to uninfested areas of the US. Currently, trap logs are used by many state inspection and regulatory agencies to detect new infestations of *T. piniperda*. However, trap logs may have limited efficacy due to (1) lack of suitability of host logs if the bark is damaged or phloem has desiccated, (2) poor placement of trap logs in the field, (3) damage of trap logs by small animals (e.g., bark feeding by mice and voles), (4) the necessity for timely removal and assessment of trap logs prior to brood emergence and deterioration of galleries by competitors and predators, and (5) difficulty in confirmation of beetle identification due to parental re-emergence and colonization by other bark beetles which are nearly identical to the

pine shoot beetle in appearance. In addition, trap logs require felling of numerous healthy trees; logs are heavy, bulky, and require debarking; and, hence, are relatively costly, labor intensive, and potentially hazardous to use.

The current alternative to trap logs is the use of funnel traps baited with the host volatile α-pinene (Haack and Lawrence 1997). Although α-pinene is attractive to *T. piniperda*, it does not compete well against freshly cut pine logs and stumps. Therefore, especially at low population levels, trap logs are usually superior to α-pinene-baited funnel traps. However, pheromones, either alone or in combination with α-pinene, may be equal or superior to trap logs for detecting *T. piniperda*. An improved *T. piniperda* trapping system, using pheromones, would improve many regulatory and management activities. For instance, an effective pheromone lure would reduce the time and effort involved in *T. piniperda* detection, survey, and inspection programs, compared with trap logs. Attractive pheromones could also be used more effectively for mass-trapping, population suppression, and reducing the spread of *T. piniperda*. The use of pheromones has not been used in the past because the *T. piniperda* pheromone system was not well understood. Although there was early evidence to support pheromone production by *T. piniperda* (Schonherr 1972), later studies reported that *T. piniperda* lacked a pheromone (Bouhot et al. 1989, Lanne et al. 1987, Loyttyniemi et al. 1988).

Despite the serious economic damage by *T. piniperda* in Europe and Asia and considerable research over the past 30 years, progress toward identifying effective pheromones has been slow. In addition to α-pinene, a number of host volatiles including (+)-3-carene, α-terpinolene (Byers et al. 1985, Schroeder and Eidmann 1987), and ethanol (Schroeder and Lindelow 1988, Vité et al. 1986) have been reported to mediate attraction of *T. piniperda* to host materials. In addtion, some insect-produced compounds have shown potential as attractants including myrtenol and *trans*-verbenol (Francke and Heeman 1976, Kangas et al. 1970, Zhou et al. 1997). Furthermore, in recent studies by Czokajlo (1998), using coupled gas-chromatographic electro-antennal detection (GC-EAD), myrtenol and *trans*-verbenol were confirmed as attractive pheromones and α-pinene-oxide and nonanal were identified as new candidate pheromones for *T. piniperda*. These four compounds have shown considerable promise for development of an effective attractive lure, increasing attraction to α-pinene by up to 400% (Czokajlo 1998). After three decades of research, development of an effective pheromone for *T. piniperda* is finally nearing fruition.

This project tested various blends of known attractive host volatiles and pheromone components for *T. piniperda* in field trapping experiments conducted in infested Scotch pine Christmas tree plantations in Michigan and Indiana.

Methods: Field trapping experiments were conducted in spring 1999 in Michigan and Indiana in Scotch pine Christmas tree plantations that were heavily infested with *T. piniperda.* Twelve-unit multiple funnel traps were set up at least 15 m apart along the grass lanes between the rows of trees in each plantation. Experiments were laid out in randomized complete blocks with 5 replicates per treatment. Traps were baited by hanging lures releasing the various test compounds from the sides of the funnel traps. Standard α-pinene baits were purchased from Phero Tech, Inc. (Delta, BC). The pheromone baits were synthesized, purified, and formulated in release devices by IPM Technologies, Inc. (Portland, OR). The three other host volatiles were

purchased from Sigma-Aldrich and released from low-density polyethylene bottles (Phero Tech, Inc.). A small piece of vapona no-pest strip was placed in the collection cup of each trap to kill all insects captured. All collected insects were stored in labeled bags and held at -16°C in the laboratory until identified, sexed, and counted.

Experiment 1 tested α-pinene alone or combined with each of the four pheromone candidates (*trans*-verbenol, myrtenol, nonanal, and α-pinene oxide) individually and all combined. Experiments 2 and 3 tested α-pinene with the six binary or four ternary combinations of the four pheromones, respectively, and compared attraction to α-pinene alone or combined with all four pheromones. Experiment 4 tested α-pinene alone or combined with each of the three other host volatiles ((+)-3-carene, α-terpinolene, and ethanol) individually and all combined. Experiment 5 tested α-pinene alone or combined with the six binary combinations of the three other host volatiles or all three combined.

Traps were set up during the first week of March 1999, prior to initial spring flight by *T. piniperda* which typically occurs in mid-March in southern Michigan and northern Indiana. However, due to cool weather conditions in early spring 1999, *T. piniperda* emergence flight was delayed until the first week of April and was very synchronous. All captured insects were collected during the first week of April and the traps were re-baited with new pheromone combinations to provide 5 additional replicates. Traps were checked frequently, however, very few additional insects were captured. Therefore, only data from the initial 5 replicates of each experiment were analyzed and additional trapping and trap log experiments testing the best combinations of pheromones and host volatiles could not be conducted in 1999.

Statistical approach: The number of *T. piniperda* captured were transformed by log (x+1) in order to meet assumptions of normality and homoscedasticity and then analyzed by ANOVA and Fisher's least-significant-difference test in order to determine differences among treatments within each experiment.

FHP PERSON LEADING THE PROJECT: James B. Hanson (NA-FHP, St. Paul)

COOPERATORS:

1. Principal investigators:

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ACCOMPLISHMENTS AND RESULTS:

In all of the trapping experiments, addition of the pheromone components and other host volatiles to α-pinene resulted in some increase in attraction of *T. piniperda*. In experiment 1, nonanal was the only individual pheromone component that increased attraction significantly compared to α-pinene alone, and resulted in a 25% increase in response (Table 1). In experiment 2, the most attractive binary combinations of pheromones were nonanal plus α-pinene-oxide, nonanal plus *trans*-verbenol, and myrtenol plus α-pinene-oxide. These three combinations resulted in trap catches that were significantly higher than the blank control, while responses to α-pinene and all other combinations were intermediate (Table 2). In experiment 3, the complete blend of all four pheromone components and α-pinene resulted in the highest trap catches. The ternary combinations of nonanal, *trans*-verbenol, plus α-pinine-oxide, and nonanal, myrtenol, plus α-pinene-oxide also increased attraction significantly compared to unbaited controls while responses to all other combinations were intermediate (Table 3).

While these results provide further evidence of attraction by *T. piniperda* to the four pheromone components, increases in trap catches compared to α-pinene alone were not as high or significant as in previous studies which demonstrated up to 400% increase in attraction (Czokajlo 1998 and unpublished data). Cool spring conditions in 1999 resulted in a delayed and synchronous emergence flight by *T. piniperda*. Although treatment positions were re-randomized to provide additional replicates of each experiment, very few additional insects were captured. Therefore, our results are based on only 5 replicates of each experiment and placement effects (of the traps) could have influenced the results. It is also possible, that pheromone release rates were lower in the present studies compared with previous studies, since baits were placed in the field approximately 5 weeks before the delayed emergence flight by *T. piniperda* occurred. Release rates may decrease somewhat over time as the amount of pheromone remaining in the vial becomes depleted. In addition, rain can interfere with release rates by blocking the capillary tube through which volatiles are released from the pheromone bait vials. Nevertheless, in all experiments, the highest trap catches were found when various combinations of the pheromone components were added to α-pinene. In most cases, combinations containing nonanal resulted in the highest attraction.

In experiments 4 and 5, the addition of other host volatiles to α-pinene, significantly increased the number of *T. piniperda* adults captured. The combination of all three host volatiles (ethanol, terpinolene, and 3-carene) significantly increased attraction by 83% compared to α-pinene alone. Responses to each of the individual host volatiles combined with α-pinene were intermediate between α-pinene alone and the full blend (Table 4). The binary combination of terpinolene plus 3-carene significantly increased trap catches by 90% compared to α-pinene alone. The other binary combinations of host volatiles increased trap catches to levels that were intermediate between α-pinene alone and α-pinene plus terpinolene and 3-carene. (Table 5).

These results show that combinations of the three host volatiles increase attraction of *T. piniperda* to α-pinene. The binary combination of terpinolene and 3-carene would be sufficient to achieve increased attraction.

Due to the delayed and synchronous flight of *T. piniperda*, additional experiments planned to test combinations of the best pheromones and host volatiles in multiple-funnel traps and on trap logs could not be conducted in 1999. Experiments will be conducted in spring 2000 testing combinations of nonanal, α-pinene-oxide, *trans*-verbenol, terpinolene and 3-carene in traps and on trap logs. All experiments will be conducted simulataneously during the initial emergence flight to avoid problems associated with a potential sudden and synchronous emergence.

Table 1: α-pinene and individual pheromone components

Treatment	**Mean No. *T. piniperda* captured/trap**	
Blank control	1.0 ± 0.7	c*
α-pinene	189.6 ± 31	ab
α-pinene + α-pinene-oxide	132.8 ± 21	b
α-pinene + *trans*-verbenol	146.4 ± 30	ab
α-pinene + myrtenol	183.8 ± 30	ab
α-pinene + all	207.6 ± 27	ab
α-pinene + nonanal	237.2 ± 40	a

*Numbers followed by the same letter are not significantly different, Fisher's least-significant-difference test on data transformed by log (x+1), $P < .05$.

Table 2: α-pinene and binary combinations of pheromone components

Treatment	**Mean No. *T. piniperda* captured/trap**	
Blank control	0.6 ± 0.4	b*
α-pinene	95.8 ± 1.5	ab
α-pinene + all	94.6 ± 10	ab
α-pinene + myrtenol + *trans*-verbenol	90.2 ± 20	ab
α-pinene + myrtenol + nonanal	104.2 ± 24	ab
α-pinene + α-pinene-oxide + *trans*-verbenol	121.2 ± 35	ab
α-pinene + nonanal + α-pinene-oxide	132.8 ± 36	a
α-pinene + nonanal + *trans*-verbenol	150.6 ± 36	a
α-pinene + myrtenol + α-pinene-oxide	163.5 ± 47	a

*Numbers followed by the same letter are not significantly different, Fisher's least-significant-difference test on data transformed by log (x+1), $P < .05$.

Table 3: α-pinene and ternary combinations of pheromone components

Treatment	**Mean No. *T. piniperda* captured/trap**	
Blank control	0.2 ± 0.2	c*
α-pinene	89.6 ± 9	ab
α-pinene + myrtenol +nonanal + *trans*-verbenol	71.8 ± 22	b
α-pinene + myrtenol + nonanal + α-pinene-oxide	114.4 ± 25	ab
α-pinene + myrtenol + α-pinene-oxide + *trans*-verbenol	102.8 ± 22	ab
α-pinene + nonanal + α-pinene-oxide + *trans*-verbenol	118.2 ± 31	ab
α-pinene + all	124.0 ± 18	a

*Numbers followed by the same letter are not significantly different, Fisher's least-significant-difference test on data transformed by log (x+1), $P < .05$.

Table 4: α-pinene and individual host volatiles

Treatment	**Mean No. *T. piniperda* captured/trap**	
Blank control	0.6 ± 0.4	c*
α-pinene	110.4 ± 12	ab
α-pinene + 3-carene	89.8 ± 11	b
α-pinene + ethanol	164.0 ± 48	ab
α-pinene + terpinolene	120.8 ± 27	ab
α-pinene + all	202.5 ± 43	a

*Numbers followed by the same letter are not significantly different, Fisher's least-significant-difference test on data transformed by log (x+1), $P < .05$.

Table 5: α-pinene and binary combinations of host volatiles

Treatment	**Mean No. *T. piniperda* captured/trap**	
Blank control	0.8 ± 0.6	c*
α-pinene	85.8 ± 25	b
α-pinene + ethanol + terpinolene	153.8 ± 32	ab
α-pinene + ethanol + 3-carene	140.2 ± 21	ab
α-pinene + all	134.2 ± 27	ab
α-pinene + terpinolene + 3-carene	163.4 ± 34	a

*Numbers followed by the same letter are not significantly different, Fisher's least-significant-difference test on data transformed by log (x+1), $P < .05$.

Implications:

These results provide further evidence that the pheromone components, nonanal, α-pinene-oxide, *trans*-verbenol, and myrtenol, and the host volatiles, ethanol, terpinolene, and 3-carene, increase attraction of *T. piniperda* to α-pinene. The most attractive individual pheromone component appeared to be nonanal, and combinations of pheromones including nonanal may provide an optimal pheromone blend. The optimal host blend appears to be terpinolene and 3-carene. Further studies will be conducted in FY2000 by NC-4501 that will test a combined blend of pheromones and host volatiles at the same and higher release rates in traps and on trap logs in order to finalize the optimal attractive lure for *T. piniperda*. The ultimate goal of an improved lure would be to enhance trapping efficiency that leads to cost-effective methods for detecting and managing *T. piniperda* populations.

DOCUMENTATION: Results from this specific study have not yet been published. We hope that after the FY2000 field season, we will have sufficient data to write a formal journal article.

FIRST YEAR FUNDED: FY 1999.

YEAR SCHEDULED TO END: FY 1999

ACTUAL YEAR TO END: Products will be delivered in FY2000 or 2001.

PRODUCTS AND DUE DATES IDENTIFIED IN ORIGINAL PROPOSAL: Research results will be accomplished by end of FY1999, with publications being published during FY2000.

STATUS OF PRODUCTS: Project results from the 1999 field season have been analyzed. Further studies will be conducted in 2000. A journal manuscript will be written and submitted in 2000 or 2001. Prior to publication, project results will be disseminated to users through various outlets.

PROGRESS ON THIS PROJECT: Acceptable.

FUNDS OBLIGATED FROM BEGINNING OF PROJECT THROUGH END OF FY 98:

Fiscal Year	STDP funding	Contribution	Source organization
1999	20K	30K	USFS, NCRS, NC-4501, Insect Unit
		1K	SUNY, staff time
		1K	IPM Technologies, staff time

FUNDS CARRIED OVER FROM FY99 TO FY00: None.

POST-PROJECT TECHNOLOGY SUPPORT: Not needed.

LOOK to the FUTURE:

The 1999 field studies demonstrated that combinations of pheromones including nonanal and the host volatiles terpinolene and 3-carene increase attraction of *T. piniperda* to α-pinene. Further studies are needed to test a combined blend of pheromones and host volatiles. In addition, the results of this study suggest that higher release rates may be required for optimal attraction. Therefore, further studies will be conducted in FY2000 to further evaluate the optimal release rate for an improved lure for *T. piniperda.*