FINAL REPORT

1999 Project Abstract
For the Period Ending August 12, 2002

TITLE: NS06 Development and Assessment of Oak Wilt Control Technologies
PROJECT MANAGER: Dr. Jennifer Juzwik
ORGANIZATION: University of Minnesota
ADDRESS: 495 Borlaug Hall, St. Paul, MN 55108
FUND: Environmental Trust Fund
LEGAL CITATION: ML 1999, Ch. 231, Sec. 16, Subd. 14(d)

APPROPRIATION AMOUNT: $ 200,000

Efficacy of the biocontrol fungus, *Gliocladium roseum* (GR), on the availability of viable oak wilt (OW) spores for overland spread of oak wilt was determined. Of twelve GR isolates that eliminated oak wilt spores on GR treated lab cultures of the OW fungus, three were further tested in field trials. Two of these isolates yielded significant reductions (14 to 20%) in incidence of OW fungus isolation from spore-mat producing trees following GR spray treatment compared to non-treated trees during two spring trials. Two models were developed to predict the critical time of spore mat production using regression and mixed effects techniques. The models indicate that the number of mats and timing of their production are influenced by tree size and cambial condition and a variety of environmental variables including late winter and early spring temperature and precipitation. Sampling of OW mats and flight behavioral studies showed that the two principal beetle vectors of OW, *Colopterus truncatus* and *Carpophilus sayi*, likely have one generation per year. *Colopterus truncatus* flies between early April and early July; a large proportion of the population (15%) carry OW spores in mid-May. Aggregation pheromones to monitor the flight activities of both insect species are now commercially available to land managers, and in order to limit the overland spread of oak wilt, pruning and other management activities that wound oaks should be avoided during the flight period. Protocols utilizing GPS and GIS technologies were developed to evaluate effectiveness of root graft barrier (RGB) line placement on underground spread of OW and to compare effects of several line placement models on the remaining oak resource using computer generated maps. For 39 residential sites with RGB lines in Ham Lake, actual use of the French Model resulted in an 80% success rate while use of two other models would have theoretically resulted in a higher success rate, but many additional trees would have been sacrificed. Thus, "trade-offs" should be considered in selecting the appropriate model for use.

Date of Report: July 1, 2001
LCMR Final Work Program Report
Date of Work Program Approval: June 16, 1999
Project Completion Date: August 12, 2002

LCMR Work Program Update Report

I. PROJECT TITLE: NS06 Development and Assessment of Oak Wilt Control Technologies

Project Manager: Dr. Jennifer Juzwik
Affiliation: Adjunct Assistant Professor, Department of Plant Pathology, University of Minnesota, and USDA Research Plant Pathologist
Mailing Address: 495 Borlaug Hall, St. Paul, MN 55108
Telephone Number: (651) 649-5114 **E-Mail:** juzwi002@tc.umn.edu **Fax:** (612)625-9728

Web Page address: none

Total Biennial Project Budget:

LCMR:	$200,000	**$ Match:**	$0
$ LCMR Amount pent:	$125,265	**- $ Match Amount Spent:**	$0
$LCMR Balance:	$ 16,044	**= $Match Balance:**	$0

Legal Citation: ML 99, Chapter 231, Section 16, Subdivision 14(d).

Appropriation Language: Continuation $100,000 the first year and $100,000 the second year are from the trust fund to the University of Minnesota to evaluate biocontrol efficacy, spore mat production, and root graft barrier guidelines for oak wilt, in cooperation with the department of agriculture.

B. Status of Match Requirement: Not applicable.

II. and III. FINAL PROJECT SUMMARY

Efficacy of the biocontrol fungus, *Gliocladium roseum* (GR), on the availability of viable oak wilt (OW) spores for overland spread of oak wilt was determined. Of twelve GR isolates that eliminated oak wilt spores on GR treated lab cultures of the OW fungus, three were further tested in field trials. Two of these isolates yielded significant reductions (14 to 20%) in incidence of OW fungus isolation from spore-mat producing trees following GR spray treatment compared to non-treated trees during two spring trials. Two models were developed to predict the critical time of spore mat production using regression and mixed effects techniques. The models indicate that the number of mats and timing of their production are influenced by tree size and cambial condition and a variety of environmental variables including late winter and early spring temperature and precipitation. Sampling of OW mats and flight behavioral studies showed that the two principal beetle vectors of OW, *Colopterus truncatus* and *Carpophilus sayi*, likely have one generation per year. *Colopterus truncatus* flies between early April and early July; a large proportion of the population (15%) carry OW spores in mid-May. Aggregation pheromones to monitor the flight activities of both insect species are now commercially available to land

managers, and in order to limit the overland spread of oak wilt, pruning and other management activities that wound oaks should be avoided during the flight period. Protocols utilizing GPS and GIS technologies were developed to evaluate effectiveness of root graft barrier (RGB) line placement on underground spread of OW and to compare effects of several line placement models on the remaining oak resource using computer generated maps. For 39 residential sites with RGB lines in Ham Lake, actual use of the French Model resulted in an 80% success rate while use of two other models would have theoretically resulted in a higher success rate, but many additional trees would have been sacrificed. Thus, "trade-offs" should be considered in selecting the appropriate model for use.

IV. OUTLINE OF PROJECT RESULTS:

A. Result 1: **Efficacy report on biocontrol of overland spread of oak wilt.**

A.1 Summary: 1) Results of field and laboratory tests of the antibiosis and hyperparasitism potential of *Gliocladium roseum* on the oak wilt fungus - Nineteen isolates of *G. roseum* were screened for their ability to reduce the number of viable *C. fagacearum* propagules from single mating-type cultures. Twelve of these isolates produced significantly more *G. roseum* propagules than the remaining seven isolates. Ten of these twelve isolates prevented recovery of any viable *C. fagacearum* propagules. Protocol for producing sexual state fruiting bodies (perithecia) and viable ascospores were developed and tested. Four isolates (two of each mating type) of *C. fagacearum* were crossed in all possible mating combinations. Three of the combinations resulted in consistent production of perithecia and ascospores in culture. Treatment of these mated cultures in petri dishes with *G. roseum* resulted in total elimination of viable *C. fagacearum* propagules for all combinations of mated isolates tested. Finally, two field trials were conducted to determine the effect of *G. roseum* on *C. fagacearum* propagule viability and recovery from naturally occurring mats. Augmentation sprays with *G. roseum* were used to achieve different levels of mat colonization by that fungus. A higher frequency of subsamples (ave. 19%) from treated mats yielded *G. roseum* than those from non-treated mats (5%). Conversely, a lower frequency (59%) of subsamples from treated mats yielded *C. fagacearum* compared to subsamples from non-treated mats (76%). Therefore, *G. roseum* is likely contributing to natural control of overland spread of *C. fagacearum* by reducing inoculum availability. 2) Recommendations, guidelines, and pilot implementation for use of this agent in community-based oak wilt control programs – No further development of this biocontrol treatment is planned because no greater than a 20% colonization rate of oak wilt mats by *G. roseum* was achieved using the high-pressure sprayer used for the augmentation treatment in the field trials. A much higher coverage would be required to justify augmentation treatment with this biocontrol fungus.

A.2 Budget: LCMR Budget $60,000
Balance $ 809

A.3 Completion Date:
Final analyses and report (M.Sc. Thesis), September 15, 2001
(A copy of the thesis was submitted to LCMR in April 2002.)

Note:

1) A manuscript based on the thesis will be submitted to a scientific journal, Biological Control, for potential publication.
2) An abstract and a MS Powerpoint presentation were prepared for the 2002 national meeting of the American Phytopathological Society. Copies of both are attached to this document.

B. Result 2: **A model to predict the critical time of spore mat production**

B.1 Summary: Two models were developed to predict the critical time of spore mat production

using multiple linear regression and mixed effects modeling techniques. The analyses emphasized development of statistical models for predicting the accumulation of mats formed with respect to time or surrogates for time (e.g. growing degree-days) and the maximum number of mats produced. The models indicate that the number of mats and timing of their production are influenced by tree size and cambial condition and a variety of environmental variables including late winter and early spring temperature and precipitation. Several general findings were noted: 1) larger trees produced greater numbers of mats; 2) warmer late winter temperatures and wetter spring conditions lead to production of greater numbers of mats; 3) mat production is initiated earlier on smaller trees but also progresses more slowly; 4) mats occurring early in the season tend to occur at greater tree heights; and 5) the variability in numbers of mats and progress of mat production for individual trees is very large and is only partially explained by the models.

B.2 Budget: LCMR Budget $20,000
Balance $ 4

B.3 Completion date:
Final report, June 30, 2001

Note: The final report was submitted to LCMR on July 17, 2001. A manuscript based on results of this modeling effort will be prepared and submitted to a scientific journal for potential publication.

C. Result 3: **Life histories of principal insect vectors of the oak wilt fungus**

C.1 Summary: General characteristics for the identification of larval and adult sap beetles (Nitidulidae) were documented photographically and summarized in an extension pamphlet for use by forestry professionals, land managers, and homeowners (copy attached to this document). Using this taxonomic knowledge, we identified and counted larval and adult sap beetles from fruiting bodies (spore mats) from groups of oak wilt-killed trees in five sites near the Minneapolis/St. Paul metropolitan area during four collection periods (Fall 1999, Spring 2000, Fall 2000, and Spring 2001). Mats were sampled from 252 red oak and northern pin oak trees, and the mat areas were calculated using digital photography and computer software; sap beetle population densities were calculated. Larvae were present in 149 (~59%) mats; the oak wilt vectors, *Colopterus truncatus* were the most abundant larvae in spring mats, while *Epuraea corticina* were the most abundant larvae in fall mats. Adults occurred in 141 (~56%) mats. *Colopterus truncatus* and *Carpophilus sayi* were equally abundant as adults in spring mats, while *Colopterus truncatus* was the most common adult nitidulid species in fall mats. We found larvae of *Colopterus truncatus* and *Carpophilus sayi* only in the spring and this suggests that these major oak wilt vectors are likely to be univoltine (one generation per year). *Epuraea corticina* larvae were found in both the spring and fall, suggesting that this species is bivoltine. Flight behavioral assays using aggregation pheromones for *Colopterus truncatus* and *Carpophilus sayi* established both the seasonal flight patterns of these species and began to reveal new information on the incidence and spore load of the oak wilt fungus with the beetles. The aggregation pheromones are now available commercially (Great Lakes IPM, Vestaburg, MI) as a management tool for land managers. During a weekly survey over two seasons in the Minneapolis/St. Paul metropolitan area, adult *Colopterus truncatus* flew in response to its three-component pheromone between early April and early-July, with the maximum responses coming on May 4 (2000) and April 20 (2001). During both years, more than 98% of the beetles were trapped between April 14 and June 1. *Colopterus truncatus* responded without loss of activity to a simplified, two-component version of the pheromone. Chipped bark, phloem, and xylem from northern pin oak was not attractive to *Colopterus truncatus* in Minnesota. During the survey of its

flight behavior between April and June, 2001 in Minnesota, we trapped a total of 548 *Colopterus truncatus* of which 243 were homogenized and assayed for incidence and spore load of *C. fagacearum*. Overall incidence of *C. fagacearum* on trapped *Colopterus truncatus* was 13.6%. The mean spore load on the 33 individuals that tested positive for *C. fagacearum* was 9,366 ± 3,719 (range = 33 to 110,000). Both incidence and spore load are higher than previously published results from dispersing nitidulids, but this study differs from previous studies in that it utilized aggregation pheromone to attract and trap the beetles and that it focused on only one species of beetle. When examined over time, the incidence of *C. fagacearum* was initially 0% (April 20/27, n = 27/25), but then rose to approximately 15% (May 11/18, n = 36/41) before the main dispersal flight of beetles ended. Preliminary studies in 2001 of the flight behavior of *Carpophilus sayi* revealed that this species responded to a one-component pheromone combined with whole wheat bread dough. Most of the *Carpophilus sayi* (80.3%) flew between June 8 and July 27, but 13 beetles were trapped in late summer and fall, suggesting a wider flight period for this vector than for *Colopterus truncatus.* In 2002, weekly trapping of *Colopterus truncatus* and *Carpophilus sayi* is continuing to validate the flight periodicities for both species and to accumulate more information on oak wilt incidence and spore loads from both sexes of the species. A number of conclusions with practical implications for monitoring the flight of sap beetles and for managing overland spread of oak wilt can be drawn from this work. A low-cost two-component pheromone blend can be used effectively as a monitoring tool for *Colopterus truncatus*. As we recorded no response of *Colopterus truncatus* or other sap beetles to oak chips, operational studies in the future might explore whether landowners, resource managers, or arborists could chip woody debris from uninfected oak trees and leave the chips on site without attracting nitidulid oak wilt vectors to the general area and accelerating overland transmission of the disease. Finally, land managers should be aware that the critical period of flight activity for *Colopterus truncatus* in Minnesota occurs conservatively between early April and early July, and in order to limit the overland spread of oak wilt, pruning and other management activities that wound oaks should be avoided during this time.

Budget: LCMR Budget $60,000
Balance $ 284

C.3 Completion date:

Final publication of findings: Information on identification of sap beetles has been published in an extension pamphlet, information on flight periodicity of sap beetles is in press in a scientific paper in the Aug. 2002 issue of the *Journal of Chemical Ecology*, information on the population density of sap beetles is in manuscript form for a scientific paper for *Environmental Entomology,* and an M.Sc. thesis in Entomology is anticipated to be filed by December 31, 2002. Final submission of the thesis has been delayed significantly due to the relocation of the graduate student to Milwaukee, Wisconsin prior to his finishing the analyses and writing of the paper on population density. The PI for this project is working with the student "long-distance" to complete both.

Note:
1) Page proofs of the scientific article for the *Journal of Chemical Ecology* are attached to this report.
2) The brochure describing how to identify nitidulids associated with oak wilt mats in Minnesota is attached to this report.
3) Reflecting the wide dissemination of these research results, abstracts from presentations at the following meetings are also attached: Multistate Research Project W-189 (July 2001, oral

presentation), Multistate Research Project W-187 (October 2001, oral presentation), the North Central Forest Pest Work Conference (October 2001, poster presentation), and the National Meeting of the American Phytopathological Society (July 2002, poster presentation).

D. Result 4: **Root graft barrier guidelines for line placement in residential settings**

D.1 Summary: Protocols utilizing GPS and GIS technologies, a digital compass, a laser range finder, and a field computer were developed to evaluate effectiveness of root graft barrier (RGB) line placement on below ground spread of OW and to compare effects of several line placement models on the remaining oak resource using computer generated maps. For 39 residential sites with RGB lines in Ham Lake, actual use of the French Model resulted in an 80% success rate while use of two other models (the Bruhn model and a Fixed Radius Model) would have theoretically resulted in a higher success rate, but many additional trees would have been sacrificed. Thus, oak wilt control specialists should consider the "trade-offs" when selecting the most appropriate model to use in different situations.

D.2 Budget: LCMR Budget $60,000
Balance $14,947

Reason for significant budget variance – Early in the 1999-2001 biennium additional, non-state funds ($27,000) were obtained to supplement the funds already appropriated by the State Legislature via LCMR. These additional funds covered salary for a seasonal technical assistant in 2000, purchase of GPS unit, and purchase of a laser range finder and digital compass, thus reducing the total encumbrances to the LCMR-based budget.

D.3 Completion date:

Final publication of findings (M.Sc. Paper, Plan B), anticipated date - December 31, 2002
Note: Submission of final thesis has been delayed significantly due to the relocation of the graduate student to Alaska prior to his finishing the analyses and writing of his final paper. The PI for this project is working with the student "long-distance" to complete both.

V. DISSEMINATION: Dissemination of research findings will be made through oral presentations to potential users (e.g. consulting foresters and arborists, pest managers), publications such as M.Sc. theses, shade tree newsletter and/or trade journal articles, and publication in peer-reviewed scientific journals. Additional dissemination through the worldwide web is anticipated. Forest Service cooperator plans to develop a national oak wilt web site in 1999. Progress and final reports of these research studies would be posted here as well.

VI. CONTEXT

A. **Significance**: Oak wilt remains the number one cause of oak mortality in Minnesota. Urban sprawl, including development of residential housing, business/industrial parks, roads and utilities in oak woods and savanna areas all are taking their toll on existing oak populations through construction damage and by accelerating the spread of oak wilt. These impacts are falling most heavily in the prime oak habitats of East Central and southeastern Minnesota. Unfortunately, gains we have made in the 7 county metro area do nothing to help stem the new wave of destruction now gaining force in our oak lands to the east and south. These research and development studies are all designed to mitigate the inevitable impacts of urbanization through improved management practices and technologies especially in these new areas where help is needed most. However, they will be equally applicable and valuable in oak wilt control in all oak habitats from urban to rural. This research builds on and expands that of LCMR Project:

Biological Control of Overland Spread of Oak Wilt - 13b.

B. Time: These projects build on and consolidate much earlier work done by Dr. David French at th University of Minnesota and recent work by the Program Manager and cooperators listed below. All products with the possible exceptions of final publications (i.e. M.Sc. thesis) for the insect life history studies and the root graft barrier development work will be completed by June 2001.

C. Budget Context:

"Biological Control of Overland Spread of Oak Wilt - 13b"

1995-97:	LCMR Budget History	$90,000	
(1997-98)	Non-LCMR Budget History	$88,000	(does not include state and federal in-kind and staff)
	Total	$178,000	

1. Budget by Results

*** Result 1 BUDGET:**

Personnel	$ 41,281 (Graduate Research Assistant, 2 yrs, 50% time);
	$11,771 seasonal Student Laboratory Assistant, 13 wk/yr)
	$ 2,000 (statistical assistant)
Equipment	$ 0
Contract	$ 1235 (professional tree climber to collect samples)
Acquisition	$ 0
Development	$ 0
Other	$ 2,664 (2,100 for spray unit; 300 for telescopic spray wand; 100 for field tools and supplies; remainder for expendable laboratory and office supplies
	$ 1049 (travel expenses for national plant pathology meeting)
TOTAL	$ 60,000

*** Result 2 BUDGET:**

Personnel	$ 19,850 (Research Fellow - 25% time)
Equipment	$ 0
Acquisition	$ 0
Development	$ 0
Other	$ 0
	$ 150 (site license for SAS, a statistical program)
TOTAL	$ 20,000

*** Result 3 BUDGET:**

Personnel	$ 9,945 (Principal Investigator, 10%, 2 yr);
	$ 26,975 (Graduate Research Assistant, 35%, 2 yr);
	$ 16,293 (Student Laboratory Assistant, 100% for 20 wk; 25% for 36 wk)
Equipment	$ 0
Acquisition	$ 0
Development	$ 0
Other	$ 3286 (Expendable laboratory supplies and field tools); $ 3,500 (Travel expenses, e.g. vehicle rental and/or mileage, lodging, meals, regional
TOTAL	$ 60,000

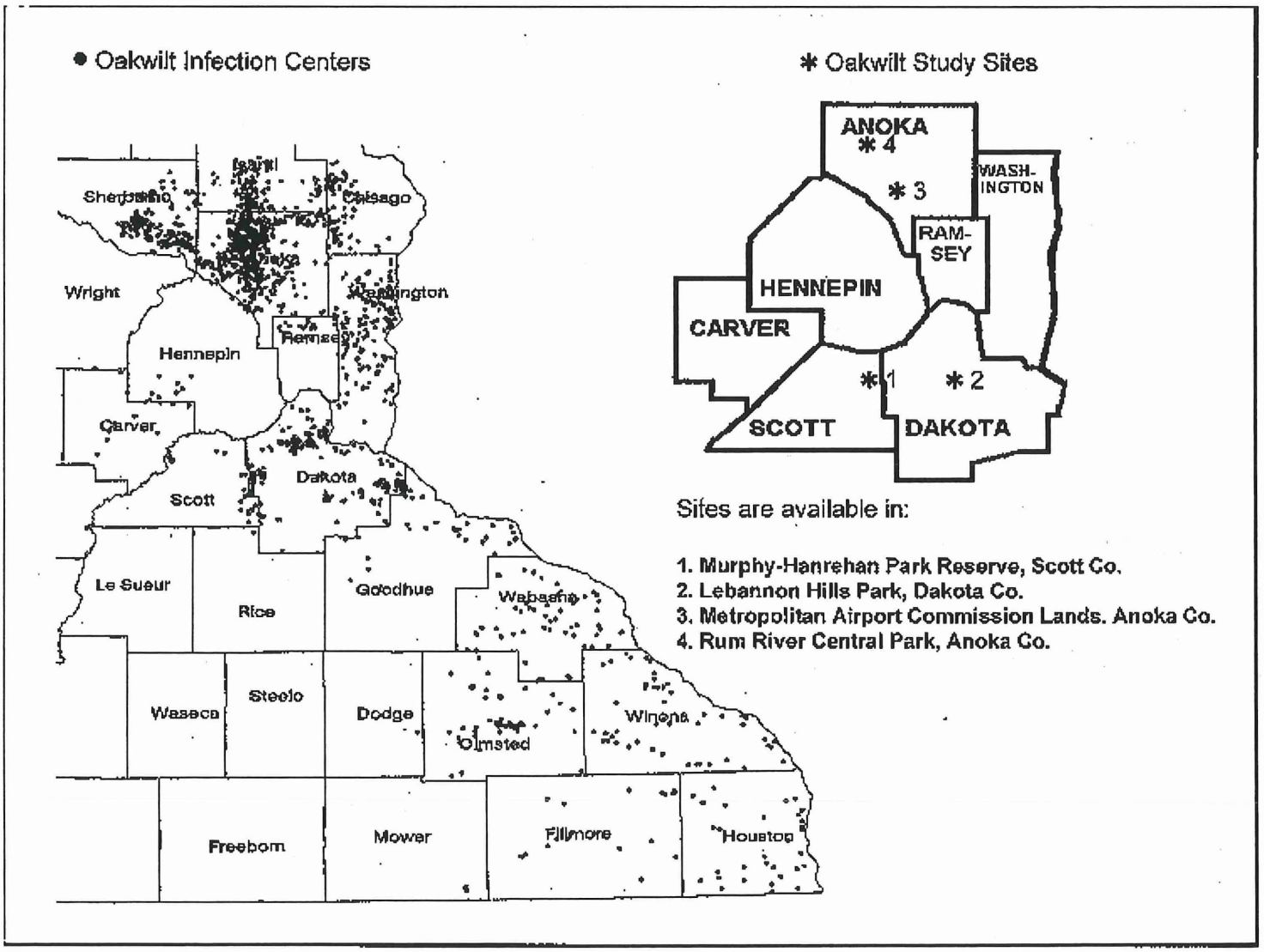
*** Result 4 BUDGET:**

Personnel	$ 39,629 (Primary Graduate Research Assistant, 50%, 2 yr); $ 4,409 (Secondary Graduate Research Assistant, 25%, 6 mo) $ 8,657 (Principal Investigator, 30%, 6 mo)
Equipment	$ 2,755 (desktop PC, printer)
Acquisition	$ 0
Development	$ 0
Other	$ 2,000 (Expendable field, laboratory, computer software, and office supplies); $ 2,600 (Travel expenses, e.g. vehicle rental and/or mileage and use charges, regional pest management meeting)
TOTAL	$ 60,000

Total Project Request (dollars requested from LCMR): $200,000

All Results: Personnel: $173,000
All Results: Equipment: $10,000
All Results: Development: $ 0
All Results: Acquisition: $ 0
All Results: Other: $17,000

2 **Budget detail summarizing for the above four results** - See attachment



Map #1, Oakwilt LCMR

X

XI. Reporting Requirements:

Periodic workprogram progress reports will be submitted not later than 31 December 1999, 30 June 2000, and 31 December 2000. A final workprogram report and associated products will be submitted by June 30, 2001, or by the completion date as set in the appropriation. Final research products (i.e. M.Sc. theses) for Result 1 and for Results 3 and 4 will be not be submitted until 15 September and 31 December 2001, respectively.

X. Research projects: See attached Addendum

Attachment A Deliverable Products and Related Budget					
LCMR Project Biennial Budget		Objective/Result			
	Result 1	Result 2	Result 3	Result 4	
Budget Item	Biocontrol of oak wilt	Predicting mat production	Vector life histories	Root graft Barriers	ROW TOTAL
Wages, salaries & benefits - Be specific on who is paid $	$ 41,281 (Graduate student) $ 11,771 (Seasonal technical assistant) $ 2,000 (statistical assistant)	$19,850(Part-time Research Fellow)	$9,945 (Principal Investigator) $ 26,975 (Graduate student) $ 16,294 (lab and field technical assistant)	$ 39,579 (Primary Graduate student) $ 4,409 (Secondary Graduate student) $ 8,657 (Principal Investigator)	$180,761
Space rental, maintenance & utilities	0	0	0	0	0
Printing & Advertising	0	0	366	0	366
Communications, telephone, mail, etc.	0	0	0	0	0
Contracts	0	0	0	0	0
Professional/technical	$ 1235	0	0	0	1235
Other contracts	0	0	0	0	0
Local automobile mileage paid	0	0	80	1,975	2,055
Other travel expenses in Minnesota	0	0	3,340	325	3,665
Travel outside Minnesota	$ 1,049	0	0	300	1,349
Office Supplies	0	0		500	500
Other Supplies	$ 2664	150	$ 3,000	$ 1,500	$ 7,314
Tools and equipment	0	0	0	0	0
Office equipment & computers	0	0	0	$ 2,755	$ 2,755
Other Capital equipment	0	0	0	0	0
Other direct operating costs	0	0	0	0	0
Land acquisition	0	0	0	0	0
Land rights acquisition	0	0	0	0	0
Buildings or other land improvement	0	0	0	0	0
Legal fees	0	0	0	0	0
COLUMN TOTAL	$60,000	$20,000	$60,000	$60,000	$200,000

ADDENDUM

Additional Information on Individual Research Projects

Result 1: **Efficacy report on biocontrol of overland spread of oak wilt**

I Abstract

Laboratory and operational level field trials are proposed to test the hypothesis that a naturally occurring fungal hyperparasite of oak wilt mats, *Gliocladium roseum*, significantly reduces the availability of the oak wilt fungus, *Ceratocystis fagacearum*, for acquisition and transmission by sap-feeding beetles. Augmentation sprays of maturing oak mats with aqueous suspensions of *G. roseum* could prove to be a valuable tool in reducing the level of pathogen inoculum on recently killed oak producing oak wilt spore mats during spring.

II Background and Hypothesis

Oak wilt is a major disease of oaks in the eastern USA and the leading cause of oak mortality in Minnesota. Oak wilt mats formed on recently killed oaks are the inoculum source for the primary insect vectors of the pathogen, *Ceratocystis fagacearum*, in the North Central states of the USA. These insects (Coleoptera: Nitidulidae) are attracted by the sweet odor of the fungal mats. After traversing and feeding on the mats, the beetles leave with pathogen spores on and in their bodies and may transmit *C. fagacearum* to fresh wounds on healthy oaks. Other organisms often colonize the fungal mats and the xylem tissue adjacent to the mats. The *Graphium* anamorph of *Ophiostoma quercus* (formerly *O. piceae*) is a common colonizer of spring mats in Minnesota and West Virginia, and has been considered a putative biocontrol agent of overland transmission of the pathogen. Recently completed research partially funded by LCMR (1995-97 biennium) demonstrated, however, that overgrowth of mats by *O. quercus* did not negatively impact acquisition of *C. fagacearum* by sap-feeding beetles associated with the mats. Other associated fungi have been shown to affect pathogen viability in laboratory studies. For example, *Gliocladium roseum* and *Trichothecium roseum* prevented growth or caused mycelial death of *C. fagacearum*. USDA Forest Service and University of Minnesota cooperators initiated laboratory studies with these two biocontrol candidates in 1997. Colonization of single mating type oak wilt cultures by certain isolates of *G. roseum* and *T. roseum* drastically reduced availability of viable *C. fagacearum* propagules in petri dish investigations. *T. roseum* isolates, however, were more unpredictable in their biocontrol ability and the dry, powdery nature of the spores render them more difficult to use in aqeous suspensions. For these reasons, efforts were then focused on promising isolates of *G. roseum*. Because of the common occurrence of the sexual stage (perithecia) of *C. fagacearum* on oak wilt mats and mucilaginous nature of the exuded sexual spores from these fruiting structures (making these spores particularly well suited for insect dispersal),

further laboratory testing of the effect of *G. roseum* on ascigerous or paired mating type cultures of *C. fagacearum* is needed.

A small-scale, experimental augmentation spray trial was undertaken in spring 1998 with the three most promising *G. roseum* biocontrol candidates. Based on positive results with two isolates in that trial, larger scale (i.e. operational level) field trials are needed to further develop *G. roseum* as a potential biocontrol tool for preventing inoculum acquisition by insects visiting oak wilt mats during the critical spring period when oaks are highly susceptible to infection.

III Methodology

Laboratory study. Isolates representing the two mating types of *Ceratocystis fagacearum* will be used to produce perithecia with ascospores in petri dish cultures. Spore suspension (0.2 ml) of selected *Gliocladium* isolates will be pipetted in a line across the diameter of each ascospore producing *C. fagacearum* colony. Following 10-12 days incubation (24°C, dark conditions), visual rating of *Gliocladium* overgrowth of *C. fagacearum* colonies will be recorded. Four 3 mm diameter mycelial plugs will be removed from two will be subjected to serial dilution plating to assess: 1) number of viable *C. fagacearum* propagules remaining in *Gliocladium* colonized area, and 2) number of viable *Gliocladium* propagules also present. Visual evaluation of spore presence will be conducted with the remaining two mycelial plugs from each petri dish culture. The number of ascospores and conidia of *C. fagacearum* in an aqueous suspension of plug washings will be determined using a hemacytometer. Data will be analyzed using standard ANOVA and categorical methods.

Field study. Candidate oak wilt study areas will be selected in summer 1999. The specific sites will depend on willingness of township, municipality or parks to participate in the operational trial. Red oaks dying from the oak wilt fungus in July and August will be marked as candidate study trees. Cambial condition of these trees will be determined in late March 2000. Those in the appropriate condition will then be monitored for onset of oak wilt mat production. The goal is to treat 15 trees with open spore mats with an aqueous suspension of *Gliocladium.* An additional 10 to 15 trees will remain untreated and serve as controls.

G *roseum* inoculum will be produced according to protocol developed for the1998 experimental trial. Timing of application will be based on peak production of oak wilt mats on the study trees. A known concentration of *G. roseum* will be mixed in the 50 gallon tank of an ATV mounted spray rig, or a spray truck depending on field conditions. The suspension will be applied to achieve run-off on the main stem and branches to a maximum of 50 feet in height. QA/QC activity will involve attaching open petri dishes with solid agar medium at different heights on study trees prior to treatment. These plates are analyzed for the number of colony-forming-units which can be compared to the predetermined concentration of the spray mixture to calculate percent coverage.

Oak wilt mats that are exposed on the spray date will be collected 7 days after spray. Sub-samples of these mats will then be analyzed through serial plating methods for both viable *C. fagacearum* and *G. roseum* propagules. Isolation of C. fagacearum and G. roseum from each sub-sample based on colony-forming units observed will be recorded. Colonization and fungal recovery data will be analyzed using standard ANOVA and categorical methods.

IV Results and Products and V. Timetable

The results of the laboratory investigation will be summarized as a data report for submission to LCMR by 30 June 2000. These results will be combined with those from the earlier single mating type culture experiments and prepared in manuscript form for submission to a peer-reviewed scientific journal. The results of the large scale field trial will be summarized as a data report for submission to LCMR by 31 December 2000. The field trial results will be combined with those of the earlier small scale trial and prepared for submission to a peer-reviewed scientific journal. It is anticipated that a final report for both laboratory and field studies will be included in a M.Sc. thesis by September 15, 2001.

VI. Budget Requirements (LCMR)

Personnel	$ 41,281 (Graduate Research Assistant, 2 yrs, 50% time); $ 11,771 (seasonal Student Laboratory Assistant, 13 wk/yr) $ 2,000 (statistical assistant)
Equipment	$ 0
Contract	$ 1235 (professional tree climber to collect samples)
Acquisition	$ 0
Development	$ 0
Other	$ 2,664 (2,100 for spray unit; 300 for telescopic spray wand; 100 for field tools and supplies; remainder for expendable laboratory supplies); $ 1049 (travel expenses for national pathology meeting)
TOTAL	$ 60,000

VII Principal Investigators and Cooperators

Principal Investigator: Dr. Jennifer Juzwik (brief resume attached)
Graduate student: Marc Neuman

CURRICULUM VITAE

Name and Title: Jennifer Juzwik, Adjunct Professor and USDA Research Plant Pathologist

Address: North Central Forest Experiment Station
USDA Forest Service
1992 Folwell Avenue
St. Paul, MN 55108

Education:

Ph.D.	1983	University of Minnesota	Plant Pathology
M.Sc.	1978	Colorado State University	Plant Pathology
B.Sc.	1976	Fairmont State College	Biology

Professional Experience:

1997 - present	Adjunct Assistant Professor, Department of Plant Pathology, University of Minnesota, St. Paul, MN
1989 - present	Research Plant Pathologist, USDA Forest Service, St. Paul, MN
1984 - 1989	Provincial Forest Pathologist, Ontario Ministry of Natural Resources, Sault Ste. Marie, Ontario, Canada
1983 - 1984	Post-Doctoral Research Fellow, University of Toronto, Canada

Professional Societies and Committees:
American Phytopathological Society, Canadian Phytopathological Society, Gamma Sigma Delta, Sigma Xi, Northeastern Nursery Association, Minnesota Shade Tree Advisory Committee, Minnesota Society of Arboriculture.

Specialization and Research Interest:
Overland transmission of oak wilt
Management of soil-borne diseases in bare-root forest nurseries
Interaction between biotic and abiotic factors in tree disease development and management

Selected Relevant Publications:

Cease, K.R.; Juzwik, J.; Skalbeck, T.C. 1997. Nitidulid species associated with oak wilt fungal mats in Minnesota. Phytopathology 87:S16.

Juzwik, J.; French, D.W. 1983. *Ceratocystis fagacearum* and *C. piceae* on the surfaces of free-flying and fungus mat-inhabiting nitidulids. Phytopathology. 73:1164-1168.

Juzwik, J.; French, D.W; Jeresek, J. 1985. Overland spread of the oak wilt fungus in Minnesota. J. Arboric. 11:232-327.

Juzwik, J.; French, D.W. 1986. Relationship between nitidulids and *Ceratocystis fagacearum* during the late summer and autumn in Minnesota. Plant Dis. 70:424-426.

Juzwik, J.; Skalbeck, T.C.; Cease, K.R. 1996. Overland spread of *Ceratocystis fagacearum* by nitidulids: a different perspective. P. 104. *In:* Proc. North American Forest Insect Work Conference, April 8-12, 1996. San Antonio, TX. Texas For. Serv. Pub. 160.

Juzwik, J.; and Meyer, J.M. 1997. Colonization of oak wilt fungal mats by *Ophiostoma piceae* during spring in Minnesota. Plant Dis. 81:410-414.

Juzwik, J.; Cease, K.R.; and Meyer, J.M. 1998. Aquisition of *Ophiostoma piceae* and *Ceratocystis fagacearum* by nitidulids from *O. piceae* oak wilt fungal mats. Plant Dis. 82:239-243.

Result 2: A model to predict the critical time of spore mat production

I Abstract

The intent of the research project is to elucidate relationships between variables related to environmental and tree size conditions and variables related to the production of oak wilt mats mats that bear the *Ceratocystis fagacearum* inoculum. This fungal inoculum is then available for insect vector acquisition and subsequent overland spread of oak wilt. The analyses will be conducted in three phases: 1) graphical analyses to discover basic relationships; 2) descriptive statistical analyses to characterize means and variances of variables related to mat production, and 3) construction of models that characterize the relationships between the two sets of variables.

II Background and hypothesis.

The underlying objective of the research is to determine the environmental conditions that trigger initial spring production of the inoculum responsible for overland spread of oak wilt. In addition, questions of amount of inoculum produced and the duration of production are also to be investigated. The primary hypothesis is that the triggering condition is related to heat with a secondary hypothesis that duration is related to tree size.

III Analytical methodology

The methodology will consist of three phases. The first phase will consist of graphical analyses. For each year and for each observation interval within years, the number of new mats observed for each tree and the total number of new mats observed for all trees will be plotted against both ambient and cambial temperature and against a measure of cumulative ambient and cambial temperature such as growing degree days. The second phase will consist of basic descriptive analyses in which means and standard deviations of the date of initial mat production, temperature and cumulative temperature at initial mat production, total number of mats, and the duration of mat production will be calculated for each tree across years. In addition, analyses of variance will be conducted to compare means with respect to factors such as location, year, and tree size. The third phase will consist of correlation and/or regression analyses for the purpose of discovering relationships among the variables related to mat production and variables related to environmental conditions and size.

IV Results and products

The primary intended products are statistical models of the relationships between variables related to mat production and environmental variables. If the models cannot be formulated in terms of mathematical relationships between variables, then the models will be formulated in terms of means, medians, and standard

deviation.

V Timetable

Graphical analyses and descriptive statistics report, June 30, 2000
Model report, December 31, 2000
Final report, June 30, 2001

VI Budget requirements

Baseline data for this modeling project was collected by cooperator, Dr. Jennifer Juzwik, between the end of March and early July in each of three years (1997-98 completed and currently underway in1999). All necessary computer hardware, graphical software, and data base software to support the research are currently available. Analytical software that is not also available in the form of statistical packages, e.g. SAS, will be developed and programmed. Thus, all expected costs will be in the form of salary and will be allocated to data base construction, data entry and editing, graphical and statistical analyses, software development, consultations, and report writing. A Masters degree level statistician working under the supervision of the Principal Investigator will accomplish the tasks. Overall, 25 weeks are anticipated for completion of the three analytical phases.

BUDGET: 1

Personnel	$ 19,850 (Research Fellow, 25% time for 2 years).
Equipment	$ 0
Acquisition	$ 0
Development	$ 0
Other	$ 150 (Site license for SAS, a statistical program)
TOTAL	$ 20,000

VII Principal Investigator

Dr. Ronald McRoberts, Mathematical Statistician, USDA Forest Service, and Adjunct Professor, Dept. of Forest Resources, University of Minnesota (resume is attached)

CURRICLUM VITAE

Name and Title: Ronald E. McRoberts, Mathematical Statistician

Address: North Central Forest Experiment Station
USDA Forest Service
1992 Folwell Avenue
St Paul, MN 55108

Education:

PhD	1984	University of Minnesota	Biostatistics
MS	1978	University of Minnesota	Biostatistics
BA	1969	University of Minnesota	Mathematics

Professional Experience:
1977 to Present - Mathematical Statistician, North Central Forest Experiment Station, USDA Forest Service, St. Paul, MN

1977 to 1980 - Instructor of Mathematics, Northwestern College, Roseville, MN

1974 to 1977 - Applications Programmer, Department of Neurosurgery, University of Minnesota

Professional Societies and Organizations:
American Statistical Association, Eastern North American Region of the International Biometric Society, Sigma Xi, Midwest Forest Mensurationists, IUFRO S4.11-00: Statistical methods, mathematics, and computers, IUFRO S6.06-02: Philosophy and methods of forest research

Selected Publications:
Juzwik, J., Stenlund, D.L., Allmaras, R.R., Copeland, S.M. and McRoberts, R.E. 1996. Incorporation of tracers and Dazomet by rotary tillers and a spading machine. Soil and Tillage Research.

Stevens, C., Palmer, M.A., Tang, A.Y. and McRoberts, R.E. 1990. Use of aminopepidase sustrate specifications and discriminant analysis to identify species of *Cylindrocladium* in Wisconsin nurseries. Mycologia 82:436-443.

Palmer, M.A., McRoberts, R.E., and Nicholls, T.H. 1988. Sources of inoculum of *Sphaeropsis sapinea* in forest tree nurseries. Phytopathology 78:831-835.

Robbins, K., Jackson, W.A., and McRoberts, R.E. 1988. White pine blister rust in the eastern upper peninsula of Michigan. No. J. Appl. For. 5.263-264.

Ostry, M.E., McRoberts, R.E., Ward, K.T. and Resendez, R. 1988. Screening hybrid poplars in vitro for resistance to leaf spot caused by *Septoria musiva*. Plant Disease (June 1988):497- 499.

Result 3: Life histories of principal insect vectors of the oak wilt fungus

I Abstract

Research is proposed to confirm and investigate the life histories of the major species of sap beetles associated with the overland transmission of the oak wilt fungus. Currently three beetle species are recognized to comprise the complex interacting with the disease in Minnesota. Information gained from an understanding of the basic biology of these beetles will be used to design vector-based strategies to curtail the overland spread of the disease. An understanding of the basic biology of these beetles is also a necessary first step for isolating host attractants and pheromones that might be important technological tools in manipulating the vector populations.

II Background and Hypothesis

In preliminary studies, adult sap beetles in the family Nitidulidae have been linked to the spread of the oak wilt fungus in Minnesota. In general order of significance as vectors, the three species identified to date are: *Colopterus truncatus*, *Epuraea corticina*, *and Carpophilus sayi*. *C. truncatus* and *C. sayi* interact with the disease by transporting fungal spores from mycelial mats on infected trees to wounds in healthy trees. *E. corticina* is important in the spermatization of perithecial initials on a mat when carrying spores of the opposite mating type of *C. fagacearum* on their bodies. Thus, this species apparently moves from mat to mat in fulfilling this role. The mucilaginous matrix of the sexual spores (ascospores) makes this spore type especially well-suited for insect transmission.

In general, the oak wilt mats provide food and shelter for larval and adult nitidulids as well as breeding locations for adults. Thus, the mats provide an aggregation site for these beetles. Previous studies have demonstrated that *C. truncatus* and *E. corticina* breed in large numbers in newly formed mats. *C. truncatus* and *C. sayi* naturally contaminated with *C. fagacearum* spores were found to be the most common visitors of fresh (24 to 72 hr old) wounds on healthy oaks. The significance of *C. truncatus* as perhaps the most significant vector is underscored by its abundance in mature mats and on fresh wounds. It is not yet known whether *C. truncatus* is important as spermatizing agent of the fungus on mats. Because of its importance, most of our life history work will focus on this species.

Our hypothesis is that the aforementioned species may have life history attributes that make them particularly good vectors of oak wilt. For example, their host location and aggregation behaviors in spring, when oaks are most susceptible to infection, may be different from later season behaviors. In addition, other life history attributes of these beetles might be exploited as weak links to interrupt the disease cycle. Understanding the attributes of a given species and the differences between species may allow us to design

management strategies to disrupt the normal host finding, aggregation, and breeding behaviors of these nitidulids.

III Methodology

We plan to use the following methods in our life history studies:

A Sampling populations of nitidulid larvae, pupae, and adults from oak wilt mats by removing sections 6 cm^2 of mat tissue from each mat. The number of sections removed will be proportionately related to the size of the individual mats (e.g. one section per 100 cm^2 of mat surface). Samples will be collected sequentially over time and at various sites to establish life history data for each species. Larvae and adults will be compared with specimens in the museum collection at the University of Minnesota and sent to specialists for verification as well. Contemporaneously with the mat sampling, newly created wounds on oaks will be sampled for nitdulids to establish whether the populations are simultaneously colonizing mats and wounds or whether there is a temporal or spatial shift in the colonization behavior of these beetles. If enough beetles can be reared in culture, the insects may be dusted with fluorescent powder and released to see if the same cohort of insects will colonize mats and fresh wounds.

B Sampling populations of nitidulid larvae, pupae, and adults from forest floor litter by removing 625 cm^2 sections of litter around oaks and sampling flying adults in flight traps. Specimens will be handled as described above. These samples will help to delineate the dispersal and flight period of these insects.

C Laboratory rearing of immatures from field-collected populations during the flight period will be conducted. Immature nitidulids will be reared on fungal mats or suitable substrates in small chambers in the lab. The purpose of this method will be to establish the developmental time of each species under controlled environmental conditions.

D Sampling and rearing data will be compiled into summary tables and analyzed statistically to look for significant differences in developmental times and life history activities between species and seasons.

IV Description of Results

A Assess sampling data of natural populations of the principal vector, *C. truncatus* (and other species as time allows), to construct a life table including mortality factors and duration of developmental stages under native conditions.

B Assess laboratory-rearing studies of *C. truncatus* (and other species where feasible) to evaluate the duration of developmental stages under controlled conditions.

C Assess the ecological relationship of the principal nitidulid species, the oak wilt fungus, and oaks in terms of season, site, and weather factors. This will be achieved by correlating the life history attributes of the

nitidulids with the disease conditions of the tree and the above-mentioned abiotic factors.

D Infer management and control strategies for the nitidulid vectors from life history and ecological attributes revealed in A through C.

V Timetable

Data report on laboratory studies, December 31, 2000
Data report on natural populations, December 31, 2000
Report on ecological relationships, June 30, 2001
Vector management and control strategies, June 30, 2001
Final work program report, June 30, 2001
Final publication report (M.Sc. thesis), December 31, 2001

VI Budget (LCMR)

Personnel	$ 9,945 (Principal Investigator); $ 26,975 (Graduate Research Assistant, 35, 2 yr); $ 16,293 (Student Laboratory Assistant, 100% for 20 wk; 25% for 36 wk)
Equipment	$ 0
Acquisition	$ 0
Development	$ 0
Other	$ 3,286 (Expendable laboratory supplies and field tools); $ 3,500 (Travel expenses, e.g. vehicle mileage, lodging, meals, regional pest management meeting)
TOTAL	$ 60,000

VII. Principal Investigators

Dr. Steven Seybold, Assistant Professor of Forest Entomology, University of Minnesota, St. Paul
Dr. Thomas Skalbeck, Visiting Assistant Professor, University of Minnesota, St. Paul

Resumes for these individuals are attached.

Sub-Project 1, NSO6 LCMK Project, 1777 - 2001

coat protein of these eight isolates showed amino acid identities between 37 and 89% and contained several conserved residues found in rod-shaped viruses like *Tobacco mosaic* and *Tobacco rattle viruses*. Phylogenetic trees based on the entire RNA-2 revealed three clusters. Two of the African PCVs appear to cluster more strongly with the Indian IPCVs than with the other African isolates. The data indicates that there is substantial divergence among the RNA-2 genomes of PCV and IPCV isolates.

Cloning and sequence analysis of the genome of beet mosaic potyvirus. L. G. NEMCHINOV and R. W. Hammond. USDA-ARS, Molecular Plant Pathology Laboratory, Beltsville, MD 20705. Phytopathology 92:S59. Publication no. P-2002-0424-AMA.

Beet mosaic virus (BtMV), a member of the economically important Potyvirus group, was first reported in *Beta vulgaris* in Germany in the late 1950s. It is distributed worldwide in all major beet growing areas. To better understand the molecular biology of the virus, we have determined the nucleotide sequence of cloned viral cDNA. BtMV has a single open reading frame typical for *Potyviruses*. Amino acid sequences of the CI RNA helicase, nuclear inclusion (NIa) protein, RNA-dependent RNA polymerase (RdRp), and capsid protein (CP) show significant similarities to homologous sequences from other potyviruses. Putative proteolytic cleavage sites for the NIa protease and the group-specific signature at the NIa-NIb junction were predicted by analogy to consensus sequences and genome arrangements of potyviruses. The RdRp identifiers common for positive-strand RNA viruses are also conserved in BtMV. The DAG motif, important for aphid transmission in *Potyviruses*, is present near the N terminus of the CP. BtMV appears to be a distinct species of genus *Potyvirus* with the most closely related species being *Peanut Mottle Virus* (approximately 60% amino acid identity).

Role of *Clonostachys rosea* in preventing the overland transmission of *Ceratocystis fagacearum*. M. F. NEUMAN and J. Juzwik. Dept. Plant Pathology, Univ. of Minnesota and USDA Forest Service, St. Paul, MN 55108. Phytopathology 92:S59. Publication no. P-2002-0425-AMA.

The overland spread of oak wilt results from acquisition and successful transmission of *Ceratocystis fagacearum* spores from fungal mats to fresh wounds on healthy oaks by insects. Secondary microbes, such as *Clonostachys rosea*, frequently colonize mats. In our previous *in vitro* studies, overgrowth of *C. fagacearum* colonies by *C. rosea* prevented recovery of viable *C. fagacearum* propagules. Two field trials were conducted to determine the effect of *C. rosea* on *C. fagacearum* propagule viability and recovery from naturally occurring mats. Augmentation sprays with *C. rosea* were used to achieve different amounts of mat colonization by that fungus. A higher frequency of subsamples (19% of 1332) from treated mats yielded *C. rosea* than those from non-treated mats (5% of 516). Conversely, a lower frequency (49% of 1332) of subsamples from treated mats yielded *C. fagacearum* compared to subsamples from non-treated mats (68% of 516). Therefore, *C. rosea* is likely contributing to natural control of overland spread of *C. fagacearum* by reducing inoculum availability.

Isolation, identification, and detection of undescribed sweetpotato viruses. S. L. New, J. A. Abad, and J. W. MOYER. Dept. Plant Pathology, North Carolina State University. Phytopathology 92:S59. Publication no. P-2002-0426-AMA.

A study was initiated to detect the presence of undescribed viruses affecting sweetpotatoes. Novel sweetpotato viruses are difficult to isolate due to the presence of SPFMV; therefore, an alternative strategy was developed. Double stranded (ds) RNA was extracted from two sweetpotato cvs, 'Beauregard' (GWB) and 'White Bunch' (WB), and three additional cvs. with 'russet crack' disease symptoms. Using dsRNA as a template, RT-PCR was performed with universal degenerate primers for poytyviruses. The amplicons were cloned into pGemT vector (Promega, Madison. WI). After screening the library, clones were sequenced and compared with known sequences. From 18 clones obtained from GWB, six were closely related to SPFMV-RC, five were homologous with SPFMV-C and seven were related to sweetpotato virus G. These results show not only the efficacy of our system but also demonstrate that strains of the same virus (SPFMV RC and C) are coinfecting the same host, suggesting that they may be different viruses. The isolation of each virus in a selected host range and the identification of undescribed viruses in WB and plants with 'russet crack' is underway.

Implication of stigmatic exudate in infection of blueberry flowers by *Monilinia vaccinii-corymbosi*. H. K. NGUGI and H. Scherm. Dept. of Plant Pathology, University of Georgia, Athens 30602. Phytopathology 92:S59. Publication no. P-2002-0427-AMA.

Infection of blueberry flowers by *Monilinia vaccinii-corymbosi*, which causes mummy berry disease, occurs when conidia germinate on the stigmatic surface, followed by hyphal ingress into the stylar canal and subsequent colonization of the ovary. We have previously shown that while no germination occurs in deionized water (dH_2O), the exudate produced on the wet stigmata of blueberry flowers strongly enhances germination of *M. vaccinii-corymbosi* conidia *in vitro*. In further experiments, germination increased up to 77.9% as exudate concentration in dH_2O was increased. Germination also occurred in tap water and in 0.2% sucrose in dH_2O but at levels significantly lower than in dH_2O containing exudate. The exudate also enhanced germination or hyphal growth rates in seven fungal species non-pathogenic to blueberry, but the effect was not as pronounced as in *M. vaccinii-corymbosi*. On detached flowers, removal of the exudate by washing reduced hyphal growth and style necrosis following inoculation with *M. vaccinii-corymbosi*. Exudate utilization and potential implications for directional guidance of hyphal growth within the stylar canal will be discussed.

Further characterization of the HG type classification test for soybean cyst nematode field populations. T. L. NIBLACK (1), R. D. Riggs (2), J. Wang (3), and R. D. Heinz (3). (1) Dept. Crop Sciences, University of Illinois, Urbana, IL 61801; (2) Dept. Plant Pathology, University of Arkansas, Fayetteville, AR 72701; (3) Dept. Plant Microbiology and Pathology, University of Missouri, Columbia, MO 65211. Phytopathology 92:S59. Publication no. P-2002-0428-AMA.

The HG Type classification system is a tool for characterizing genetically diverse populations of *Heterodera glycines*, the soybean cyst nematode (SCN). HG Type classification is based on a bioassay very similar to that used for race classification except in two important aspects: 1) 'Pickett' is not included in the list of soybean lines used for HG Type designation; and 2) HG Types are defined by SCN development on seven soybean lines rather than four. Both HG Types and races are determined on the basis of a Female Index (FI), calculated as follows: (mean number of females on test soybean line / mean number of females on standard susceptible) × 100. We compared HG Type and race classification on over 100 SCN populations to determine their relative utility for making cultivar recommendations to farmers. We tested the use of alternative susceptible cultivars and found that designation of one standard, Lee 74, is essential for repeatability of HG Type tests. We also tested the use of eggs per cyst and cysts per gram root as an alternative to the FI and found that neither is generally worth the additional effort involved in obtaining such numbers.

Weaponry revealed: secreted proteins of *Erwinia amylovora*. R. M. NISSINEN (1), K. J. van Wijk (2), J. A. Ytterberg (2), T. Thannhauser (3), and S. V. Beer (1). (1) Dept. Plant Pathology; (2) Dept. Plant Biology; (3) Biotechnology Center, Cornell University, Ithaca, NY 14853. Phytopathology 92:S59. Publication no. P-2002-0429-AMA.

The pathogenicity of *Erwinia amylovora*, the causal agent of fire blight, depends on the chromosomal *hrp/dsp* gene cluster, located on a pathogenicity island of 66 kbp. We aimed to isolate and identify proteins secreted via the Hrp pathway by a microproteomics approach. We isolated secreted proteins from culture supernatants of several *E. amylovora* strains and mutants grown under *hrp*-inducing conditions. The secreted proteins were resolved by 2-D-PAGE, and proteins were identified by MALDI-TOF-MS. Clear differences were seen between protein maps of wild-type strain Ea273 and mutants impaired in Hrp-secretion or in *hrp*-gene induction indicating that at least 15 proteins are secreted via the *E. amylovora* Hrp pathway under apoplast-mimicking conditions. A promoter trap library was developed to isolate genes that are induced in apoplast-mimicking conditions, including the genes encoding the secreted proteins.

Comparison of the spatial pattern of two foliar diseases of strawberry. M. NITA and L. V. Madden. Department of Plant Pathology, The Ohio State University, OARDC Wooster, OH 44691. Phytopathology 92:S59. Publication no. P-2002-0430-AMA.

Spatial distributions were determined over 3 yr for two foliar diseases, Phomopsis leaf blight (causal agent: *Phomopsis obscurans*), and powdery mildew (causal agent: *Sphaerotheca macularis*), occurring in the same commercial strawberry fields. Incidence of each disease was recorded from n = 15 leaflets in each of N = 100 (10 by 10 grid) evenly spaced sampling units. Degree of heterogeneity was measured with the *theta* parameter of the beta-binomial distribution (an indication of small-scale pattern), and degree of spatial correlation among sampling units was measured with the SADIE *Ia* index (an indication of larger scale patterns). Tests of *Ia* showed significant aggregation for all powdery mildew data sets, but for less than half of the Phomopsis data sets. Both *theta* and *Ia* were higher for powdery mildew than for Phomopsis in all data sets. Combined statistical results indicated that:

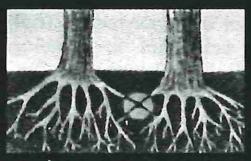
Slides from Oral Presentation
American Phytopathological Society Meeting
July 2002

Sub-Project 1
NSCG, LCMR
Project
1999-2001

Role of *Clonostachys rosea* in Preventing the Overland Transmission of *Ceratocystis fagacearum*

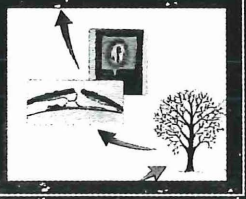
Marc F. Neuman, Dept. Plant Pathology, Univ. Of Minnesota, and
JENNIFER JUZWIK, North Central Research Station, USDA-Forest Service

Transmission of Oak Wilt

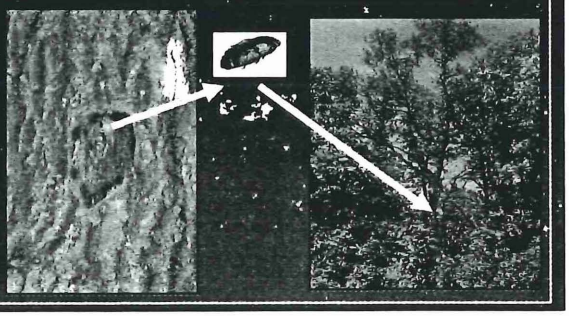


Below-ground

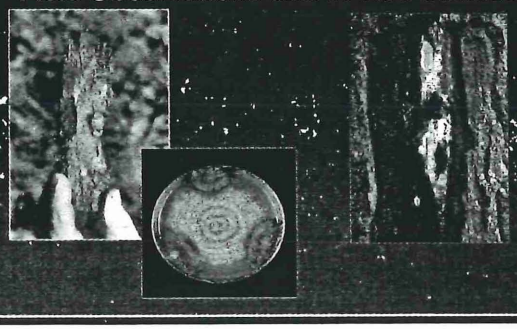
Above-ground



Overland Transmission of Oak Wilt



Field Observations and *In Vitro* Studies



Research Objective

- To determine the effect of *Clonostachys rosea* on *Ceratocystis fagacearum* viability and recovery from naturally occurring oak wilt mats.

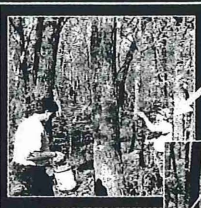
METHODS

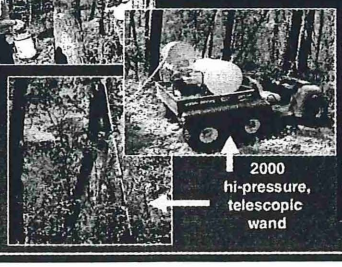
DESIGN

- 2700 acre area with several sites
- Three *C. rosea* isolates
- Two year study

TREE SELECTION

- Potential mat producing trees





TREATMENTS

- Three *C. rosea* isolate from *in vivo* study
- Control

•APPLICATION

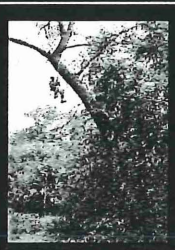
- Low pressure, hand-held sprayer
- High pressure, ATV mounted sprayer

SAMPLE COLLECTION & STORAGE

- Oak wilt mats mechanically excised
- Wrapped and stored at 2 C

LAB PROCESSING

- Sub-samples taken
- Serial dilution plating
- Resulting colonies of CR and CF recorded



RESULTS

Extent of CR colonization of mats achieved with sprays

Treatment	No. Trees producing mats	Total No. Mats	No. mats with 0 to 6 CR positive subsamples				Statistical result
			0	1 to 2	3 to 4	5 to 6	
1998							
CR 171	4	44	18	18	5	3	a
CR 362	3	45	23	16	4	2	a
CR 10622	3	23	12	5	5	1	a
none	4	50	40	8	2	0	b
2000							
CR 171	4	60	43	9	8	0	ab
CR 362	3	31	17	6	1	7	a
CR 10622	4	25	19	6	0	0	bc
none	3	36	40	4	0	1	c

Frequency of CR isolation from mat subsamples

Treatment	Mat Sub-Samples	
	Total no.	% positive
1998		
CR 171	264	22 a
CR 362	270	16 a
CR 10622	138	22 a
Control	300	5 b
2000		
CR 171	330	12 b
CR 362	180	22 a
CR 10622	150	4 c
Control	216	5 c

Extent of CF recovery from oak wilt mats in study

Treatment	No. Trees producing mats	Total No. Mats	No. mats with 0 to 6 CF positive subsamples				Statistical result
1998			0	1 to 2	3 to 4	5 to 6	
CR 171	4	44	4	15	15	10	a
CR 362	3	48	3	6	13	23	a
CR 10622	3	23	2	10	8	3	a
none	4	50	3	3	16	28	b
2000							
CR 171	4	41	20	7	6	8	ab
CR 362	3	17	9	3	4	1	a
CR 10622	4	19	12	0	3	4	bc
none	3	17	6	3	5	3	c

Frequency of CF isolation from mat subsamples

Treatment	Mat Sub-Samples	
	Total no.	% positive
1998		
CR 171	264	49 d
CR 362	270	67 b
CR 10622	138	62 c
Control	300	76 a
2000		
CR 171	330	36 b
CR 362	180	44 b
CR 10622	150	38 b
Control	216	56 a

Numbers of CFU of *C. rosea* and *C. fagacearum*

Treatment	*Clonostachys rosea*		*Ceratocystis fagacearum*	
	No. mats	Ave. cfu/cm^2	No. mats	Ave. cfu/cm^2
1998		(x 10^5)		(x 10^5)
CR 171	26	258 a	40	191 a
CR362	22	127 b	42	240 a
CR 10622	11	125 b	21	205 a
Control	10	52 c	48	315 a
2000				
CR 171	17	601 ab	35	135 a
CR362	14	792 a	22	263 a
CR 10622	6	113 c	17	144 ab
Control	5	181 bc	30	78 b

CONCLUSIONS

- Different levels of mat colonization by CR were achieved, but actual coverage was low.
- Higher frequencies of sub-samples from treated mats yielded more CR and less CF compared to controls.
- Therefore, CR is likely contributing to natural control of overland spread of CF by reducing inoculum availability.

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SEMIOCHEMICAL-MEDIATED FLIGHT RESPONSES OF SAP BEETLE VECTORS OF OAK WILT, *Ceratocystis fagacearum*

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Abstract—The sap beetle, *Colopterus truncatus* (Coleoptera: Nitidulidae), is one of the primary vectors of the oak wilt pathogen, *Ceratocystis fagacearum*, in the north-central United States. Field behavioral assays utilizing various release rates and blends of three methyl-branched hydrocarbon aggregation pheromone components showed that flight responses of this beetle were similar in Illinois and Minnesota populations. In both locations, both sexes of the beetle responded synergistically to a combination of the three-component pheromone and fermenting whole-wheat bread dough. Further, *Colopterus truncatus* preferred a high release rate over a low release rate of the three-component blend. In both locations, the response of *C. truncatus* to a simplified version of the pheromone consisting of (2*E*,4*E*,6*E*)-3,5-dimethyl-2,4,6-octatriene (**1**) and (2*E*,4*E*,6*E*,8*E*)-3,5,7-trimethyl-2,4,6,8-decatetraene (**3**) was not significantly different from the response to the three component blend. An experiment in Illinois with all possible combinations of the components demonstrated that the decatetraene (**3**) was the crucial component in the blend; of all treatments, the maximal response was elicited by **3** + dough. Chipped bark, phloem, and xylem from northern pin oak, *Quercus ellipsoidalis*, was not attractive to *C. truncatus* in Minnesota. During a weekly survey over two seasons in Minnesota, *C. truncatus* flew in response to the three-component pheromone between early April and early July, with the maximum responses coming on May 4, 2000 and April 20, 2001. During

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both years, more than 98% of the beetles were trapped between April 14 and June 1. During the same survey, *Glischrochilus* spp. (Nitidulidae) flew during longer periods of the summer, particularly in 2001. The sex ratio of *C. truncatus* responding during all experiments was female-biased (1.8:1, female–male), which is characteristic of other male-produced coleopteran aggregation pheromones. Other sap beetles that play a minor role in the pathobiology of *C. fagacearum* also responded in experiments conducted in Minnesota. *Carpophilus brachypterus* Say was cross-attracted to the two- and three-component blends of the *C. truncatus* pheromone and dough, whereas two *Glischrochilus* spp. were attracted to all treatments that contained dough.

Key Words—*Colopterus truncatus*, oak wilt, *Ceratocystis fagacearum*, aggregation pheromone, Coleoptera, Nitidulidae, sap beetle, *Glischrochilus* spp., *Carpophilus brachypterus*, monitoring, phenology.

INTRODUCTION

Oak wilt, *Ceratocystis fagacearum*, Bretz (Hunt), is a fungal disease that annually kills many oak trees (Fagaceae: *Quercus* spp.) across a large area in the eastern and north-central United States (Tainter and Baker, 1996). For example, by the mid-1990s, aerial photographs recorded 3000 oak wilt infection centers (areas) in Minnesota that contained 100,000 recently killed trees (French, 1995). This disease has had a significant effect on the vegetative composition of natural, periurban, and urban forests and is the disease of most concern to forest health specialists in 5 of the 22 states in which it occurs (Billings, 2000).

One way in which new oak wilt infection centers are established in the landscape is through the dispersal of oak wilt spores during the feeding and reproductive activities of sap beetles (Coleoptera: Nitidulidae). These insects are attracted to and aggregate on oak wilt fungal fruiting tissues (mats) that occur on oaks recently killed by the pathogen (Lin and Phelan, 1992; Cease and Juzwik, 2001). The beetles feed, mate, and oviposit on the mats from which oak wilt fungal propagules are ingested and accumulate on the cuticular surfaces of the adults and larvae (Curl, 1955; Jewell, 1956; Juzwik, 1983). Spore-laden sap beetles also aggregate, feed, mate, and oviposit in sap fluxes associated with fresh wounds on uninfected oak trees, thereby propagating the disease (Jewell, 1956). The response of sap beetles to wounded oak tissue can be remarkably rapid; some species may be attracted to experimentally created wounds within 15 min (J.J., personal observation). In one study, up to 78% of sap beetles that responded to wounds carried viable oak wilt spores in or on their bodies (Juzwik et al., 1999). Behaviors associated with exposure to inoculum and the visitation of fresh wounds have led to the identification of two sap beetles, *Colopterus truncatus* (Randall) and *Carpophilus sayi* (Parsons), as the primary vectors of oak wilt in the north central United States (Juzwik et al., 1999; Cease and Juzwik, 2001).

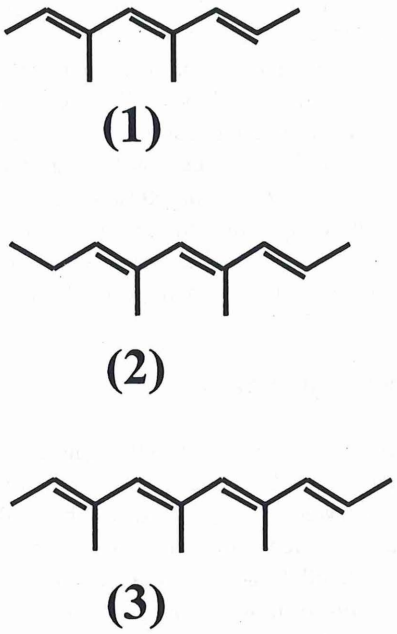

FIG. 1. Hydrocarbon components of the male-produced *Colopterus truncatus* aggregation pheromone: (**1**) (2*E*,4*E*,6*E*)-3,5-dimethyl-2,4,6-octatriene, (**2**) (2*E*,4*E*,6*E*)-4,6-dimethyl-2,4,6-nonatriene, and (**3**) (2*E*,4*E*,6*E*,8*E*)-3,5,7-trimethyl-2,4,6,8-decatetraene.

In order to improve our understanding of the life history of *C. truncatus*, we investigated the response of this insect and associated sap beetles to semiochemicals in oak forests of Illinois and Minnesota. Little is known about the chemical ecology of the response of *C. truncatus* to oak wilt mats or to wounded oaks in the field. However, a three-component, male-produced aggregation pheromone was recently isolated and identified from *C. truncatus* (Cossé and Bartelt, 2000). Three methyl-branched hydrocarbons, (2*E*,4*E*,6*E*)-3,5-dimethyl-2,4,6-octatriene (**1**), (2*E*,4*E*,6*E*)-4,6-dimethyl-2,4,6-nonatriene (**2**), and (2*E*,4*E*,6*E*,8*E*)-3,5,7-trimethyl-2,4,6,8-decatetraene (**3**) (Figure 1), elicited electroantennographic activity from male and female antennae, and when combined with fermenting whole-wheat bread dough as a trap bait, synthetic hydrocarbon **2**, the combination of **1** and **3**, and the combination of **1**, **2**, and **3**, all elicited flight responses from both sexes that significantly exceeded the response to dough alone (Cossé and Bartelt, 2000). The nonatriene (**2**) was the most abundant of the three components; volatiles collected from males indicated emission rates of 1.8:100:3.3 for **1**, **2**, and **3**, respectively. However, Cossé and Bartelt (2000) did not unequivocally establish the relative significance of the components for the flight response of *C. truncatus*,

particularly in light of the differences in volatility of components **2** and **3** after they had been loaded on rubber septa for slow release in the field.

In this study, we report new information on the chemical ecology of *C. truncatus* and other sap beetles associated with oak wilt. The specific goals of this study were to: (1) determine and compare the responses of *C. truncatus* to its pheromone at two different locations in the north-central United States; (2) optimize the blend and release rate of the *C. truncatus* synthetic aggregation pheromone components and explore the potential of specific synergy of the pheromone with oak tissue; and (3) determine the flight period of *C. truncatus* in oak forests in Minnesota to more clearly define the high risk seasonal period for overland transmission of oak wilt.

METHODS AND MATERIALS

Semiochemicals and Baits. As attractants, we used the synthetic aggregation pheromone components for *C. truncatus* [**1**, **2**, and **3** (Cossé and Bartelt, 2000) (Figure 1)] combined with fermenting whole-wheat bread dough or oak chips. Our standard attractant for *C. truncatus* consisted of dough in a plastic cup (see below) and 3.5, 500, and 280 μg each of the all *E* isomers of **1**, **2**, and **3**, respectively, applied in hexane to a rubber septum. Solution concentrations were quantified by capillary GC using an internal standard, and all pheromone components were formulated at the USDA-ARS laboratory in Peoria (Cossé and Bartelt, 2000). With these initial amounts of **1**, **2**, and **3** loaded on a septum, laboratory analyses have indicated that the proportions released during the first day from a trap are very close to those emitted by male beetles (Cossé and Bartelt, 2000). In two experiments, the treated septa were placed within 1.5 ml polyethylene Eppendorf microcentrifuge tubes each with a single pinhole in the lid (low release rate). Otherwise, the treated septa were pinned directly to the trap (high release rate).

The synthetic compounds had ca. 80% chemical purity and after passage through silica gel with hexane to remove polar contaminants, the remaining impurities were small amounts of *Z* isomers. The *Z* isomers result both as artifacts of synthesis and from slow isomerization of the *E* isomers, and there is presently no simple, large-scale method available for their removal. However, with other nitidulids, small amounts of *Z* isomers have not significantly reduced trap catch (Bartelt, 1999).

When compound **3** is synthesized (Bartelt et al., 1990), about 1–2% of the product consistently is compound **1** (which arises because a Wittig-Horner condensation does not quite go to completion). When a 100:1 mixture of **3** and **1** is applied to a rubber septum, **3** and **1** are released with an initial ratio of about 5:1 instead of 100:1 because of the impurity and because of their different boiling points (Cossé and Bartelt, 2000). Thus, synthetic **3** on a rubber septum will deliver both **3** and **1** into the air at approximately the same ratio as from the male beetles

(3.3:1.8). No complication of this sort arises with **2** because neither **3** nor **1** are produced in its synthesis, nor is **2** produced in the synthesis of **1** or **3**.

For one experiment (IV), samples of **1**, **2**, and **3** were purified further so that they could be tested individually and in all possible combinations. Synthetic **3** was purified by HPLC on a $AgNO_3$-coated silica column (Heath and Sonnet, 1980), using 0.5% 1-hexene in hexane as the solvent. The procedure removed all traces of **1** and a substantial fraction of the *Z* isomers [final chemical purity 90%, by capillary GC, instrumentation as in Cossé and Bartelt (2000)]. The major triene **2** was similarly purified to >90% by HPLC; the purity of minor triene **1** was 81%. After HPLC purification, this sample was free of compounds **2** and **3**.

Fermenting whole-wheat bread dough (dough) was used in these experiments because it is an attractive food bait to a variety of nitidulid species and because it has also been shown to act as a synergist for all nitidulid pheromones tested (Bartelt, 1997). Dough was prepared in the laboratory between 2 and 6 hr before use and consisted of whole-wheat flour, table sugar, water, and baker's yeast (3:1:3–4:0.1). Approximately 10–15 ml of dough was placed in a 35-ml plastic cup, and the cup was placed in the trap.

To test for the possibility of attraction of *C. truncatus* to volatiles produced by oaks, tissue was collected from a healthy northern pin oak, *Quercus ellipsoidalis* (E. J. Hill), in mid-April 2001. Live branches (<6 cm diam.) were cut and chipped using a chipper/shredder. All branch chips were mixed together and stored in heavy plastic bags in a −55°C freezer until they were placed in the field.

Traps and Field Sites. All studies used wind-oriented funnel traps (Dowd et al., 1992), which were hung from tree branches or posts ca. 1–1.5 m above the ground. Traps were placed at least 10 m apart. In one experiment, the traps were slightly modified to hold approximately 300 ml of oak chips. In Illinois, all studies were conducted in a single mixed hardwood forest near East Peoria (40.7°N latitude, 89.8°W longitude), while in Minnesota, four separate northern red/white oak (*Quercus rubra* L./*Quercus alba* L.) forests near Minneapolis-St. Paul were used as field sites. All four Minnesota field sites were separated by at least 5 km: Carlos Avery Wildlife Management Area (45.4°N latitude, 93.0°W longitude), a private residence in Columbus township (45.2°N latitude, 93.1°W longitude), Katherine Abbott Park in Mahtomedi (45.1°N latitude, 93.0°W longitude), and a private residence in Lake Elmo (45.0°N latitude, 92.9°W longitude). If more than one block was located at a site, blocks were separated by at least 100 m.

Field Bioassays. Six experiments were conducted in this study. They took place in Illinois in 2000 and in Minnesota in 2000 and 2001.

Experiment I. A study was conducted from late March to mid-April, 2000 in Illinois to establish the importance of the dough coattractant and to determine which of two pheromone release rates was most attractive to *C. truncatus*. The experiment consisted of: (1) a high release rate of **1** + **2** + **3**, (2) a low release rate of **1** + **2** + **3**, (3) dough, (4) a high release rate of **1** + **2** + **3** + dough, (5) a low

release rate of **1** + **2** + **3** + dough, and (6) an unbaited control. The experiment was initiated on March 24 with two completely randomized blocks (six traps per block), and the traps were emptied semiweekly until April 18. Treatment locations were rerandomized within blocks and dough baits were replaced after each trap was emptied. Pheromone baits were replaced weekly, but the replacement was staggered so that it was done in just one of the blocks after each trap check. Thus, after March 28 pheromone baits of two ages were always present, and in principle, changes in bait attractiveness over time would not be completely confounded with day effects.

Experiment II. Experiment I (Illinois) was duplicated in 2000 in Minnesota with the additional goal of monitoring the seasonal flight periodicity of *C. truncatus*. The same six treatments and the same trap type from experiment I were used. Experiment II was initiated on April 14 in a completely randomized block design in each of four field sites. Traps were emptied, rerandomized, and rebaited weekly until November 3. Traps were not baited in a staggered fashion as described in experiment I.

Experiment III. In 2001, the response to different treatments and the seasonal flight periodicity of *C. truncatus* were again monitored in Minnesota. This experiment consisted of: (1) a high release rate of **1** + **2** + **3** + dough, (2) dough, and (3) an unbaited control. The experiment was initiated on April 5 and continued until November 9. Sites, rebaiting, and rerandomization schedules were the same as in 2000.

Experiment IV. In 2000, in a second experiment in Illinois, the effects of **1**, **2**, and **3** on the response of *C. truncatus* were studied. In this case, the compounds were released individually and in all possible combinations. The amounts of each component loaded on the septa were 3.5, 500, and 280 μg, respectively, no matter what combination was tested. Based on the results of experiment I, high release rates of **1**, **2**, and **3** were always combined with dough. The experiment consisted of: (1) **1** + dough, (2) **2** + dough, (3) **3** + dough, (4) **1** + **2** + dough, (5) **1** + **3** + dough, (6) **2** + **3** + dough, (7) **1** + **2** + **3** + dough, and (8) dough alone control. The experiment was established as two completely randomized blocks, each with eight traps, in the same woodlot used for experiment I. Traps were set on posts at a height of 1 m and were baited fresh at ca. 07:00 AM on mornings with a favorable weather forecast (i.e., temperature >15°C, with minimal wind and cloud cover). As peak flight activity of *C. truncatus* occurs in mid- to late afternoon (R.J.B., personal observation), traps were emptied either near dusk or early the following morning. Treatment locations were rerandomized within blocks each day. Seven replications of the experiment were run between April 21 and May 5, 2000, and each replication was of short duration to ensure that the component ratios and emission rates of the pheromone components would be essentially stable throughout the study.

Experiment V. Based on the results of experiment IV in Illinois, an experiment was conducted in 2001 in Minnesota (same four sites) to compare responses of

C. truncatus to the high release rate of **1** + **2** + **3** and to the high release rate of **1** + **3**. As in experiment IV, the amounts of **1**, **2**, and **3** loaded on the septa were always 3.5, 500, and 280 μg, respectively. However, in this instance, component **3** also consisted of 1–2% of component **1**. The experiment consisted of: (1) **1** + **2** + **3** + dough, (2) **1** + **3** + dough, (3) dough, and (4) an unbaited control. Traps were hung from trees at a height of 1.5 m, baited fresh between 07:00 and 10:00 AM on days with a favorable weather forecast (as above), and emptied 24 hr later. This experiment was conducted for eight days between April 28 and May 31.

Experiment VI. An experiment was conducted in 2001 in Minnesota to compare responses of *C. truncatus* to oak chips (i.e., their volatiles) with responses to oak chips plus the high release rate of **1** + **3**. The experiment consisted of: (1) **1** + **3** + 300 ml oak chips, (2) 300 ml oak chips, and (3) an unbaited control. The experiment was set up as completely randomized blocks in each of the four previously described sites. Traps were placed, emptied, rebaited, and rerandomized on the same schedule and dates as in experiment V. The oak chips were replaced daily, and the previous day's chips were always moist when they were removed from the traps, increasing the likelihood that volatiles were still being released.

Statistical Analyses. Data were tabulated by species and sex, with the exception of experiment II, in which the sexes of responding *C. truncatus* were not determined. Nitidulid beetles other than *C. truncatus* were also tallied in the Minnesota experiments. Data from all experiments were analyzed by Friedman's nonparametric two-way ANOVA. Data from experiment IV also were analyzed by standard two-way ANOVA, after transformation of the trap-catch counts by log $(X + 1)$, because several linear contrasts were of particular interest. When F statistics were significant, means were compared using LSD (Bonferroni's method) with an experiment-wise $\alpha = 0.05$. Since trap catches of *C. truncatus* dropped off dramatically after mid-June in Minnesota, statistical analyses of responses of this species in 2000 and 2001 were only carried out on data collected during the mid-April to the mid-June periods. In some cases, analyses of trap catches of other nitidulid species spanned the entire trapping season. Voucher specimens from Minnesota trap catches were deposited in the University of Minnesota Insect Collection.

RESULTS

Experiment I. The high release rate of **1** + **2** + **3** + dough was the most attractive treatment to *C. truncatus* (Friedman's $\chi^2 = 367.1$, $df = 5$, $P < 0.001$; Figure 2A). The high release rate of **1** + **2** + **3** and dough were clearly synergistic; without the dough, *C. truncatus* did not respond to the high release rate of **1** + **2** + **3**, and dough alone attracted about one sixth the mean number of *C. truncatus* that responded to the combined treatments. There was no difference between the responses of *C. truncatus* to dough alone and to dough combined with **1** + **2** + **3** released at the low rate. Overall, trap catches were too low to take

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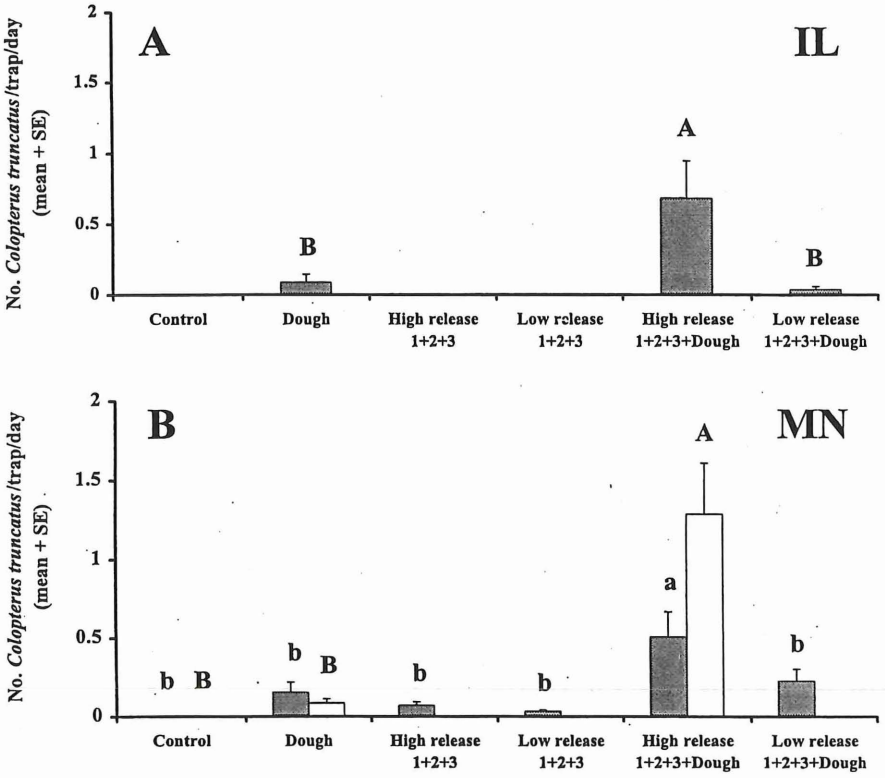

FIG. 2. Flight response of *Colopterus truncatus* to high and low release rates of synthetic aggregation pheromone components **1** + **2** + **3**, with and without dough, from (A) March 24 to April 18, 2000 in Illinois (experiment I) and from (B) April 4 to June 15, 2000, (dark bars) and April 5 to June 15, 2001 (open bars) in Minnesota (experiments II and III). Responses to treatments are significantly different if histogram bars within a year are marked with different letters.

advantage of the information from staggered pheromone replacement. Both sexes of *C. truncatus* (31 females and 21 males) responded in this experiment, and the sex ratio was not different among treatments ($\chi^2 = 0.07$, $df = 5$, $P = 0.97$). No *C. truncatus* were collected in control traps.

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Experiments II and III. Colopterus truncatus responded to the two release rates of its aggregation pheromone in Minnesota as it did in Illinois (Figure 2B). In 2000, the high release rate of **1** + **2** + **3** combined with dough was clearly the most attractive treatment to *C. truncatus* (Friedman's $\chi^2 = 3{,}414.9$, $df = 5$, $P < 0.001$; Figure 2B). In experiments II, III, V, and VI, there were no effects for site or the site × treatment interaction for any of the taxa that responded. In experiments

I and IV, only one site was used. As in Illinois, the responses of *C. truncatus* to dough alone and to the high release rate of **1** + **2** + **3** alone were low and did not differ from the response to dough alone or to the unbaited trap. However, when the dough and high release rate of aggregation pheromone were combined, the response increased more than threefold above the response to the high release rate of **1** + **2** + **3** alone, indicating that the treatments were synergistic. Both treatments involving the low release rate of **1** + **2** + **3** elicited responses that were not different from the response to the unbaited trap. However, when the low release rate of **1** + **2** + **3** was combined with the dough, the response was only exceeded by the comparable high release rate treatment. In 2001, the high release rate of **1** + **2** + **3** + dough was the most attractive treatment to *C. truncatus* (Friedman's $\chi^2 = 363.7$, $df = 2$, $P < 0.001$; Figure 2B). Although the numbers of trapping days were nearly identical, overall *C. truncatus* trap catch was nearly twice as great in 2001 (385) when compared to 2000 (206). Both sexes of *C. truncatus* responded in 2001 (the only year sex ratio data were collected); 247 females and 138 males were captured, and the sex ratio did not vary across treatments ($\chi^2 = 0.28$, $df = 2$, $P = 0.59$). No *C. truncatus* were collected in control traps during either year.

Two other nitidulid beetles, *Glischrochilus quadrisignatus* (Say) and *G. fasciatus* (Olivier), responded to the treatments in experiments II and III in a different way than did *C. truncatus* (Figure 3). In both years, these species responded to all traps containing dough at a higher level than those without dough (2000: Friedman's $\chi^2 = 3{,}722$, $df = 5$, $P < 0.001$; 2001: Friedman's $\chi^2 = 588.1$, $df = 2$, $P < 0.001$). No synergism was found, and there was no discernible response to either release rate of **1** + **2** + **3** alone. Hence, there was no evidence for cross-attraction of *G. quadrisignatus* and *G. fasciatus* to the pheromone of *C. truncatus*. Two other sap beetles, *Carpophilus sayi*, and *Epuraea corticina* Erichson were trapped in experiments II and III, but not in sufficiently high enough numbers to analyze.

Seasonality in Experiments II and III. There was a definite seasonality to the flight activity of *Colopterus truncatus* in Minnesota. In both years, adult activity was initiated in mid- to late April, peaked between late April and early May, and largely disappeared by early and mid-June (Figure 4A). Continued monitoring until November caught only three additional *C. truncatus*, one on September 15, 2000, and one each on August 24 and 31, 2001. The maximum flight responses came on May 4 (2000) and April 20 (2001), and during both years, more than 98% of the beetles were trapped between April 14 and June 1.

The seasonal pattern of flight activity of *G. quadrisignatus* and *G. fasciatus* (both species pooled) was different than *C. truncatus* (Figure 4B). In 2000, these species were abundant in the spring and their presence continued at a low, but discernible level throughout the rest of the year, showing a pattern somewhat similar to *C. truncatus*. In 2001, there was no large spring peak, and the abundance varied throughout the remainder of the year, peaking in mid- to late July.

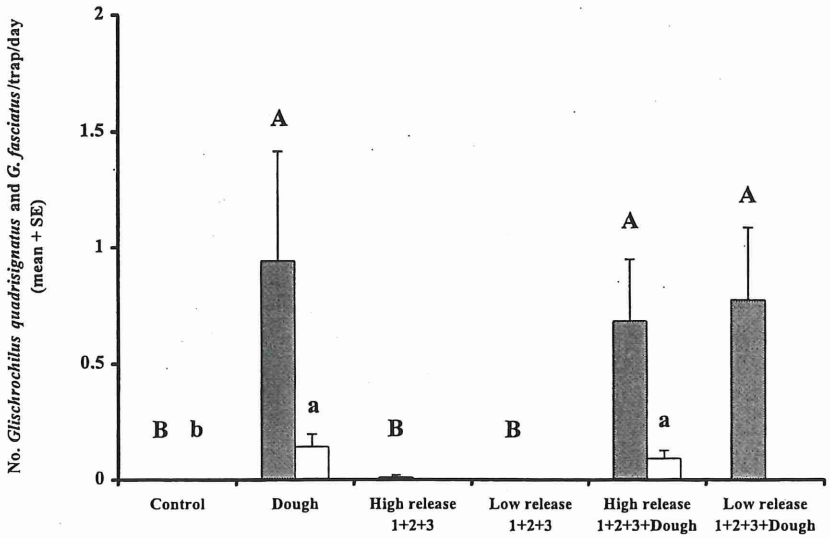

FIG. 3. Pooled flight responses of *Glischrochilus quadrisignatus* and *G. fasciatus* to high and low release rates of synthetic *Colopterus truncatus* aggregation pheromone components **1** + **2** + **3**, with and without dough, from April 4 to June 15, 2000 (dark bars) and April 5 to June 15, 2001 (open bars) in Minnesota (experiments II and III). Responses to treatments are significantly different if histogram bars within a year are marked with different letters.

While there is a large amount of variation in trap catches during both years of the study, *G. quadrisignatus* and *G. fasciatus* were generally more abundant than *C. truncatus*, and they did not show the same distinct limited seasonality in Minnesota.

Experiment IV. The effect of all possible combinations of **1** + **2** + **3** on *C. truncatus* in Illinois, showed a strong treatment effect, using either the Friedman's nonparametric two-way ANOVA (Friedman's $\chi^2 = 2{,}104.2$, $df = 7$, $P < 0.001$) or the standard two-way ANOVA with data transformed using $\log(X + 1)$ ($F = 21.7$, $df = 7{,}49$, $P < 0.001$) (Figure 5). The day × treatment interaction was not significant ($F = 1.3$, $df = 42{,}49$, $P < 0.185$), indicating that relationships among treatments were fairly consistent from day to day. Individual block effects were allowed to vary from day to day in the analysis (i.e., the day × block interaction was fitted); this precaution allowed for the possibility that the *C. truncatus* population density changed in different ways over time in different areas of the site. The dominant effect in the experiment was that trap catches were dramatically higher whenever compound **3** was present in the bait (the linear contrast for the presence versus absence of **3** was significant, $F = 135.7$, $df = 1{,}49$, $P < 0.001$). On the other hand, the corresponding

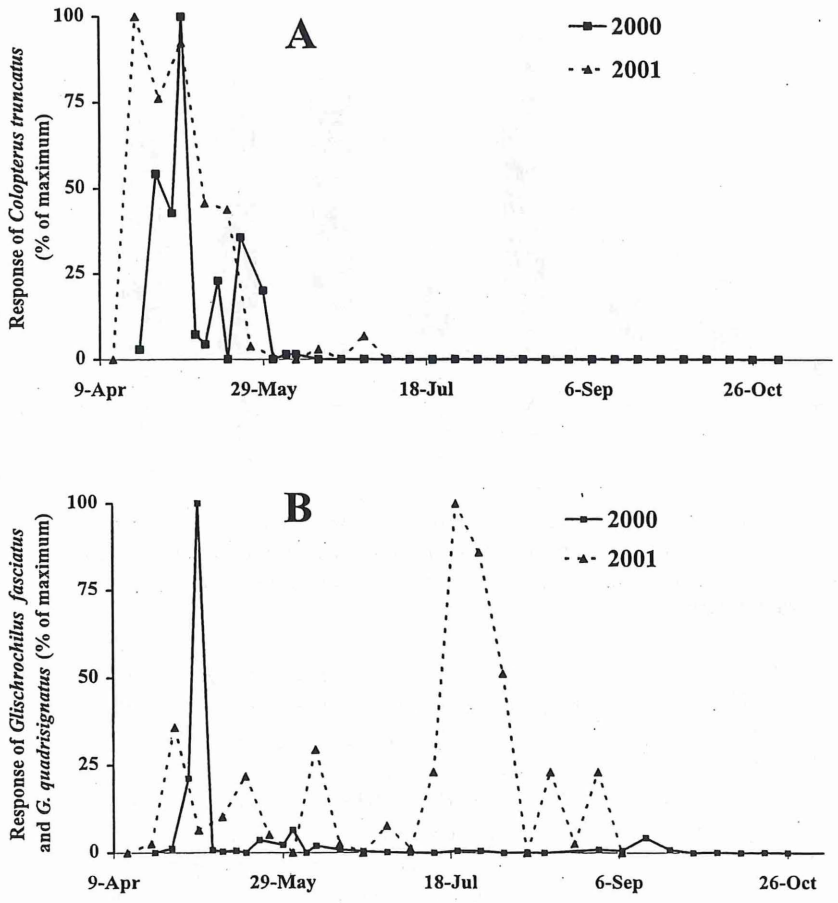

FIG. 4. Relative seasonal flight responses of (A) *Colopterus truncatus* to all treatments in experiments II and III from April 4 to November 3, 2000, and April 5 to November 9, 2001, in Minnesota and (B) *Glischrochilus quadrisignatus* and *G. fasciatus* to all treatments in experiments II and III from April 4 to November 3, 2000, and April 5 to November 9, 2001, in Minnesota. Of the 591 *C. truncatus* trapped in 2000 and 2001, only three beetles were trapped after early July (September 15, 2000; and August 24 and 31, 2001—both females). Maximum trap catch per week in 2000 was 40 beetles (May 4), whereas maximum trap catch per week in 2001 was 97 beetles (April 20). For *Glischrochilus* spp., maximum trap catch per week in 2000 was 136 beetles (May 11), whereas the maximum trap catch per week in 2001 was 76 beetles (July 18).

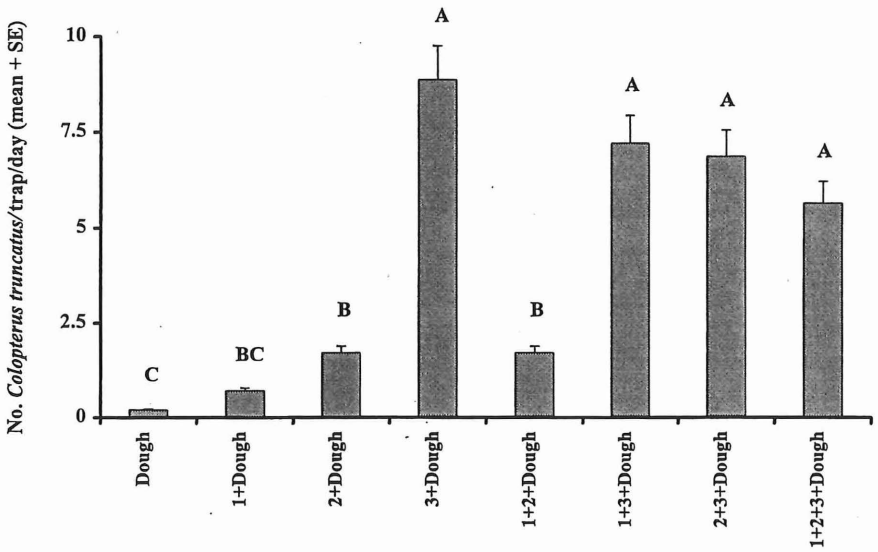

FIG. 5. Flight response of *Colopterus truncatus* to high release rate of all possible combinations of synthetic aggregation pheromone components **1**, **2**, and **3** with dough (experiment IV) in Illinois. Responses to treatments are significantly different if histogram bars are marked with different letters.

contrasts for **1** and **2** were not significant ($F = 0.0009$, $df = 1,49$, $P = 0.973$; $F = 1.932$, $df = 1,49$, $P = 0.170$, respectively), although the response to **2** plus dough was nominally more attractive than and different from the response to the dough control (Figure 5). The higher-order effects for interactions among treatments were significant ($F = 3.595$, $df = 4,49$, $P = 0.012$), but these were minor when compared with the contrast for **3**. That the interaction was significant at all was due to catches for **3** being somewhat lower when **2** was also present versus when **2** was absent, for unknown reasons. This trend was not significant when the means were considered just two at a time with LSD tests. Overall, 291 female and 171 male *C. truncatus* were captured in experiment IV. The sex ratio did not differ among treatments ($\chi^2 = 6.2$, $df = 7$, $P = 0.52$).

Experiment V. In Minnesota, the responses of *C. truncatus* to the high release rate of **1** + **2** + **3** + dough and to the high release rate of **1** + **3** + dough were not different (Friedman's $\chi^2 = 1{,}453.2$, $df = 3$, $P < 0.001$; Figure 6A). Both pheromone-containing treatments were more attractive than the dough alone or unbaited control treatments. Of the 182 beetles in the traps, there were 117 females and 65 males, and the sex ratio was not different among treatments ($\chi^2 = 0.48$, $df = 3$, $P = 0.79$).

Two other nitidulids, *Carpophilus brachypterus* (Say) and *Glischrochilus fasciatus*, also responded to treatments in experiment V (Figure 6B). *Carpophilus*

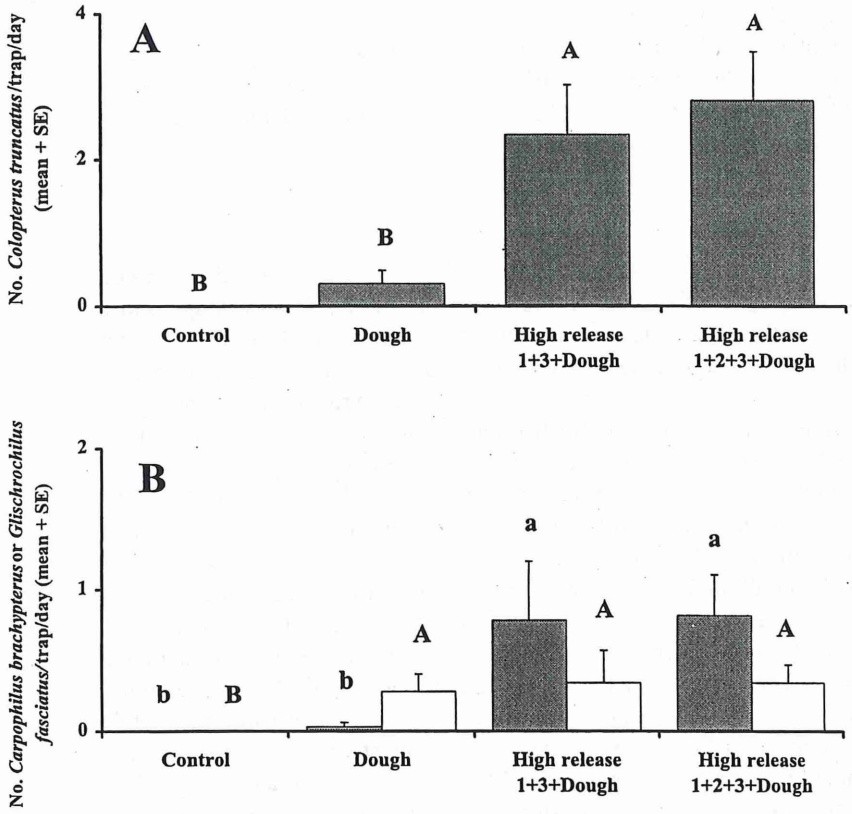

FIG. 6. Flight responses of (A) *Colopterus truncatus* and (B) *Carpophilus brachypterus* (shaded bars) and *Glischrochilus fasciatus* (open bars) to high release rate of synthetic *Colopterus truncatus* aggregation pheromone components **1** + **2** + **3** + dough, high release rate of **1** + **3** + dough, dough alone, and unbaited control in Minnesota (experiment V). Responses to treatments are significantly different if histogram bars within a species are marked with different letters.

brachypterus responded to the treatments similarly to *Colopterus truncatus* (albeit at lower levels), with a significant and similar response to both pheromone–dough combinations (Friedman's $\chi^2 = 813.8, df = 3, P < 0.001$; Figure 6B). *Glischrochilus fasciatus* responded to the treatments differently than *C. truncatus* and *C. brachypterus*. With *G. fasciatus*, there were no differences among the responses to traps containing dough (Friedman's $\chi^2 = 269.1, df = 3, P < 0.001$; Figure 6B). Although some *G. quadrisignatus* were collected in this study, the catches (eight beetles) were too low to discern any pattern in the response.

Experiment VI. The high release rate of **1** + **3** + oak chips was the most attractive treatment to *C. truncatus* (Friedman's $\chi^2 = 147.0, df = 2, P < 0.001$).

Overall trap catches in this experiment were quite low: no beetles were caught in either the control or oak chips-alone treatments, and more than 75% of the traps baited with **1** + **3** + oak chips were empty. Of the 16 *C. truncatus* caught in the traps baited with **1** + **3** + oak chips, there were 12 females and 4 males. No other sap beetles were caught in this experiment.

DISCUSSION

Both Illinois and Minnesota populations of *C. truncatus* responded similarly to the low- and high-release rate formulations of **1** + **2** + **3** with dough and to dough alone. Both populations preferred the high release rate formulation. Since the low release rate pheromone formulation keeps the ratios of emitted volatiles constant for a longer period of time than the high release rate (i.e., the unenclosed septum) formulation (R.J.B., unpublished data), our trapping results suggest that the sustained emission of the constant ratio of pheromone components is not the most important factor in the attraction of *C. truncatus*. Rather, they suggest that the total amount of components emitted was the most important factor in trap catch.

In both Illinois and Minnesota populations, there was no difference in the response to the combination of **1** + **2** + **3** with dough or the combination of **1** + **3** with dough. In contrast to earlier work in Illinois on *C. truncatus* (Cossé and Bartelt, 2000), this study showed directly in Illinois, and by inference in Minnesota, that component **2** was not necessary for, and in fact perhaps reduced, the attraction of *C. truncatus*. Even though component **2** was the most abundantly produced of the three male-specific hydrocarbons investigated in this study, it was component **3** that dominated the response. Component **3** had not been tested alone in previous behavioral studies, but it did show considerable electrophysiological activity when presented alone to male and female antennal preparations (Cossé and Bartelt, 2000). Although there were no apparent differences in the responses of Illinois and Minnesota populations of *C. truncatus* to various release rates and blends of the aggregation pheromone, there may be differences in the seasonal pattern of response to aggregation pheromone (see below).

Since *C. truncatus* and other nitidulids have a relatively rapid and strong response to fresh wounds in oak trees (Juzwik et al., 1999; J.J., personal observation), we hypothesized that the volatiles produced by oak tissue would synergize the *C. truncatus* aggregation pheromone. Based on the evidence shown here (experiment VI), volatiles produced from chipped oak tissue are not attractive, and these volatiles likely do not synergize the pheromone. Trap catches in experiment VI were quite low, and are probably explained by a low-level response to the pheromone without the dough synergist as observed in the experiments I and II. The oak tissue in our experiments might not have been attractive to *C. truncatus* because the process of chipping might produce different volatiles (or different amounts of volatiles) that are not normally encountered by nitidulids

when searching for fresh sap-laden wounds on living trees. Further work is needed to identify and characterize the volatiles of wounded oaks, how they change over time, and when they are most attractive to nitidulids searching for feeding, mating, and ovipositional sites. Preliminary chemical analyses, however, show that volatiles from individuals within an oak species are quite variable and suggest that this work will be difficult (R.J.B., unpublished data).

Since sap beetles aggregate on oak wilt mats (Cease and Juzwik, 2001), it is possible that oak wilt mat volatiles synergize the aggregation pheromone emitted by *C. truncatus* and other species while on mat tissue. Volatiles from laboratory cultures of oak wilt have been described and are attractive to sap beetles (*C. truncatus* was not tested) in wind-tunnel bioassays (Lin and Phelan, 1992). Further study on the relationship between oak wilt mat volatiles and nitidulid pheromones will be conducted when a stable *Ceratocystis fagacearum* volatile formulation is available for field tests.

The distinct seasonality shown by *C. truncatus* in our study is very important in understanding and managing the overland spread of oak wilt. Peak production of oak wilt mats (Campbell and French, 1955) and the window of peak susceptibility to infection (Prey and Kuntz, 1995) both occur during the spring in Minnesota. Our results show that in Minnesota, *C. truncatus* is attracted to pheromone + dough-baited traps in April, May, and June, but very few *C. truncatus* are caught in the same traps with the same baits later in the summer and in the fall. Skalbeck (1976), when trapping with fermenting dough and *Ceratocystis fimbriata* baits over two years in three Minnesota forest types, also found *C. truncatus* only in the spring and early summer, with the peak abundance in April and May. In another instance in Minnesota, of 40 *C. truncatus* trapped in response to combinations of dough and (2*E*,4*E*,6*E*,8*E*)-7-ethyl-3,5-dimethyl-2,4,6,8-decatetraene, 95% of the trap catch flew before July 6 (one male and one female were trapped in September) (R.J.B., unpublished data). However, McMullen and Shenefelt (1961), Levesque and Levesque (1992), Dowd and Nelsen (1994), and Cossé and Bartelt (2000) have all reported trapping *C. truncatus* in flight during the fall to various stimuli in Wisconsin, Quebec (Canada), Illinois, and Illinois, respectively. Climatic differences between Minnesota and these other north-central areas may influence the life history, and, specifically, the flight behavior of *C. truncatus*.

Sampling *C. truncatus* from oak wilt mats in Minnesota has revealed that it is univoltine. Adults disperse in flight for mating in the spring and early summer, larvae only occur in the mats in spring and early summer, and adult densities are approximately eight times higher in the mats in the fall than in the spring (J.F.K., unpublished data). As the fall cohort of oak wilt mats is newly formed each fall, it is likely that *C. truncatus* has flown to the mats at this time. These adults are also likely to feed on these mats and are less likely to disperse from them again through flight. Rather, they are more likely to drop to the litter layer on the forest floor to overwinter. Further work in Minnesota needs to be done to determine whether

C. truncatus flies selectively in the spring and early summer in response to synergized synthetic pheromone (as a mating and feeding response) and selectively in the early fall to mat odors (as a feeding and preoverwintering response). Alternatively, *C. truncatus* may fly in response to pheromone in the fall, but at much lower densities or it may reach the mats in the fall by crawling to them. It should be noted that the seasonally restricted flight behavior of *C. truncatus* in Minnesota is not unique among sap beetles. Other species in the genera *Carpophilus* also show strong seasonal flight activity periods in California (Bartelt et al., 1994), South Carolina (Bartelt et al., 1995), and Australia (James et al., 1994), while species of *Colopterus* and *Cryptarcha* show a similar seasonal pattern in Texas (Appel et al., 1986).

As with other nitidulid pheromones (Bartelt, 1997; Zilkowski and Bartelt, 1999), the *C. truncatus* aggregation pheromone is cross-attractive to other species. In our experiments in Minnesota, as was the case in previous work in Illinois (Cossé and Bartelt, 2000), *Carpophilus brachypterus* responded to the synergized *Colopterus truncatus* pheromone. Since the decatetraene (**3**) is the main component of the aggregation pheromone of *Carpophilus brachypterus* (Williams et al., 1995), the response of *Carpophilus brachypterus* is most likely due to pheromone component similarity, reflecting the close phylogenetic relationship between the genera *Colopterus* and *Carpophilus* within the subfamily Carpophilinae (Downie and Arnett, 1996). The effect could also be interpreted as a kairomonal response. *Carpophilus brachypterus* may benefit by locating wounds in oaks or mats originally colonized by *Colopterus truncatus* and signaled by aggregation pheromone production by *C. truncatus*. Since *Carpophilus brachypterus* has been collected from oak wounds (Vogt, 1950; Norris, 1956), this chemically guided ecological relationship seems plausible. However, our review of the literature and a reexamination of summary data in Skalbeck (1976) suggests that *C. brachypterus* has not been collected to date from oak wilt mats (T.C. Skalbeck, personal communication). *Glischrochilus quadrisignatus* and *G. fasciatus* (subfamily Cryptarchinae) responded equally to all treatments containing dough. Thus, these species respond to food volatiles (Lin and Phelan, 1991), but do not show a kairomonal response to the *C. truncatus* aggregation pheromone.

One interesting outcome from these experiments is that female *C. truncatus* consistently responded to the *C. truncatus* aggregation pheromone in both Illinois and Minnesota at nearly twice the rate that males did. The sex ratio of *C. truncatus* trapped in all experiments from both locations was approximately 1.8:1 (female–male) (Minnesota 1.8:1; Illinois 1.7:1). In the previous study with aggregation pheromone components of *C. truncatus* in Illinois, Cossé and Bartelt (2000) also reported female-biased sex ratios between 1.1 and 1.7:1. In other studies with sap beetles, Petroski et al. (1994) recorded a sex ratio of 1.2:1 in *Carpophilus obsoletus* Erichson responding to its aggregation pheromone in the field, whereas Bartelt et al. (1994) reported sex ratios "close to" 1:1 in five *Carpophilus* spp. and in *Haptoncus luteolus* (Erichson) responding to aggregation pheromones in

the field. In the latter study, the sex ratios for four of the *Carpophilus* spp. and for *H. luteolus* were nearly 1:1 in date fruits, which are the hosts colonized by the beetles. The sex ratio of a sample of *C. truncatus* collected in oak wilt mats in Minnesota was also nearly 1:1 (1.1:1, $N = 128$; J.F.K., unpublished data).

In other coleopteran aggregation pheromone systems with a male-produced attractant, the sex ratio of beetles responding behaviorally to pheromone or the magnitude of the electrophysiological response to pheromones also tends to be female biased. Indeed, aggregation pheromones may, in part, serve a sex pheromone function (Borden, 1985). Female-biased responses have been reported in bark beetles (Scolytidae), e.g., the California five-spined ips, *Ips paraconfusus* Lanier (Wood et al., 1967, 1968; Byers, 1983), and the pine engraver, *Ips pini* (Say) (Seybold et al., 1992; Miller et al., 1990, 1995, 1996; Miller and Borden, 1990, 2000; Miller, 2000) and in grain beetles (Bostrichidae, Cucujidae, and Silvanidae), e.g., the larger grain borer, *Prostephanus truncatus* (Horn) (Birkenshaw and Smith, 2000), the rusty and flat grain beetles, *Cryptolestes ferrugineus* (Stephens) and *C. pusillus* (Schönherr) (Chambers et al., 1990), and the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (White and Chambers, 1989). See Millar et al. (1985 a,b) and Pierce et al. (1988) for counter-examples with grain beetles. In the initial case of the isolation and identification of an aggregation pheromone from an insect (*I. paraconfusus*, Wood et al., 1967, 1968), the sex-specific production and the female-biased responses caused the investigators to view the signals as sex attractants. Since female *C. truncatus* are attracted at a greater rate than males in our study, this nitidulid example lends additional support to the hypothesis that male-produced aggregation pheromones evolved secondarily from their initial roles as sex pheromones to attract females (Raffa et al., 1993; Phillips, 1997; Plarre and Vanderwel, 1999).

A parallel can be made between sap beetles that colonize, develop on, and emerge from oak wilt mats (or wounds) and bark beetles that undergo similar activities in phloem in tree stems and branches. In the cases of both *C. truncatus* and *I. paraconfusus*, the sex ratios of the arriving insects (simulated by response to baited traps) do not appear to match the sex ratios of those that emerge from the developmental substrate. For example, the sex ratio of *I. paraconfusus* responding to the immediate source of naturally produced or synthetic pheromones in various field studies ranges from 1.8 to 5.6:1 (Struble and Hall, 1955; Gara, 1963; Wood et al., 1967, 1968; Byers, 1983), whereas the sex ratio of insects emerging from Ponderosa pine logs has been reported as approximately 1.2:1 (Gara, 1963; Cameron and Borden, 1967) and 1.3:1 (Struble and Hall, 1955). Similarly, with *C. truncatus* we have an arriving sex ratio of 1.8:1 and a sex ratio of 1.1:1 in the colonized oak wilt mats. Further investigation is needed to determine the sex ratio of *C. truncatus* that develop and emerge from oak wilt mats and wounds in oaks. However, higher differential mortality of males during dispersal has been offered as a potential explanation for this sex ratio difference in *I. paraconfusus* (Cameron

and Borden, 1967), and this hypothesis may apply to the sex ratio difference for *C. truncatus* as well.

Finally, a number of conclusions with practical implications for monitoring the flight of *C. truncatus* and for managing overland spread of oak wilt can be drawn from this work. Experiments I and II demonstrated that the most attractive bait for *C. truncatus* is the high release rate of pheromone compounds with the bread dough coattractant. Further, experiment V showed that it is unnecessary to add synthetic **2** to the pheromone baits for monitoring *C. truncatus*. Thus, the simplest effective approach to preparation of pheromone baits would be to use synthetic **3**, without bothering to remove the **1** that is invariably present as a synthetic artifact. Since the two- and three-component blends of the *C. truncatus* aggregation pheromone are equally attractive, the less expensive two-component blend can be used effectively as a monitoring tool. As we recorded no response of *C. truncatus* or other sap beetles to 300-ml aliquots of oak chips (experiment VI), operational studies in the future might explore whether landowners, resource managers, or arborists could chip woody debris from uninfected oak trees and leave the chips on site without attracting nitidulid oak wilt vectors to the general area and accelerating overland transmission of the disease. Finally, land managers should be aware that the critical period of flight activity for *C. truncatus* in Minnesota occurs conservatively between early April and early July, and in order to limit the overland spread of oak wilt, pruning and other management activities that wound oaks should be avoided during this time. In a future study, a similar critical period needs to be defined for the other primary oak wilt vector, *Carpophilus sayi*.

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REFERENCES

APPEL, D. N., ANDERSEN, K., and LEWIS, R., JR. 1986. Occurrence of nitidulid beetles (Coleoptera: Nitidulidae) in Texas oak wilt centers. *J. Econ. Entomol.* 79:1276–1279.

BARTELT, R. J. 1997. Aggregation pheromones of *Carpophilus* spp. (Coleoptera: Nitidulidae): Review of chemistry and biology. *Recent Res. Dev. Entomol.* 1:115–129.

BARTELT, R. J. 1999. Sap beetles, pp. 69–89, *in* J. Hardie and A. K. Minks (eds.). Pheromones of Non-Lepidopteran Insects Associated with Agricultural Plants. CABI Publishing, Wallingford, UK.

BARTELT, R. J., WEISLEDER, D., and PLATTNER, R. D. 1990. Synthesis of nitidulid beetle pheromones: alkyl-branched tetraene hydrocarbons. *J. Agric. Food Chem.* 38:2192–2196.

BARTELT, R. J., VETTER, R. S., CARLSON, D. G., and BAKER, T. C. 1994. Responses to aggregation pheromones for five *Carpophilus* species (Coleoptera: Nitidulidae) in a California date garden. *Environ. Entomol.* 23:1534–1543.

BARTELT, R. J., WEAVER, D. K., and ARBOGAST, R. T. 1995. Aggregation pheromone of *Carpophilus dimidiatus* (F.) (Coleoptera: Nitidulidae) and responses to *Carpophilus* pheromones in South Carolina. *J. Chem. Ecol.* 21:1763–1779.

BILLINGS, R. F. 2000. State forest health programs: A survey of state foresters. *J. For.* 98:20–25.

BIRKENSHAW, L. A., and SMITH, R. M. 2000. Function of aggregation pheromone in the larger grain borer *Prostephanus truncatus*: variation in response to individuals as evidence for a role in sexual selection. *J. Chem. Ecol.* 26:1325–1339.

BORDEN, J. H. 1985. Aggregation pheromones, pp. 257–285, *in* G. A. Kerkut and L. I. Gilbert (eds.). Comprehensive Insect Physiology, Biochemistry, and Pharmacology. Vol. 9. Pergamon Press, Oxford.

BYERS, J. A. 1983. Sex-specific responses to aggregation pheromone: Regulation of colonization density of the bark beetle *Ips paraconfusus*. *J. Chem. Ecol.* 9:129–142.

CAMERON, E. A., and BORDEN, J. H. 1967. Emergence patterns of *Ips confusus* (Coleoptera: Scolytidae) from ponderosa pine. *Can. Entomol.* 99:236–244.

CAMPBELL, R. N., and FRENCH, D. W. 1955. A study of mycelial mats of oak wilt. *Phytopathology* 45:485–489.

CEASE, K. R., and JUZWIK, J. 2001. Predominant nitidulid species (Coleoptera: Nitidulidae) associated with spring oak wilt mats in Minnesota. *Can. J. For. Res.* 31:635–643.

CHAMBERS, J., MORGAN, C. P., WHITE, P. R., MORI, K., FINNEGAN, D. E., and PINNIGER, D. B. 1990. Rust-red grain beetle, *Cryptolestes ferrugineus*, and flat grain beetle, *Cryptolestes pusillus*: antennal and behavioral responses to synthetic components of their aggregation pheromones. *J. Chem. Ecol.* 16:3353–3372.

COSSÉ, A. A., and BARTELT, R. J. 2000. Male-produced aggregation pheromone of *Colopterus truncatus*: structure, electrophysiological, and behavioral activity. *J. Chem. Ecol.* 26:1735–1748.

CURL, E. A. 1955. Natural availability of oak wilt inocula. *Ill. Nat. Hist. Surv. Bull.* 26:277–323.

DOWD, P. F., and NELSEN, T. C. 1994. Seasonal variation of sap beetles (Coleoptera: Nitidulidae) populations in central Illinois cornfield-oak woodland habitat and potential influence of weather patterns. *Environ. Entomol.* 23:1215–1223.

DOWD, P. F., BARTELT, R. J., and WICKLOW, D. T. 1992. Novel insect trap useful in capturing sap beetles (Coleoptera: Nitidulidae) and other flying insects. *J. Econ. Entomol.* 85:772–778.

DOWNIE, N. M., and ARNETT, R. H., JR. 1996. The Beetles of Northeastern North America, Volume II: Polyphaga, Superfamily Bostrichoidea through Curculionoidea. The Sandhill Crane Press, Gainesville, Florida.

FRENCH, D. W. 1995. Oak wilt management in Minnesota, pp. 117–120, *in* D. N. Appel and R. F. Billings (eds.). Oak Wilt Perspectives: The Proceedings of the National Oak Wilt Symposium. **Au: Where held?**

GARA, R. I. 1963. Studies on the flight behavior of *Ips confusus* (LeC.) (Coleoptera: Scolytidae) in response to attractive material. *Contrib. Boyce Thompson Inst.* 22:51–66.

HEATH, R. R., and SONNET, P. E. 1980. Technique for *in situ* coating of Ag+ onto silica gel in HPLC columns for the separation of geometrical isomers. *J. Liq. Chromatogr.* 3:1129–1135.

JAMES, D. G., BARTELT, R. J., and FAULDER, R. J. 1994. Attraction of *Carpophilus* spp. (Coleoptera: Nitidulidae) to synthetic aggregation pheromones and host-related coattractants in Australian stone fruit orchards: beetle phenology and pheromone dose studies. *J. Chem. Ecol.* 20:2805–2819.

JEWELL, F. F. 1956. Insect transmission of oak wilt. *Phytopathology* 46:244–257.

JUZWIK, J. 1983. Factors affecting the overland transmission of *Ceratocystis fagacearum* in Minnesota. PhD dissertation. University of Minnesota, St. Paul, 96 pp.

Au: Note: not cited in text should this be deleted?

JUZWIK, J., FRENCH, D. W., and JERESEK, J. 1985. Overland spread of the oak wilt fungus in Minnesota. *J. Arboric.* 11:323–327.

JUZWIK, J., SKALBECK, T. C., and NEUMAN, M. F. 1999. Nitidulid species associated with fresh wounds on red oaks during spring in Minnesota. *Phytopathology* 89:S38.

LEVESQUE, C., and LEVESQUE, G. Y. 1992. Epigeal and flight activity of Coleoptera in a commercial raspberry plantation and adjacent sites in southern Quebec (Canada): introduction and Nitidulidae. *Great Lakes Entomol.* 25:271–285.

LIN, H. V., and PHELAN, P. L. 1991. Identification of food volatiles attractive to *Glischrochilus quadrisignatus* and *Glischrochilus fasciatus* (Coleoptera: Nitidulidae). *J. Chem. Ecol.* 17:2469–2480.

LIN, H. V., and PHELAN, P. L. 1992. Comparison of volatiles from beetle-transmitted *Ceratocystis fagacearum* and non-insect-dependent fungi. *J. Chem. Ecol.* 18:1623–1632.

MCMULLEN, L. H., and SHENEFELT, R. D. 1961. Nitidulidae collected from banana bait traps in Wisconsin. *Trans. Wisc. Acad. Sci., Arts Lett.* 50:233–237.

MILLAR, J. G., PIERCE, H. D., JR., PIERCE, A. M., OEHLSCHLAGER, A. C., and BORDEN, J. H. 1985a. Aggregation pheromones of the grain beetle, *Cryptolestes turcicus* (Coleoptera: Cucujidae). *J. Chem. Ecol.* 11:1071–1081.

MILLAR, J. G., PIERCE, H. D., JR., PIERCE, A. M., OEHLSCHLAGER, A. C., BORDEN, J. H., and BARAK, A. V. 1985b. Aggregation pheromones of the flat grain beetle, *Cryptolestes pusillus* (Coleoptera: Cucujidae). *J. Chem. Ecol.* 11:1053–1069.

MILLER, D. R. 2000. Vertical displacement of *Ips latidens* and *Ips pini* (Coleoptera: Scolytidae) by semiochemical interruption. *Can. Entomol.* 132:789–797.

MILLER, D. R., and BORDEN, J. H. 1990. β-Phellandrene: kairomone for pine engraver, *Ips pini* (Say) (Coleoptera:Scolytidae). *J. Chem. Ecol.* 16:2519–2531.

MILLER, D. R., and BORDEN, J. H. 2000. Dose-dependent and species-specific responses of pine bark beetles (Coleoptera: Scolytidae) to monoterpenes in association with pheromones. *Can. Entomol.* 132:183–195.

MILLER, D. R., GRIES, G., and BORDEN, J. H. 1990. *E*-myrcenol: a new pheromone for the pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae). *Can. Entomol.* 122:401–406.

MILLER, D. R., BORDEN, J. H., and LINDGREN, B. S. 1995. Verbenone: dose-dependent interruption of pheromone based attraction of three sympatric species of pine bark beetles (Coleoptera: Scolytidae). *Environ. Entomol.* 24:692–696.

MILLER, D. R., BORDEN, J. H., and SLESSOR, K. N. 1996. Enantiospecific pheromone production and response profiles for populations of pine engraver *Ips pini* (Say) (Coleoptera: Scolytidae) in British Columbia. *J. Chem. Ecol.* 22:2157–2172.

NORRIS, D. M. 1956. Associations of insects with the oak tree and *Endoconidiophora fagacearum* Bretz. PhD dissertation. Iowa State University, Ames, 284 pp.

Au: Ames?

PETROSKI, R. J., BARTELT, R. J., and VETTER, R. S. 1994. Male-produced aggregation pheromone of *Carpophilus obsoletus* (Coleoptera: Nitidulidae). *J. Chem. Ecol.* 20:1483–1493.

PIERCE, H. D., JR., PIERCE, A. M., JOHNSTON, B. D., OEHLSCHLAGER, A. C., and BORDEN, J. H. 1988. Aggregation pheromone of square-necked grain beetle, *Cathartus quadricollis* (Guér.). *J. Chem. Ecol.* 14:2169–2184.

PHILLIPS, T. W. 1997. Semiochemicals of stored-product insects: research and applications. *J. Stored Prod. Res.* 33:17–30.

PLARRE, R., and VANDERWEL, D. 1999. Stored product beetles, pp. 149–198, *in* J. Hardie and A. K. Minks (eds.). Pheromones of Non-Lepidopteran Insects Associated with Agricultural Plants. CABI Publishing, Wallingford, UK.

PREY, A. J., and KUNTZ, J. E. 1995. Epidemiology of oak wilt outside Texas, pp. 15–20, *in* D. N. Appel and R. F. Billings (eds.). Oak Wilt Perspectives: The Proceedings of the National Oak Wilt Symposium. **Au: where held?**

RAFFA, K. F., PHILLIPS, T. W., and SALOM, S. M. 1993. Strategies and mechanisms of host colonization by bark beetles, pp. 103–128, *in* T. D. Showalter and G. M. Filip (eds.). Beetle–Pathogen Interactions in Conifer Forests. Academic Press, London.

SEYBOLD, S. J., TEALE, S. A., WOOD, D. L., ZHANG, A., WEBSTER, F. X., LINDAHL, K. Q., JR., and KUBO, I. 1992. The role of lanierone in the chemical ecology of *Ips pini* (Coleoptera: Scolytidae) in California. *J. Chem. Ecol.* 18:2305–2329.

SKALBECK, T. C. 1976. The distribution of Nitidulidae in deciduous forests in Minnesota. PhD dissertation. University of Minnesota, St. Paul, 260 pp.

STRUBLE, G. R., and HALL, R. C. 1955. The California five-spined engraver, its biology and control. United States Department of Agriculture Circ. No. 964, 21 pp.

TAINTER, F. H., and BAKER, T. C. 1996. Principles of Forest Pathology. John Wiley Sons, New York.

VOGT, G. B. 1950. Occurrence and records of Nitidulidae. *Coleop. Bull.* 5:4–11.

WHITE, P. R., and CHAMBERS, J. 1989. Saw-toothed grain beetle *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae): Antennal and behavioral responses to individual components and blends of aggregation pheromone. *J. Chem. Ecol.* 15:1015–1031.

WILLIAMS, R. N., ELLIS, M. S., and BARTELT, R. J. 1995. Efficacy of *Carpophilus* aggregation pheromones in nine species in northeastern Ohio, and identification of the pheromone of *C. brachypterus*. *Entomol. Exp. Appl.* 77:141–147.

WOOD, D. L., STARK, R. W., SILVERSTEIN, R. M., and RODIN, J. O. 1967. Unique synergistic effects produced by the principal sex attractant compounds of *Ips confusus* (LeConte) (Coleoptera: Scolytidae). *Nature* 215:206.

WOOD, D. L., BROWNE, L. E., BEDARD, W. D., TILDEN, P. E., SILVERSTEIN, R. M., and RODIN, J. O. 1968. Response of *Ips confusus* to synthetic sex pheromones in nature. *Science* 159:1373–1374.

ZILKOWSKI, B. W., and BARTELT, R. J. 1999. Cross-attraction of *Carpophilus humeralis* to pheromone components of other *Carpophilus* species. *J. Chem. Ecol.* 25:1759–1770.

Sub-Project 3, NS06 LCMR Project, 1999-2001, Meeting Abstracts from Four Meetings

Chemical Ecology of Sap Beetles (Coleoptera: Nitidulidae): Vectors of Oak Wilt Disease in Minnesota

An abstract of a presentation to the Annual Meeting of Multistate Research Project W-189, July 6, 2001, Lake Tahoe, CA

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Since fall of 1999, we have conducted field trials to study the flight response of *Colopterus truncatus* (Rand.) and other nitidulid beetles implicated in the overland spread of oak wilt to the *C. truncatus* aggregation pheromone. Oak wilt is a vascular wilt disease that kills hundreds of thousands of red oaks in the United States each year, and is of great concern to forest managers and property owners alike. As a part of a larger study of the life history of these beetles, we used the *C. truncatus* aggregation pheromone to determine the seasonal flight period of *C. truncatus* and associated nitidulids. The pheromone is a 3 compound blend of methyl branched hydrocarbons isolated and identified by Cossé and Bartelt: (2*E*, 4*E*, 6*E*)-3,5-dimethyl-2,4,6-octatriene; (2*E*, 4*E*, 6*E*)-4,6-dimethyl-2,4,6-nonatriene; and (2*E*, 4*E*, 6*E*,8*E*)-3,5,7-trimethyl-2,4,6,8-decatetraene.

We have monitored the flight of *C. truncatus* from early April until early November in 2000 and 2001 (ongoing) using various combinations of whole wheat bread dough (WWBD) and male-produced *C. truncatus* aggregation pheromone as trap baits. The traps have been placed in four different sites ("blocks") around the Twin Cities area and have been emptied and re-randomized within the blocks weekly. Eleven different species of nitidulids were caught in the traps so far in 2001. As expected, *C. truncatus* was the most common beetle in the traps, followed by *Glischrochilus quadrisignatus,* which responded largely to WWBD. Results from 2001 have resembled results from 2000 in that there is a large peak of flight activity in late April and early May, trailing off to low levels in June. Of the treatments, the combination of pheromone and WWBD was the most attractive to *C. truncatus* during both years.

We carried out an additional study testing for response of *C. truncatus* to oak volatiles and oak volatiles combined with *C. truncatus* pheromone. We used slightly modified traps to hold chipped oak tissue, and placed them in four field sites. An experiment in 2000 showed that pheromone in the absence of WWBD is slightly attractive to *C. truncatus*. This year, we found that oak chips by themselves are not attractive to *C. truncatus*. Further, while *C. truncatus* did respond to the oak chips + pheromone treatment the response was quite low and likely due to the pheromone alone. Due to limitations in the amount of pheromone available, we were not able to include the pheromone alone treatment. We also performed an experiment testing for response of *C. truncatus* to two different blends of the *C. truncatus* pheromone, the naturally occurring 3-component pheromone and a 2-component pheromone blend (the octatriene and decatetraene). In the experiment both blends were combined with WWBD. We found that *C. truncatus* responded to both pheromone treatments at a higher rate than to WWBD alone, and that there was no significant difference between responses to either blend.

This work has several implications for management of oak wilt. First, an effective pheromone-based monitoring system can provide important information on the activity period of oak wilt vectors. Land managers and forest health professionals can time pruning activities to avoid this activity period. Secondly, the use of the 2-component *C. truncatus* pheromone blend and bread dough provides a more cost effective monitoring tool. Finally, our results show that piles of chipped oak near uninfected trees will not likely attract oak wilt vectors. Although *C. truncatus* and other nitidulid beetles have responded to fresh wounds on live oak trees in other studies, our studies show that oak chips are not attractive to these beetles. As a result, piles of chipped oak trees do not pose an additional risk of attracting potential nitidulid vectors of oak wilt.

Population Densities and Life Histories of Larval and Adult Nitidulid Beetles (Coleoptera: Nitidulidae) on Oak Wilt (*Ceratocystis fagacearum*) Spore Mats in Minnesota

Poster Abstract for North Central Forest Insect and Disease Workshop, October 1-4, 2001, Big Bay, MI

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Sap beetles (Nitidulidae) are known vectors of oak wilt, *Ceratocystis fagacearum*, but the population biology and life history of these beetles in oak woodlands are poorly understood. The principal nitidulid oak wilt vectors are *Colopterus truncatus*, *Carpophilus sayi*, and *Epuraea corticina*. To study the population densities and seasonal occurrences of these vectors, we removed fruiting bodies (spore mats) from groups of oak wilt-killed trees in five sites near the Minneapolis/St. Paul metropolitan area during four collection periods (Fall 1999, Spring 2000, Fall 2000, and Spring 2001). Larval and adult nitidulids were identified and counted, and mat areas were calculated using digital photography and computer software. After beetle abundances and mat areas were measured, population densities were calculated.

During this study, we collected 252 mats from all sides of the bole of *Quercus rubra* and *Q. ellipsoidalis* at heights ranging from ground level to 3.5m. Larvae were present in 149 (~59%) mats. Larvae of all three oak wilt vector species were found in the mats, as were larvae from another nitidulid genus, *Glischrochilus* spp. As many as four different species of nitidulid larvae occurred on a single mat, and as many as 163 larvae were recovered from a single mat. The density of larval nitidulids varied by species from 0.0 to 1.7 larvae/cm^2 of mat tissue, with an average of ~ 0.11 larvae/cm^2 of mat tissue. Larval density varied among species, among

years, between seasons, and among sites. *Colopterus truncatus* were the most abundant larvae in spring mats, while *Epuraea corticina* were the most abundant larvae in fall mats.

Adults occurred in 141 (~56%) mats. Adults of all three oak wilt vector species were found on the mats, as were adults of an additional seven nitidulid species. As many as six different species of nitidulid adults occurred on a single mat, and as many as 68 adults were recovered from a single mat. The density of adult nitidulids varied by species from 0.0 to 11 beetles/cm^2 of mat tissue, with an average of ~ 0.012 adults/cm^2 of mat tissue. Like larvae, adult density varied among species, among years, between seasons, and among sites. *Colopterus truncatus* and *Carpophilus sayi* were equally abundant as adults in spring mats, while *Colopterus truncatus* was the most common adult nitidulid species in fall mats.

To carry out this work, we developed a novel technique for oak wilt mat removal to maximize insect collection (using cordless power tools). By identification of larvae during both spring and fall, we found larvae of *Colopterus truncatus* and *Carpophilus sayi* only in the spring. Thus, these species are likely to be univoltine. *Epuraea corticina* larvae were found in both the spring and fall, suggesting that this species is bivoltine. Eggs were commonly observed in spring mats, but were not seen in fall mats, and were not identified to species. No nitidulid pupae were observed in oak wilt mats during this study.

Oak Wilt, *Ceratocystis fagacearum*, Incidence and Spore Load of Dispersing Nitidulid Beetles/Interaction of Wildland Fire, Oak Wilt, and Xylophagous and Phloeophagous Beetles in Minnesota Oak Forests

Abstract for Presentation at Annual Meeting of W-187, October 12-13, 2001, Portland, OR

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The sap beetle, *Colopterus truncatus*, is considered among the most important vectors of oak wilt in the North Central United States. Using various combinations of three hydrocarbons isolated from males we have found that *C. truncatus* flies and responds to aggregation pheromone primarily between April and June. The most cost effective bait investigated consists of two of the hydrocarbons combined with whole wheat bread dough. Although these beetles respond to freshly wounded oaks, chipped oak tissue is not attractive to flying adults. In three experiments conducted between April and June, 2001 in Minnesota, we trapped a total of 548 *C. truncatus* of which 243 were homogenized and assayed for incidence and spore load of *C. fagacearum*. Overall incidence of *C. fagacearum* on trapped *C. truncatus* was 13.6%. The mean spore load on the 33 individuals that tested positive for *C. fagacearum* was 9,366 ± 3,719 (range = 33 to 110,000). Both incidence and spore load are higher than previously published results from dispersing nitidulids, but this study differs from previous studies in that it utilized aggregation pheromone to attract and trap the beetles and that it focused on only one species of beetle. When examined over time, the incidence of *C. fagacearum* was initially 0% (April 20/27, n = 27/25), but then rose to approximately 15% (May 11/18, n = 36/41) before the main dispersal flight of beetles ended. The sex ratio of *C. truncatus* that responded in all experiments was approximately 2:1 (female: male). No information was obtained regarding the association of sex of *C. truncatus* and incidence or load of *C. fagacearum*. Other nitidulids that also responded in these experiments included *Glishcrochilus* spp. (to dough) and *Carpophilus brachypterus* (to *C. truncatus* pheromone = cross attraction). In 2002, weekly trapping of *C. truncatus* and another nitidulid, *Carpophilus sayi*, will continue followed by fungal isolation from known sexes of each beetle species.

A new study is proposed to investigate the three-way interactions among wildfire, oak wilt, and xylophagous and phloeophagous beetles. In October 2000, a wildfire burned approximately 4,000 acres in the Carlos Avery Wildlife Management Area (Anoka and

Chisago Cos., MN). Numerous oak wilt infection centers occur in this northern red oak, *Quercus rubra*, "savannah" habitat that experienced the fire. In this area we plan to establish 16 experimental plots representing 4 each of burned/unburned and oak wilt/non oak wilt conditions. In each plot, fuel loads will be measured and monitored, level of oak wilt will be measured and monitored, and xylophagous and phloeophagous beetles will be sampled. From preliminary sampling in August and September 2001, *Monarthrum mali* (Coleoptera: Scolytidae) appears to be strongly and preferentially attracted to burned oaks. The responding *M. mali* are being assayed for incidence and spore load of *C. fagacearum*. Other targeted insects, *Pseudopityophthorus* spp. (Coleoptera: Scolytidae) and the two-lined chestnut borer, *Agrilus bilineatus* (Coleoptera: Buprestidae) will be sampled in branches and stems and using a variety of trapping methods. Ultimately, the two-way interactions between fire-insects, fire-oak wilt, and oak-wilt-insects will be described and reported from measurements on these experimental plots.

ABSTRACT

Use of aggregation pheromones of sap beetles to study overland transmission of *Ceratocystis fagacearum*. J. F. Kyhl (1), J. JUZWIK (2), R. J. Bartelt (3), and S. J. Seybold (1). (1) Dept. Entomology, Univ. of Minnesota, St. Paul, MN 55108; (2) USDA Forest Service, 1561 Lindig St., St. Paul, MN 55108; (3) USDA-ARS, Peoria, IL 61604. Phytopathology 92:S43. Publication no. P-2002-0313-AMA..

Two sap beetles, *Colopterus truncatus* (Cot) and *Carpophilus sayi* (Cas) (Coleoptera: Nitidulidae), are considered the principal insect species responsible for overland spread of the oak wilt pathogen, *Ceratocystis fagacearum* (Cf), in Minnesota. Flight traps baited with Cot and Cas aggregation pheromones were used to study seasonal flight activity of vectors in oak stands and the incidence and levels of Cf on the vectors. During a weekly survey over two growing seasons (2000, 2001), greater than 98 percent of the Cot obtained in Cot pheromone baited traps were caught between 14 April and 1 June. Of the 243 Cot collected during this period in 2001, 14 percent yielded Cf based on fungal bioassays. The mean Cf popagule load per beetle on the Cf positive Cot was 9.4×10^3. Similar studies were initiated in June 2001 using Cas baited traps; greater than 80 percent of Cas obtained during 2001 were caught between 8 June and 27 July.

Sub-Project 3
NSOG CCMR
Project
1999 - 2001

USE OF AGGREGATION PHEROMONES OF SAP BEETLES TO STUDY OVERLAND TRANSMISSION OF *CERATOCYSTIS FAGACEARUM*

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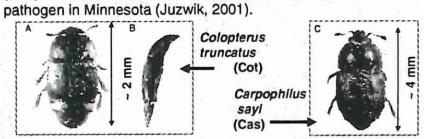
INTRODUCTION

Background

- Oak wilt is a major disease of oaks in the Eastern USA.
- The causal organism, *Ceratocystis fagacearum*, is transmitted from diseased to healthy trees in two ways: 1) below-ground via common root systems, and 2) above-ground via insect vectors.

- Two sap beetles, *Colopterus truncatus* (Cot) and *Carpophilus sayi* (Cas) are considered to be the principal insect vector species of the pathogen in Minnesota (Juzwik, 2001).



- Aggregation pheromones (insect produced chemicals) were recently identified for these two beetle species and synthesized in the laboratory (Cosse and Bartelt, 2000; Bartelt, unpub.).
- These pheromones are potentially useful tools for studying overland transmission of the oak wilt pathogen.

Research Objectives

- Determine flight activities for each species between early April and early November using pheromone baits, and
- Determine the incidence and levels of *C. fagacearum* on the collected adult Cot and Cas individuals.

MATERIALS AND METHODS

Experimental Parameters

Pheromone Bait Used	Year	Time Period Monitored	No. Sites Monitored	Description of sites*
Cot	2000	4/21 - 11/9	4	2 in oak wilt infection centers 2 in oak wilt free stands
	2001	4/13 - 11/7	4	2 in oak wilt infection centers 2 in oak wilt free stands
Cas	2001	6/8 - 10/18	2	2 in oak wilt infection centers

*** Note: All sites located in east-central Minnesota.**

Pheromone Bait Traps

Cot or Cas pheromone loaded on rubber septa and suspended in trap.

Isolation of *C. fagacearum*

Flight-trapped Beetle
↓
Homogenize in sterile water by sonication
↓
Serially dilute aqueous suspension and plate 0.5 ml on each of three replicate plates of acidified PDA.
↓
Incubate for 12 days in the dark at 24°C

ACKNOWLEDGEMENTS

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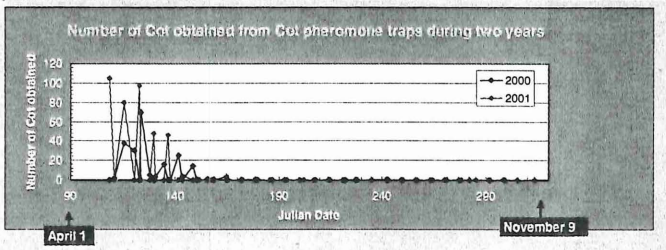
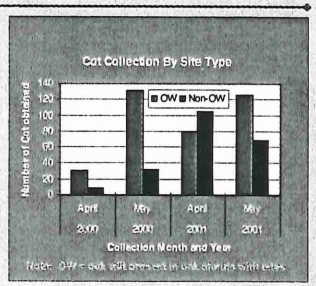
RESULTS

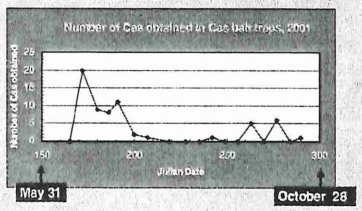
A. Flight Activities of Cot and Cas

Cot from Cot Pheromone Traps





Cas from Cas pheromone Traps

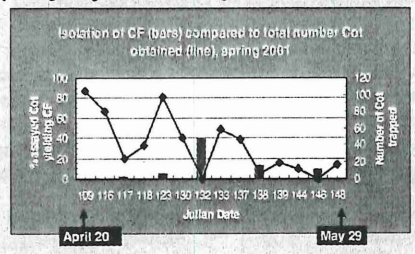


B. Isolation of *C. fagacearum* from beetles

Incidence of *C. fagacearum* (CF) on collected Cot, 2001

Collection Dates	Fungal isolation from beetles: No. assayed	% yielding *CF*	Ave. no. cfu/beetle ($\times 10^3$)
4/20	27	0	--/a
4/2 - 4/29	47	2	0.2
5/4	43	5	0.1
5/11 - 5/14	56	39	11
5/18 - 5/20	54	13	3
5/25 - 5/29	16	6	3

Cot yielding *C. fagacearum* according to date beetles were collected.



DISCUSSION

- In east-central Minnesota, Cot disperses almost exclusively during early and mid-spring. In 2000 and 2001, > 98% of the Cot obtained in Cot pheromone traps were caught between 14 April and 1 June.
- The flight activity of Cas in east-central Minnesota is inconclusive at this time, but data collection is on-going in 2002. Based on 2001 data collected between 8 June and 18 October, > 80% of Cas obtained were caught between 8 June and 27 July.
- Of 243 assayed beetles collected on different dates between mid-April and late May, 14% yielded *C. fagacearum.* This frequency of fungus isolation from dispersing Cot is much higher than isolation rates (< 4%) for free-flying nitidulids (several species) obtained in whole-wheat dough and fruit bait traps in earlier studies (Juzwik and French 1983).
- The average *C. fagacearum* propagule loads (0.1 to 11 x 10^3 CFU/beetle) on the fungus-positive beetles would be sufficient to initiate infection of a healthy tree if such a beetle visited a fresh, xylem-penetrating wound. The number is also similar to the levels found (< 0.1 to 6.1 x 10^3) on fungus-positive beetles in the previous study mentioned above.
- Cot and Cas pheromone bait traps could be useful tools for annually monitoring when the two principal vector species are dispersing in oak stands during the critical infection period in east central Minnesota.

Sub-Project 3
NC504 Lcmk Project
1999-2001

Poster presentation
American Phytopathological
Society Meetings, July 2002

June 2001 Final Report
Sub-Project 4, NS06 LCMR Project
Dr. Joseph O'Brien, Principal Investigator

ROOT GRAFT BARRIER GUIDELINES FOR LINE PLACEMENT IN RESIDENTIAL SETTINGS

Barrier Line Placement Study

A total of 70 plots were located on two sites in the Minneapolis-St. Paul metropolitan area. Site 1 was the city of Ham Lake (HL), which is a growing community with mainly sandy soils in the Anoka series. Site 2 was Murphy-Hanrehan (M-H) Park Reserve, which has varied soils that tend to be less porous than those in Ham Lake. These soils correspond loosely to the Grayling sand and Pemene loamy sand soils on the sites used by Johann Bruhn in his study of movement of *Ceratocystis fagacearum* through the roots of red oak in Michigan. The study design involved creating stem maps and measuring trees on 10 plots per year, beginning with 1996. Ten sites for each year that had been plowed in 1996, 1997, 1998, and 1999 were measured at HL, and ten sites plowed in 1997, 1998 and 1999 were examined at M-H. One site was dropped at HL because of data corruption after the field season, so only 9 sites were recorded for the plowing year 1997.

It is the model for barrier placement developed by Bruhn that was used to evaluate the effectiveness of the method generally used by vibratory plow contractors in Minnesota. The method used by these contractors is sometimes referred to as the "French" method, after Dr. David W. French, who pioneered much of the research in oak wilt control in Minnesota. This method suggests placing vibratory plow lines distal to the first ring of living trees that lie outside an oak wilt infection center. The method described by Bruhn relies on a probit-based model that uses as variables the diameters of infected and healthy trees, and the distance between them.

Results

Summary information for HL is provided in Table X1. Of 39 sites analyzed, red oak trees determined to be wilting, or having been previously killed by oak wilt were present on eight, for a total "success" rate of 79.5%. The practical result of a primary barrier line failure is that additional vibratory plowing or other control is required to limit the spread of disease. However, a homeowner may be willing to accept an 80% chance of control, as well as the attendant costs should additional plowing be necessary, if it means that additional high-value oak trees might be saved. For example, in almost every case in HL, the actual placement of vibratory plow lines was inside that suggested by Bruhn's model, indicating that the chance of successful control in these cases was probably less than the 95% predicted by the model. However, when barrier lines are placed farther from the infection center, additional trees that appear healthy at the time of plowing are left inside the primary barrier line, where they are much more likely to contract oak wilt and die. The trade-off that must be made is thus between the chance of successful control in the first attempt at vibratory plowing, and the sacrifice of trees that might not contract oak wilt if the barrier line is placed between them and the infection center.

In Table 1, the number of residual healthy trees at the time of plowing is the number of oak trees that were actually inside the primary barrier line at the time of plowing. In the 39 plots, 419 total oak trees were inside the primary barrier lines, but if Bruhn's 95% model had been followed, the number would have been 771, a difference of 352 trees. Most of these are high-value red oak or pin oak trees, which would have been unprotected from root graft transmission if the more conservative model developed by Bruhn had been followed. Of the 419 trees left inside the primary barrier line, 143 of them were actually dead or dying by the time of this study, that is, from 2-5 years after plowing. It isn't possible to say how many trees would have been killed if the plowing method suggested by the Bruhn model had been followed, but it would likely have been higher. Balanced against the sacrifice of these trees is the need to re-plow a site that had barrier "jumps" or "skips," that is, trees that became infected outside of the barrier line intended to control the disease. Several other comparisons can also made from the data in Table X, including comparison of the effects of the Bruhn model at 90% and 99% confidence, and fixed

radius barrier lines at 50' and 75'. In almost every case, each of these schemes places more trees inside the primary barrier line than the actual plow lines established by the vibratory plow contractors.

Eight plots of 39 installed at HL had skips (barrier line failures) that were attributed to root graft transmission of the pathogen, indicating that the barrier line failed to limit spread of the pathogen. Three possibilities can account for such barrier line failure: 1) the fungus was already distal to the point where the barrier line was placed; 2) the root grafts between healthy and infected trees were not disrupted by the plow line; or 3) other site factors that limited the effectiveness of the barrier line were present. The vibratory plow blade extends 5' below the soil line, and most oak roots will be disrupted at that depth, but it is possible that deeper roots are formed. If the pathogen was already in the roots distal to the point where the barrier line was placed, this indicates that the placement of the line was too liberal—that is, placed too closely to the infection center. Plowing in urban situations such as those encountered in HL is often complicated by the presence of objects and structures that limit where vibratory plow lines can be placed. These include sidewalks, driveways, underground utility lines, septic fields and tanks, and flower beds and landscaped areas. Compromises are almost always necessary in such settings, which can severely limit the successful placement of plow lines.

There is no current information on the ability of oaks to produce roots at depths greater than 5', and other than Bruhn's model, no information on rate of spread of the oak wilt pathogen in roots. Thus, it is not possible to assume that one or another factor was responsible for the failure of these root grafts.

However, at M-H, of the 30 plots evaluated at this site, not a single primary barrier line failure was observed. The reasons for this are probably many, but the primary factor probably involves a lack of the impediments that make plowing in urban situations difficult. Plow operators at M-H in most cases can place the plow lines exactly where they wish, without having to worry about structures or utility lines. And because individual oak trees are not quite as valuable as those in the landscapes near dwellings, plow line placement can be more conservative than that used in urban areas. In fact, it appears that many of the plow lines established at M-H are more conservative than the placement recommended by the Bruhn model, but this is tempered by the tendency of plow operators at M-H to utilize more secondary barriers—plow lines closer to the infection center than the primary barrier—to save trees. Because of the lack of impediments, it is much easier to place both primary and secondary lines in such settings. Data from M-H is still being analyzed, and will be published in Graham Mahal's Master's thesis, sometime later this year.

Additional data analysis is forthcoming. Each of the plots established in the study comprises a stem map similar to that reproduced in Appendix X1. Applying each of the various models under consideration results in a coded stem map such as that reproduced in Appendix X2, which allows comparison of the effects of each model with the other models under investigation, and with the actual results of vibratory plowing on each plot. Each plot generates several graphs and data tables, and analysis of these data have proven more difficult than was assumed.

One of the analyses still to be completed is an examination of Bruhn's model at 80% for the HL plots. Because it is now known that the actual success rate was about 80% for the plots at HL, it is possible to compare this situation with the results predicted by Bruhn's model for the same level of confidence. If the analysis suggests that the number of trees inside/outside the primary barrier line are similar if the 80% model is applied and compared to the observed data, it will provide very good evidence that the Bruhn model is applicable to the sandy soils in Minnesota.

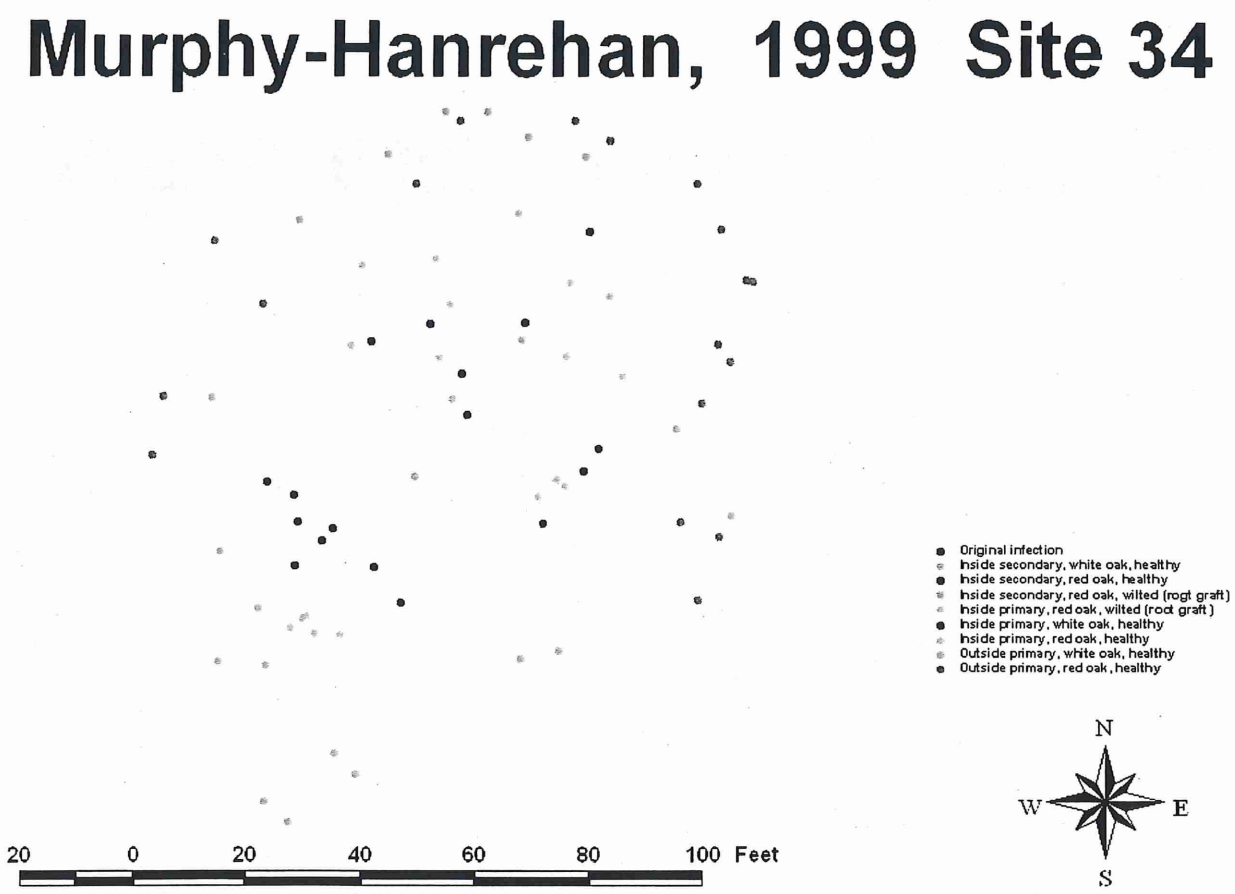
Analysis of the M-H data may indicate that the plow operators in that location are already following a procedure that closely approaches that suggested by the model or may show that the procedures at that location are more conservative than those suggested by the model.

Summary data from the study are reproduced in Appendix X3 and X4. The information provided in these appendixes is still being analyzed; the analysis and results will be published as soon as possible.

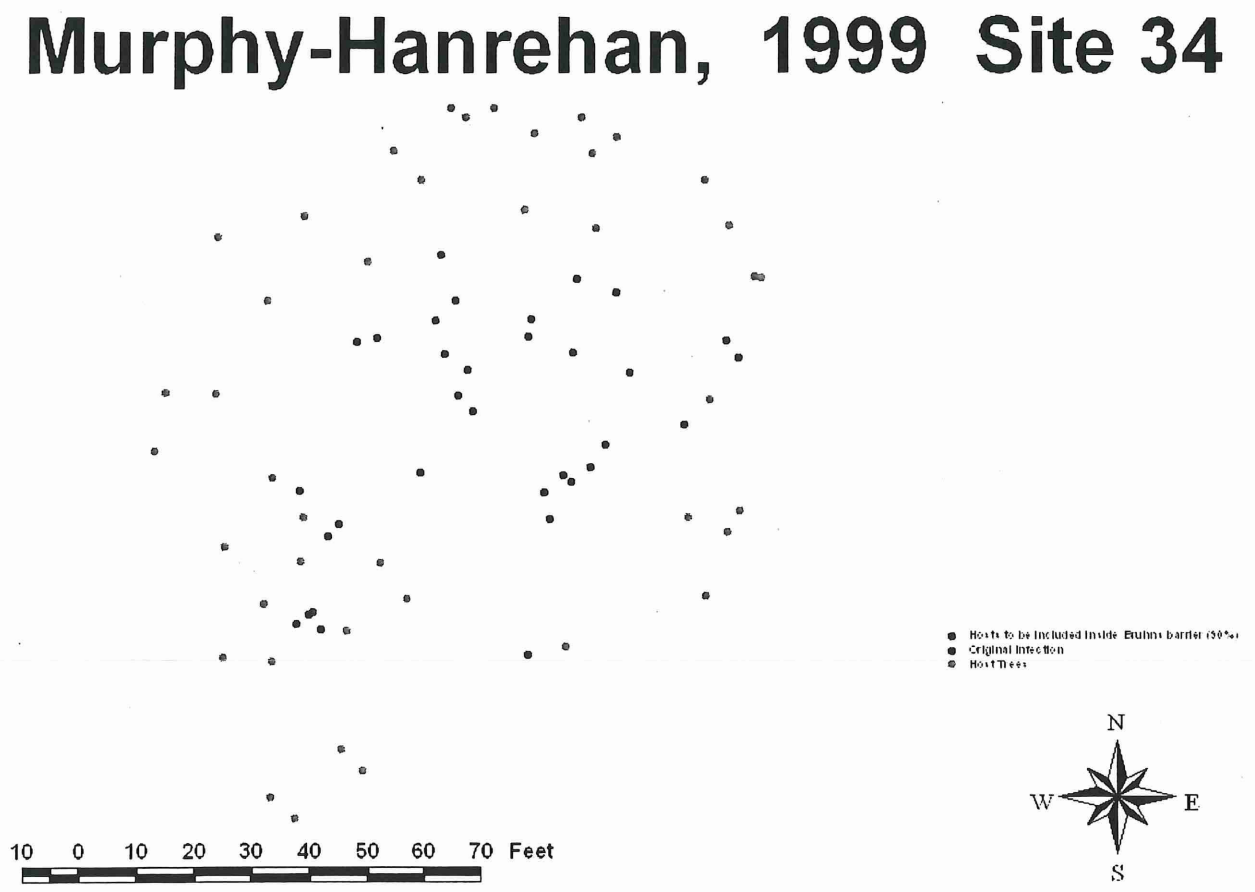
Conclusions:

- The barrier line models developed by Bruhn appear to be appropriate and acceptable for use in Minnesota, pending final analysis of the data collected.
- As a corollary, the rate of spread of the oak wilt pathogen through roots in sandy soils in Minnesota appears to be similar to that encountered by Bruhn on similar soils in Michigan.
- Confirmation of the rate of spread of the oak wilt pathogen through roots in loamy sand soils will be dependent upon further analysis of the data collected at Murphy Hanrehan Park Reserve.
- Homeowners may prefer a less "conservative" approach than that suggested by the Bruhn 95% model, if doing so results in saving potentially valuable landscape trees. Additional vibratory plow or other treatments are likely in such situations, but may be cost-effective and acceptable.
- Current practices appear to achieve about 80% success (in one visit) over time in urban areas, and somewhat higher (approaching 100%) in park-like landscapes.
- The techniques developed in the conduct of this study were found to achieve considerable time savings in the development of stem maps, and will be published as a separate article.
- The full analysis of the barrier line placement portion of this study will be achieved in Graham Mahal's Master's thesis, which should be completed by the end of 2002.

Appendix X1. Stem Map of Murphy-Hanrehan Site 34, showing distribution of trees inside and outside of the primary barrier line.



Appendix X2. Placement of barrier line suggested by Bruhn's model. Blue trees should be inside the barrier line. Note that it is impossible to fully implement the solution to the model because some smaller trees that are not at immediate risk are "trapped" inside the line because a larger tree distal to the infection center should be placed inside the line.



Key to Appendix X3 and X4.

IN = Inside the line
OUT = Outside the line
Pri = Primary barrier line
Sec = Secondary barrier line
Original Infection = Trees killed by oak wilt at the time of plowing
RO = Red oak
WO = White oak
Hlthy = Healthy
Dead = Dead
NI = New infection (overland spread)
RG = Root graft infection (local spread)
Wlt = Actively wilting tree

Appendix X3. Summary of Barrier Line Plot Data

Ham Lake

1996

Site Number:	15a	1996 Ham Lake	
In_Pri_RO_Hlthy		3	
Original Infection		1	
Out_Pri_RO_Hlthy		27	
Total number of trees at site:		31	
Average DBH of trees at site:		13.4	
Site Number:	15b	1996 Ham Lake	
In_Pri_RO_Hlthy		13	
In_Pri_RO_Wlt_RG		1	
Original Infection		2	
Out_Pri_RO_Hlthy		25	
Out_Pri_RO_Wlt_NI		3	
Out_Pri_RO_Wlt_RG		2	Primary Line Failure
Total number of trees at site:		46	
Average DBH of trees at site:		13.2	
Site Number:	15c	1996 Ham Lake	
In_Pri_RO_Wlt_RG		4	
Original Infection		2	
Out_Pri_RO_Hlthy		2	
Out_Pri_RO_Wlt_RG		12	Primary Line Failure
Total number of trees at site:		20	
Average DBH of trees at site:		14.6	

Site Number: 20	1996 Ham Lake
IN_Pri_RO_Dead	1
In_Pri_RO_Hlthy	4
In_Pri_WO_Hlthy	9
Original Infection	1
Out_Pri_RO_Dead	1
Out_Pri_RO_Hlthy	13
Out_Pri_WO_Dead	1
Out_Pri_WO_Hlthy	5
Total number of trees at site:	35
Average DBH of trees at site:	14.1

Site Number: 28	1996 Ham Lake
IN_Pri_RO_Dead	3
In_Pri_RO_Hlthy	6
In_Pri_RO_Wlt_RG	4
In_Pri_WO_Hlthy	1
Original Infection	2
Out_Pri_RO_Dead	1
Out_Pri_RO_Hlthy	10
Out_Pri_WO_Hlthy	4
Total number of trees at site:	31
Average DBH of trees at site:	10.1

Site Number: 30	1996 Ham Lake
In_Pri_RO_Hlthy	9
In_Pri_RO_Wlt_RG	14
IN_Sec_RO_Dead_R	4
Original Infection	3
Out_Pri_RO_Dead	3
Out_Pri_RO_Hlthy	28

Out_Pri_WO_Hlthy	1	
Total number of trees at site:	62	
Average DBH of trees at site:	15.3	

Site Number: 34 1996 Ham Lake

In_Pri_RO_Wlt_RG	5	
Original Infection	2	
Out_Pri_RO_Hlthy	24	
Out_Pri_RO_Wlt_RG	6	Primary Line Failure
Out_Pri_WO_Hlthy	1	
Out_Pri_WO_Wlt_NI	1	
Total number of trees at site:	39	
Average DBH of trees at site:	13.7	

Site Number: 42a 1996 Ham Lake

In_Pri_RO_Wlt_RG	4	
Original Infection	1	
Out_Pri_RO_Dead	1	
Out_Pri_RO_Hlthy	10	
Out_Pri_WO_Dead	1	
Out_Pri_WO_Hlthy	4	
Total number of trees at site:	21	
Average DBH of trees at site:	16.2	

Site Number: 42b 1996 Ham Lake

IN_Pri_RO_Dead	1	
In_Pri_RO_Hlthy	3	
In_Pri_RO_Wlt_RG	2	
In_Pri_WO_Hlthy	1	
Original Infection	1	

Out_Pri_RO_Hlthy	8	
Out_Pri_RO_Wlt_RG	3	Primary Line Failure
Out_Pri_WO_Hlthy	6	
Total number of trees at site:	25	
Average DBH of trees at site:	15.2	

Site Number: 51 1996 Ham Lake

IN_Pri_RO_Dead	1
In_Pri_RO_Hlthy	6
In_Pri_RO_Wlt_RG	2
In_Pri_WO_Hlthy	1
In_Sec_RO_Wlt_RG	2
Original Infection	1
Out_Pri_RO_Hlthy	5
Out_Pri_WO_Hlthy	5
Total number of trees at site:	23
Average DBH of trees at site:	16.3

1997

Site Number:	1	1997 Ham Lake
In_Pri_RO_Hlthy		5
In_Pri_RO_Wlt_RG		7
Original Infection		3
Out_Pri_RO_Hlthy		9
Out_Pri_WO_Hlthy		2
Total number of trees at site:		26
Average DBH of trees at site:		19.6

Site Number:	14a	1997 Ham Lake
In_Pri_RO_Hlthy		5
In_Sec_RO_Hlthy		1
Original Infection		1
Out_Pri_RO_Hlthy		3
Total number of trees at site:		10
Average DBH of trees at site:		11.0

Site Number:	14b	1997 Ham Lake
In_Pri_RO_Hlthy		7
In_Pri_WO_Hlthy		1
In_Sec_RO_Hlthy		2
In_Sec_WO_Hlthy		2
Original Infection		1
Out_Pri_RO_Hlthy		18
Out_Pri_WO_Hlthy		3
Total number of trees at site:		34
Average DBH of trees at site:		9.0

Site Number: 15 1997 Ham Lake

IN_Pri_RO_Dead	7
In_Pri_RO_Hlthy	2
IN_Pri_WO_Dead	1
In_Pri_WO_Hlthy	5
IN_Sec_RO_Dead	1
In_Sec_RO_Hlthy	1
In_Sec_WO_Hlthy	2
Original Infection	1
Total number of trees at site:	20
Average DBH of trees at site:	13.4

Site Number: 16 1997 Ham Lake

In_Pri_RO_Hlthy	7
In_Pri_RO_Wlt_RG	3
In_Pri_WO_Hlthy	5
Original Infection	1
Out_Pri_RO_Hlthy	5
Out_Pri_WO_Hlthy	1
Total number of trees at site:	22
Average DBH of trees at site:	15.6

Site Number: 21 1997 Ham Lake

In_Pri_RO_Hlthy	1
In_Sec_RO_Wlt_RG	3
Original Infection	3
Out_Pri_RO_Hlthy	11
Total number of trees at site:	18
Average DBH of trees at site:	18.1

Site Number: 29	1997 Ham Lake	
IN_Pri_RO_Dead	1	
In_Pri_RO_Hlthy	5	
In_Sec_RO_Wlt_RG	1	
In_Sec_WO_Hlthy	1	
Original Infection	1	
Out_Pri_RO_Dead	1	
Out_Pri_RO_Hlthy	12	
Out_Pri_RO_Wlt_NI	2	
Out_Pri_WO_Hlthy	9	
Total number of trees at site:	33	
Average DBH of trees at site:	14.6	

Site Number: 35	1997 Ham Lake	
In_Pri_RO_Hlthy	16	
In_Pri_RO_Wlt_RG	3	
In_Sec_RO_Wlt_RG	1	
In_Sec_WO_Hlthy	2	
Original Infection	2	
Out_Pri_RO_Hlthy	6	
Out_Pri_RO_Wlt_RG	1	Primary Line Failure
Total number of trees at site:	31	
Average DBH of trees at site:	13.8	

Site Number: 37	1997 Ham Lake
In_Pri_RO_Hlthy	3
In_Pri_RO_Wlt_RG	4
In_Pri_WO_Hlthy	2
Original Infection	1
Out_Pri_RO_Hlthy	13
Out_Pri_WO_Hlthy	2

Total number of trees at site:	25
Average DBH of trees at site:	12.5

Site Number: 46 1997 Ham Lake

In_Pri_RO_Hlthy	2
In_Pri_WO_Hlthy	3
Original Infection	2
Out_Pri_RO_Hlthy	7
Out_Pri_WO_Hlthy	5
Total number of trees at site:	19
Average DBH of trees at site:	9.5

1998

Site Number: 10	1998 Ham Lake	
In_Pri_RO_Hlthy	11	
In_Pri_RO_Wlt_RG	5	
In_Sec_RO_Hlthy	2	
In_Sec_RO_Wlt_RG	3	
Original Infection	2	
Out_Pri_RO_Dead	2	
Out_Pri_RO_Hlthy	35	
Total number of trees at site:	60	
Average DBH of trees at site:	14.0	

Site Number: 12	1998 Ham Lake	
In_Pri_RO_Hlthy	3	
In_Pri_RO_Wlt_RG	11	
In_Pri_WO_Hlthy	2	
Original Infection	2	
Out_Pri_RO_Dead	2	
Out_Pri_RO_Hlthy	30	
Out_Pri_RO_Wlt_RG	5	Primary Line Failure
Out_Pri_WO_Hlthy	12	
Total number of trees at site:	67	
Average DBH of trees at site:	13.3	

Site Number: 14	1998 Ham Lake	
In_Pri_RO_Hlthy	1	
In_Pri_RO_Wlt_RG	1	
Original Infection	2	
Out_Pri_RO_Hlthy	3	
Total number of trees at site:	7	
Average DBH of trees at site:	17.7	

Site Number:	15	1998 Ham Lake
IN_Pri_RO_Dead		3
In_Pri_RO_Hlthy		14
In_Pri_RO_Wlt_RG		4
In_Pri_WO_Hlthy		2
Original Infection		1
Out_Pri_RO_Dead		8
Out_Pri_RO_Hlthy		85
Out_Pri_RO_Wlt_NI		1
Out_Pri_WO_Hlthy		3
Total number of trees at site:		121
Average DBH of trees at site:		10.9

Site Number:	21a	1998 Ham Lake
In_Pri_RO_Hlthy		1
In_Pri_RO_Wlt_RG		1
Original Infection		1
Out_Pri_RO_Hlthy		4
Out_Pri_WO_Hlthy		6
Total number of trees at site:		13
Average DBH of trees at site:		21.0

Site Number:	21b	1998 Ham Lake
In_Pri_RO_Hlthy		1
In_Pri_RO_Wlt_RG		1
In_Pri_WO_Hlthy		1
Original Infection		1
Out_Pri_RO_Hlthy		3
Out_Pri_WO_Hlthy		3
Total number of trees at site:		10
Average DBH of trees at site:		22.7

Site Number:	31	1998 Ham Lake
IN_Pri_RO_Dead		1
In_Pri_RO_Hlthy		4
IN_Pri_WO_Dead		1
In_Pri_WO_Hlthy		1
IN_Sec_RO_Dead		1
IN_Sec_RO_Dead2		1
In_Sec_RO_Wlt_RG		4
In_Sec_WO_Hlthy		1
Original Infection		4
Out_Pri_RO_Hlthy		15
Out_Pri_WO_Hlthy		2
Total number of trees at site:		35
Average DBH of trees at site:		10.9

Site Number:	33	1998 Ham Lake
In_Pri_RO_Hlthy		2
Original Infection		3
Out_Pri_RO_Dead		1
Out_Pri_RO_Hlthy		53
Out_Pri_WO_Hlthy		1
Total number of trees at site:		60
Average DBH of trees at site:		12.9

Site Number:	35	1998 Ham Lake
In_Pri_RO_Hlthy		6
Original Infection		1
Out_Pri_RO_Dead		3
Out_Pri_RO_Hlthy		42
Out_Pri_WO_Hlthy		7
Total number of trees at site:		59

Average DBH of trees at site:	13.3

Site Number: 40 1998 Ham Lake

In_Pri_RO_Hlthy	3
Original Infection	1
Out_Pri_RO_Dead	1
Out_Pri_RO_Hlthy	18
Total number of trees at site:	23
Average DBH of trees at site:	13.4

1999

Site Number:	14	1999 Ham Lake	
In_Pri_RO_Hlthy		2	
In_Pri_WO_Hlthy		8	
Original Infection		4	
Out_Pri_RO_Hlthy		21	
Out_Pri_WO_Hlthy		8	
Total number of trees at site:		43	
Average DBH of trees at site:		9.0	

Site Number:	19	1999 Ham Lake	
In_Pri_RO_Hlthy		3	
In_Pri_WO_Hlthy		3	
Original Infection		1	
Out_Pri_RO_Dead		1	
Out_Pri_RO_Hlthy		8	
Out_Pri_WO_Hlthy		6	
Total number of trees at site:		22	
Average DBH of trees at site:		14.8	

Site Number:	24	1999 Ham Lake	
IN_Pri_RO_Dead		1	
In_Pri_RO_Hlthy		7	
In_Pri_RO_Wlt_RG		13	
Original Infection		2	
Out_Pri_RO_Hlthy		29	
Out_Pri_RO_Wlt_RG		1	Primary Line Failure
Total number of trees at site:		53	
Average DBH of trees at site:		12.8	

Site Number:	30	1999 Ham Lake
In_Pri_RO_Hlthy		4
In_Pri_RO_Wlt_RG		1
In_Sec_RO_Hlthy		2
Original Infection		1
Out_Pri_RO_Dead		1
Out_Pri_RO_Hlthy		29
Total number of trees at site:		38
Average DBH of trees at site:		12.5

Site Number:	34	1999 Ham Lake
In_Pri_RO_Hlthy		2
In_Pri_RO_Wlt_RG		4
Original Infection		1
Out_Pri_RO_Hlthy		5
Total number of trees at site:		12
Average DBH of trees at site:		8.4

Site Number:	4	1999 Ham Lake
IN_Pri_RO_Dead		1
In_Pri_RO_Hlthy		2
In_Pri_RO_Wlt_RG		1
In_Pri_WO_Hlthy		2
Original Infection		1
Out_Pri_RO_Hlthy		15
Out_Pri_WO_Hlthy		7
Total number of trees at site:		29
Average DBH of trees at site:		10.4

Site Number:	40	1999 Ham Lake
In_Pri_WO_Hlthy		3
Original Infection		1

Out_Pri_RO_Dead	2	
Out_Pri_RO_Hlthy	12	
Out_Pri_WO_Hlthy	2	
Total number of trees at site:	20	
Average DBH of trees at site:	19.5	

Site Number: 49 1999 Ham Lake

In_Pri_RO_Hlthy	8	
In_Pri_WO_Hlthy	9	
In_Sec_RO_Hlthy	1	
In_Sec_WO_Hlthy	2	
Original Infection	1	
Out_Pri_RO_Hlthy	5	
Out_Pri_WO_Hlthy	16	
Total number of trees at site:	42	
Average DBH of trees at site:	12.7	

Site Number: 6 1999 Ham Lake

In_Pri_RO_Hlthy	4	
In_Pri_RO_Wlt_RG	2	
In_Sec_RO_Hlthy	1	
Original Infection	2	
Out_Pri_RO_Hlthy	20	
Out_Pri_RO_Wlt_RG	2	Primary Line Failure
Total number of trees at site:	31	
Average DBH of trees at site:	12.1	

Site Number: 9 1999 Ham Lake

IN_Pri_RO_Dead	3	
In_Pri_RO_Hlthy	19	
In_Pri_RO_Wlt_RG	28	
Original Infection	7	
Out_Pri_RO_Dead	5	

Out_Pri_RO_Hlthy	24
Out_Pri_WO_Hlthy	5
Total number of trees at site:	91
Average DBH of trees at site:	8.5

Murphy-Hanrehan

1997

Site Number: 1	1997 Murphy-Hanrehan
In_Pri_RO_Hlthy	2
In_Pri_RO_Wlt_RG	2
In_Sec_RO_Wlt_RG	1
In_Sec_WO_Hlthy	1
Original Infection	1
Out_Pri_RO_Dead	1
Out_Pri_RO_Hlthy	5
Total number of trees at site:	13
Average DBH of trees at site:	15.7

Site Number: 15	1997 Murphy-Hanrehan
IN_Pri_RO_Dead	3
In_Pri_RO_Hlthy	9
IN_Pri_WO_Dead	3
In_Pri_WO_Hlthy	9
IN_Sec_RO_Dead	1
In_Sec_RO_Hlthy	5
IN_Sec_WO_Dead	2
In_Sec_WO_Hlthy	6
Original Infection	1
Total number of trees at site:	39
Average DBH of trees at site:	12.2

Site Number: 21	1997 Murphy-Hanrehan
IN_Pri_RO_Dead	1
In_Pri_RO_Hlthy	9
In_Sec_RO_Hlthy	2
In_Sec_RO_Wlt_RG	1

In_Sec_WO_Hlthy	1
Original Infection	1
Out_Pri_RO_Dead	13
Out_Pri_RO_Hlthy	64
Out_Pri_WO_Dead	4
Out_Pri_WO_Hlthy	12
Total number of trees at site:	108
Average DBH of trees at site:	10.4

Site Number: 22 1997 Murphy-Hanrehan

In_Pri_RO_Hlthy	4
In_Pri_WO_Hlthy	3
In_Sec_WO_Hlthy	3
Original Infection	2
Out_Pri_RO_Hlthy	34
Out_Pri_WO_Hlthy	7
Total number of trees at site:	53
Average DBH of trees at site:	10.7

Site Number: 30 1997 Murphy-Hanrehan

IN_Pri_RO_Dead	1
In_Pri_RO_Hlthy	8
In_Pri_RO_Wlt_RG	3
In_Pri_WO_Hlthy	4
IN_Sec_RO_Dead	2
IN_Sec_RO_Dead2	1
In_Sec_RO_Hlthy	1
In_Sec_RO_Wlt_RG	5
Original Infection	2
Out_Pri_RO_Dead	6
Out_Pri_RO_Hlthy	20
Out_Pri_RO_Wlt_NI	1

Total number of trees at site:	54
Average DBH of trees at site:	13.6

Site Number: 36 1997 Murphy-Hanrehan

In_Pri_RO_Hlthy	5
In_Pri_RO_Wlt_RG	2
IN_Pri_WO_Dead	1
In_Pri_WO_Hlthy	5
In_Sec_RO_Wlt_RG	2
Original Infection	2
Out_Pri_RO_Dead	7
Out_Pri_RO_Hlthy	42
Out_Pri_WO_Dead	3
Out_Pri_WO_Hlthy	26
Total number of trees at site:	95
Average DBH of trees at site:	13.0

Site Number: 37 1997 Murphy-Hanrehan

In_Pri_RO_Wlt_NI	5
In_Pri_RO_Wlt_RG	1
In_Pri_WO_Hlthy	5
Original Infection	2
Out_Pri_RO_Dead	1
Out_Pri_RO_Hlthy	10
Out_Pri_RO_Wlt_NI	1
Out_Pri_WO_Hlthy	13
Total number of trees at site:	38
Average DBH of trees at site:	12.4

Site Number: 38	1997 Murphy-Hanrehan
In_Pri_RO_Hlthy	10
In_Pri_WO_Hlthy	5
In_Sec_WO_Hlthy	1
Original Infection	2
Out_Pri_RO_Dead	3
Out_Pri_RO_Hlthy	16
Out_Pri_WO_Hlthy	3
Total number of trees at site:	40
Average DBH of trees at site:	16.2

Site Number: 4	1997 Murphy-Hanrehan
In_Pri_RO_Hlthy	6
In_Pri_RO_Wlt_RG	2
In_Pri_WO_Hlthy	1
Original Infection	1
Out_Pri_RO_Dead	1
Out_Pri_RO_Hlthy	12
Out_Pri_WO_Hlthy	6
Total number of trees at site:	29
Average DBH of trees at site:	16.7

Site Number: 5	1997 Murphy-Hanrehan
In_Pri_RO_Hlthy	2
IN_Pri_WO_Dead	2
In_Pri_WO_Hlthy	1
Original Infection	1
Out_Pri_RO_Dead	3
Out_Pri_RO_Hlthy	12
Out_Pri_WO_Hlthy	20
Total number of trees at site:	41

Average DBH of trees at site:	13.4

1998

Site Number:	14	1998 Murphy-Hanrehan	
IN_Pri_RO_Dead			3
In_Pri_RO_Hlthy			15
IN_Pri_WO_Dead			1
In_Pri_WO_Hlthy			4
In_Sec_WO_Hlthy			3
Original Infection			1
Out_Pri_RO_Dead			1
Out_Pri_RO_Hlthy			19
Out_Pri_RO_Wlt_NI			1
Out_Pri_WO_Hlthy			10
Total number of trees at site:			58
Average DBH of trees at site:			12.3

Site Number:	15	1998 Murphy-Hanrehan	
IN_Sec_RO_Dead_R			1
In_Sec_WO_Hlthy			3
Original Infection			2
Out_Pri_RO_Dead			7
Out_Pri_RO_Hlthy			19
Out_Pri_WO_Dead			1
Out_Pri_WO_Hlthy			13
Total number of trees at site:			46
Average DBH of trees at site:			12.9

Site Number:	16	1998 Murphy-Hanrehan	
IN_Pri_RO_Dead			1
In_Pri_RO_Hlthy			11

IN_Pri_WO_Dead	1
In_Pri_WO_Hlthy	4
In_Sec_RO_Wlt_RG	4
In_Sec_WO_Hlthy	3
Original Infection	1
Out_Pri_RO_Dead	3
Out_Pri_RO_Hlthy	31
Out_Pri_WO_Dead	6
Out_Pri_WO_Hlthy	23
Total number of trees at site:	88
Average DBH of trees at site:	12.4

Site Number: 21 1998 Murphy-Hanrehan

IN_Pri_RO_Dead	1
In_Pri_RO_Hlthy	2
IN_Pri_WO_Dead	1
In_Pri_WO_Hlthy	12
In_Sec_WO_Hlthy	1
Original Infection	1
Out_Pri_RO_Dead	5
Out_Pri_RO_Hlthy	24
Out_Pri_WO_Dead	2
Out_Pri_WO_Hlthy	28
Total number of trees at site:	77
Average DBH of trees at site:	15.5

Site Number: 25 1998 Murphy-Hanrehan

In_Pri_RO_Hlthy	4
In_Pri_RO_Wlt_RG	4
In_Pri_WO_Hlthy	4
Original Infection	3
Out_Pri_RO_Hlthy	13

Out_Pri_WO_Hlthy	3
Total number of trees at site:	31
Average DBH of trees at site:	13.4

Site Number: 28 1998 Murphy-Hanrehan

IN_Pri_RO_Dead	1
In_Pri_RO_Hlthy	8
In_Sec_RO_Wlt_RG	1
Original Infection	2
Out_Pri_RO_Dead	7
Out_Pri_RO_Hlthy	39
Out_Pri_WO_Hlthy	9
Total number of trees at site:	67
Average DBH of trees at site:	12.7

Site Number: 29 1998 Murphy-Hanrehan

IN_Pri_RO_Dead	4
In_Pri_RO_Hlthy	3
In_Pri_RO_Wlt_RG	3
In_Pri_WO_Hlthy	2
IN_Sec_RO_Dead	1
Original Infection	1
Out_Pri_RO_Dead	9
Out_Pri_RO_Hlthy	38
Out_Pri_WO_Hlthy	3
Total number of trees at site:	64
Average DBH of trees at site:	14.6

Site Number: 3 1998 Murphy-Hanrehan

IN_Pri_RO_Dead	3
In_Pri_RO_Hlthy	7
In_Pri_RO_Wlt_NI	5
In_Sec_RO_Hlthy	1

Original Infection	1
Out_Pri_RO_Dead	1
Out_Pri_RO_Hlthy	21
Out_Pri_WO_Dead	1
Out_Pri_WO_Hlthy	23
Total number of trees at site:	63
Average DBH of trees at site:	11.3

Site Number: 30 1998 Murphy-Hanrehan

IN_Pri_RO_Dead	4
In_Pri_RO_Hlthy	17
In_Pri_RO_Wlt_RG	3
IN_Pri_WO_Dead	2
In_Pri_WO_Hlthy	7
In_Sec_RO_Wlt_RG	1
IN_Sec_WO_Dead	1
In_Sec_WO_Hlthy	3
Original Infection	1
Out_Pri_RO_Dead	3
Out_Pri_RO_Hlthy	18
Out_Pri_WO_Dead	2
Out_Pri_WO_Hlthy	7
Total number of trees at site:	69
Average DBH of trees at site:	13.8

Site Number: 4 1998 Murphy-Hanrehan

IN_Pri_RO_Dead	2
In_Pri_RO_Hlthy	4
In_Pri_RO_Wlt_NI	3
In_Pri_RO_Wlt_RG	2
In_Pri_WO_Hlthy	5
In_Sec_RO_Wlt_RG	2

IN_Sec_WO_Dead	1
Original Infection	1
Out_Pri_RO_Dead	10
Out_Pri_RO_Hlthy	40
Out_Pri_WO_Dead	3
Out_Pri_WO_Hlthy	24
Total number of trees at site:	97
Average DBH of trees at site:	11.4

1999

Site Number: 1 1999 Murphy-Hanrehan

IN_Pri_RO_Dead	4
In_Pri_RO_Hlthy	4
In_Pri_WO_Hlthy	1
In_Sec_RO_Wlt_RG	2
Original Infection	1
Out_Pri_RO_Dead	5
Out_Pri_RO_Hlthy	25
Out_Pri_WO_Hlthy	8
Total number of trees at site:	50
Average DBH of trees at site:	17.5

Site Number: 14 1999 Murphy-Hanrehan

IN_Pri_RO_Dead	2
In_Pri_RO_Hlthy	2
IN_Pri_WO_Dead	1
In_Pri_WO_Hlthy	5
In_Sec_RO_Wlt_RG	4
Original Infection	1
Out_Pri_RO_Dead	6
Out_Pri_RO_Hlthy	12
Out_Pri_RO_Wlt_NI	1

Out_Pri_WO_Hlthy	2
Total number of trees at site:	36
Average DBH of trees at site:	17.5

Site Number: 21 1999 Murphy-Hanrehan

IN_Pri_RO_Dead	2
In_Pri_RO_Hlthy	14
IN_Pri_WO_Dead	5
In_Pri_WO_Hlthy	3
Original Infection	1
Out_Pri_RO_Dead	7
Out_Pri_RO_Hlthy	42
Out_Pri_WO_Dead	2
Out_Pri_WO_Hlthy	17
Total number of trees at site:	93
Average DBH of trees at site:	14.0

Site Number: 28 1999 Murphy-Hanrehan

In_Pri_RO_Hlthy	15
In_Pri_WO_Hlthy	9
Original Infection	2
Out_Pri_RO_Dead	10
Out_Pri_RO_Hlthy	39
Out_Pri_WO_Dead	2
Out_Pri_WO_Hlthy	9
Total number of trees at site:	86
Average DBH of trees at site:	12.7

Site Number: 30 1999 Murphy-Hanrehan

In_Pri_RO_Hlthy	7
In_Pri_WO_Hlthy	12
In_Sec_WO_Hlthy	4
Original Infection	1

Out_Pri_RO_Hlthy	8
Out_Pri_WO_Hlthy	10
Total number of trees at site:	42
Average DBH of trees at site:	13.8

Site Number: 33 1999 Murphy-Hanrehan

Original Infection	1
Out_Pri_RO_Hlthy	31
Out_Pri_WO_Hlthy	9
Total number of trees at site:	41
Average DBH of trees at site:	10.8

Site Number: 34 1999 Murphy-Hanrehan

In_Pri_RO_Hlthy	18
In_Pri_RO_Wlt_RG	1
In_Pri_WO_Hlthy	15
In_Sec_RO_Hlthy	1
In_Sec_RO_Wlt_RG	1
In_Sec_WO_Hlthy	1
Original Infection	1
Out_Pri_RO_Hlthy	18
Out_Pri_WO_Hlthy	19
Total number of trees at site:	75
Average DBH of trees at site:	8.8

Site Number: 4 1999 Murphy-Hanrehan

IN_Pri_RO_Dead	1
In_Pri_RO_Hlthy	3
In_Pri_WO_Hlthy	9
Original Infection	2
Out_Pri_RO_Hlthy	6
Out_Pri_WO_Hlthy	6

Total number of trees at site:	27
Average DBH of trees at site:	13.9

Site Number:	45a	1999	Murphy-Hanrehan
IN_Pri_RO_Dead			7
In_Pri_RO_Hlthy			8
In_Pri_WO_Hlthy			1
In_Sec_RO_Hlthy			4
Original Infection			1
Out_Pri_RO_Dead			3
Out_Pri_RO_Hlthy			20
Out_Pri_WO_Hlthy			6
Total number of trees at site:			50
Average DBH of trees at site:			14.3

Total Number of Trees:	3077

Appendix X4. Summary of point data for oak wilt vibraline study by year and location.

Ham Lake

1996

IN_Pri_RO_Dead	6
In_Pri_RO_Hlthy	44
In_Pri_RO_Wlt_RG	36
In_Pri_WO_Hlthy	12
IN_Sec_RO_Dead_R	4
In_Sec_RO_Wlt_RG	2
Original Infection	16
Out_Pri_RO_Dead	6
Out_Pri_RO_Hlthy	152
Out_Pri_RO_Wlt_NI	3
Out_Pri_RO_Wlt_RG	23
Out_Pri_WO_Dead	2
Out_Pri_WO_Hlthy	26
Out_Pri_WO_Wlt_NI	1

1997

IN_Pri_RO_Dead	8
In_Pri_RO_Hlthy	53
In_Pri_RO_Wlt_RG	17
IN_Pri_WO_Dead	1
In_Pri_WO_Hlthy	16
IN_Sec_RO_Dead	1
In_Sec_RO_Hlthy	4
In_Sec_RO_Wlt_RG	5
In_Sec_WO_Hlthy	7
Original Infection	16
Out_Pri_RO_Dead	1
Out_Pri_RO_Hlthy	84
Out_Pri_RO_Wlt_NI	2

Out_Pri_RO_Wlt_RG	1
Out_Pri_WO_Hlthy	22
1998	
IN_Pri_RO_Dead	4
In_Pri_RO_Hlthy	46
In_Pri_RO_Wlt_RG	23
IN_Pri_WO_Dead	1
In_Pri_WO_Hlthy	6
IN_Sec_RO_Dead	1
IN_Sec_RO_Dead2	1
In_Sec_RO_Hlthy	2
In_Sec_RO_Wlt_RG	7
In_Sec_WO_Hlthy	1
Original Infection	18
Out_Pri_RO_Dead	17
Out_Pri_RO_Hlthy	288
Out_Pri_RO_Wlt_NI	1
Out_Pri_RO_Wlt_RG	5
Out_Pri_WO_Hlthy	34
1999	
IN_Pri_RO_Dead	5
In_Pri_RO_Hlthy	51
In_Pri_RO_Wlt_RG	49
In_Pri_WO_Hlthy	25
In_Sec_RO_Hlthy	4
In_Sec_WO_Hlthy	2
Original Infection	21
Out_Pri_RO_Dead	9
Out_Pri_RO_Hlthy	168
Out_Pri_RO_Wlt_RG	3
Out_Pri_WO_Hlthy	44

Murphy-Hanrehan Park Reserve

1997

IN_Pri_RO_Dead	5
In_Pri_RO_Hlthy	55
In_Pri_RO_Wlt_NI	5
In_Pri_RO_Wlt_RG	10
IN_Pri_WO_Dead	6
In_Pri_WO_Hlthy	33
IN_Sec_RO_Dead	3
IN_Sec_RO_Dead2	1
In_Sec_RO_Hlthy	8
In_Sec_RO_Wlt_RG	9
IN_Sec_WO_Dead	2
In_Sec_WO_Hlthy	12
Original Infection	15
Out_Pri_RO_Dead	35
Out_Pri_RO_Hlthy	215
Out_Pri_RO_Wlt_NI	2
Out_Pri_WO_Dead	7
Out_Pri_WO_Hlthy	87

1998

IN_Pri_RO_Dead	19
In_Pri_RO_Hlthy	71
In_Pri_RO_Wlt_NI	8
In_Pri_RO_Wlt_RG	12
IN_Pri_WO_Dead	5
In_Pri_WO_Hlthy	38
IN_Sec_RO_Dead	1
IN_Sec_RO_Dead_R	1
In_Sec_RO_Hlthy	1
In_Sec_RO_Wlt_RG	8

	IN_Sec_WO_Dead	2
	In_Sec_WO_Hlthy	13
	Original Infection	14
	Out_Pri_RO_Dead	46
	Out_Pri_RO_Hlthy	262
	Out_Pri_RO_Wlt_NI	1
	Out_Pri_WO_Dead	15
	Out_Pri_WO_Hlthy	143
1999		
	IN_Pri_RO_Dead	16
	In_Pri_RO_Hlthy	71
	In_Pri_RO_Wlt_RG	1
	IN_Pri_WO_Dead	6
	In_Pri_WO_Hlthy	55
	In_Sec_RO_Hlthy	5
	In_Sec_RO_Wlt_RG	7
	In_Sec_WO_Hlthy	5
	Original Infection	11
	Out_Pri_RO_Dead	31
	Out_Pri_RO_Hlthy	201
	Out_Pri_RO_Wlt_NI	1
	Out_Pri_WO_Dead	4
	Out_Pri_WO_Hlthy	86
	Total Number of Trees:	3077

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Cover photos by:

Top left: Nitidulid eggs on a mature oak wilt mat collected during midspring.

Center: Nitidulid larva: *Epuraea corticina* Erichson.

Bottom left: Nitidulid adult: *Glischrochilus sanguinolentus* (Olivier).

Illustrations by:

The authors are grateful to Julie Martinez for her expert drawings of nitidulid larvae.

HOW to Identify Common Nitidulid Beetles Associated with Oak Wilt Mats in Minnesota

Contents *Page*

Purpose

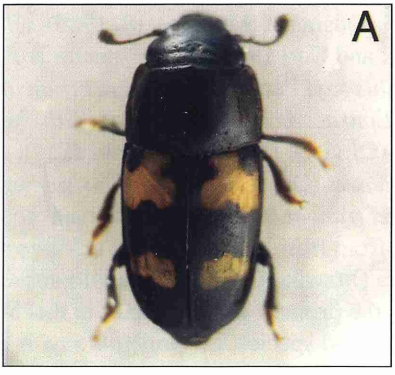
We developed this handbook for forestry professionals, land managers, and homeowners to help them identify the most common adult and larval sap beetles found in oak wilt mats in the North Central States. Although the photographs depict the natural color of adults, preserved specimens may not have exactly the same color as those in the pictures. All sizes given are length ranges and mean lengths based on measurements of our specimens using a light microscope with an ocular micrometer. The brief written descriptions are not intended to be taxonomically complete, but to pinpoint the features most useful in distinguishing species from one another. The descriptions are largely based on those of Parsons (1943).

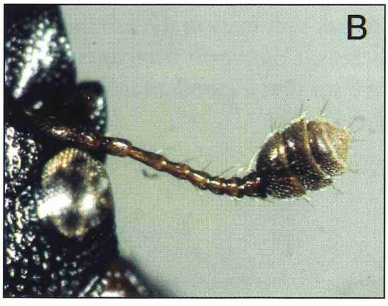
Introduction

Oak wilt, caused by the fungus *Ceratocystis fagacearum* (Bretz) Hunt, is an important disease of oaks throughout the Eastern United States. Thousands of native oaks, particularly those in the red oak group (Section Lobatae = *Erythrobalanus*), succumb to the disease each year across the Midwest. The pathogen is spread in two ways: underground through root grafts and overland by insect vectors. Insect transmission is the primary means of establishing new oak wilt infection centers, and the principal vectors in the North Central States are sap beetles (Coleoptera: Nitidulidae) (Juzwik 2001). Although there are many nitidulid species associated with oaks, six species make up the majority of those typically collected from oak wilt mats in Minnesota (Cease and Juzwik 2001), Wisconsin (McMullen *et al.* 1960), and Illinois (Himelick and Curl 1958). These species are *Carpophilus sayi* Parsons, *Colopterus truncatus* Randall, *Epuraea corticina* Erichson, *Glischrochilus fasciatus* (Olivier), *G. quadrisignatus* (Say), and *G. sanguinolentus* (Olivier). *Carpophilus sayi* and *Colopterus truncatus* are the predominant species collected from fresh wounds on red oak trees in Minnesota (Juzwik *et al.* 1999), making these two nitidulids the primary vector species in this State. Of the six nitidulid species, *Carpophilus sayi* is more abundant in oak wilt mats in the spring than in the fall, the three *Glischrochilus* species are more abundant in oak wilt mats in the fall than in the spring, whereas, *Colopterus truncatus* and *Epuraea corticina* are similarly abundant in oak wilt mats in the spring and fall.

Characteristics of General Nitidulid Adult

Adult sap beetles are generally small (~2 to 12 mm long) with flattened and broadly oval to somewhat elongate bodies (see **A** below). The body color of the adults ranges from reddish brown to black, in some cases with red, orange, or yellow patterns on the elytra (wing covers). Certain species have short elytra that expose the upper surface of the last two or three abdominal segments. Adult sap beetles are distinguished from other beetles visiting oak wilt mats by having 11-segmented antennae that end in a 3-segmented, ball-like club (**B**). Adults are attracted to and live in fermenting plant sap, decaying fruit, or fungi (Downie and Arnett 1996).

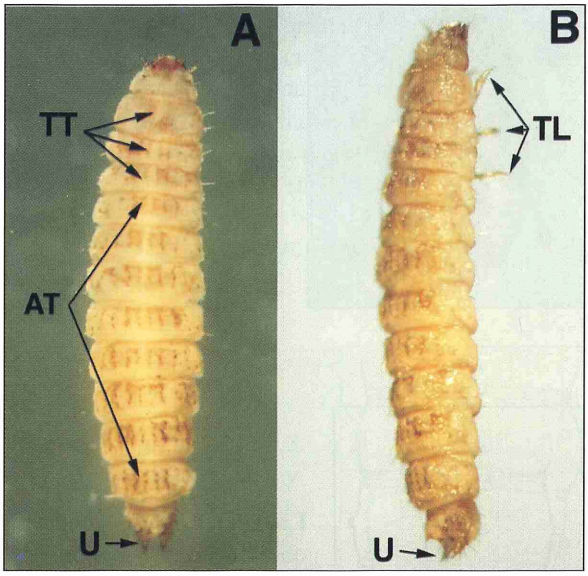




Adult sap beetle, *Glischrochilus fasciatus* (**A**) and enlarged 11-segmented right antenna showing the 3-segmented club (**B**).

Characteristics of General Nitidulid Larva

Larval sap beetles are elongate and cylindrical, range from ~3 to 8 mm in length, and have three pairs of well-developed thoracic legs (see below). The first thoracic tergum (hardened plate on upper surface of the body segment bearing the first pair of legs) is usually pigmented, but in some species terga on both the thorax and abdomen are pigmented. The ninth abdominal tergum is especially important for identifying the larvae. This tergum usually has paired urogomphi (fixed, hornlike structures) that may be simple or branched and often have paired pregomphi located anteriorly (in front of them).

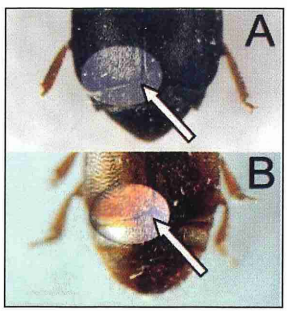


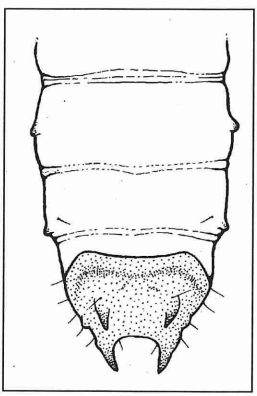
Larval sap beetle, *Epuraea corticina*, showing (**A**) dorsal view with plate-like and pigmented thoracic and abdominal terga (TT and AT) and urogomphi (U) on the last abdominal tergum. Thoracic legs (TL) are evident in (**B**) the lateral view.

Species Descriptions

***Carpophilus sayi* Parsons**

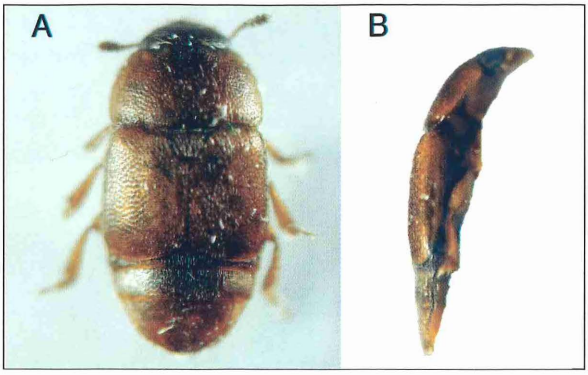
Adults: Small (3.5 to 5.1 mm, mean = 4.2 mm, n = 15), very dark brown to black, sometimes with pronotum (shoulders) and pronotal margins rufous (pale red). Sparsely pubescent ("fuzzy") and punctate. Elytra short, revealing last two abdominal segments. Nearly perpendicular angle formed by median suture (line where two elytra meet) and tip of elytron (see **A** below). For comparison, an obtuse angle is formed by median suture and tip of elytron in *Colopterus truncatus* (see **B** below).





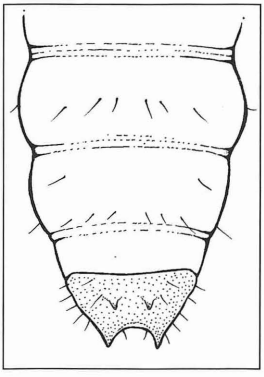
Larvae: Length, based on one specimen, 4.2 mm. Very similar to *Colopterus truncatus*, but pregomphi are longer, and the space between urogomphi is more angular or deeply truncate.

Colopterus truncatus **Randall**



Adults: Very small (1.9 to 2.6 mm, mean = 2.2 mm, n = 24), medium brown, sparsely pubescent; head, pronotum, and elytra punctate; elytra short, revealing last three abdominal segments (**A**, dorsal view). Obtuse angle formed by median suture and tip of elytron (see **B** on previous page). Lateral view (**B**, this page) illustrates how extremely flattened adult *Colopterus truncatus* can be.

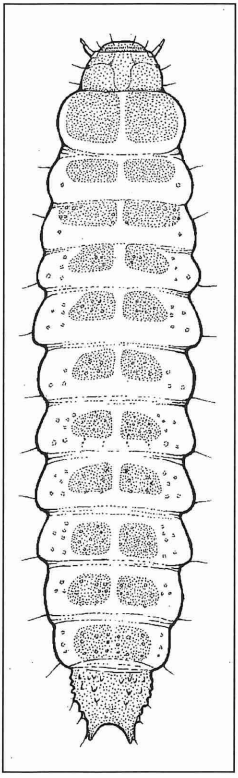
Larvae: Length 3.2 to 5.3 mm. Body entirely creamy white. Head and prothorax sclerotized (hardened) and golden brown. Ninth abdominal segment sclerotized dorsally, with one small pair of pregomphi and one pair unbranched urogomphi.



Epuraea corticina
Erichson

Adults: Small (2.8 to 4.0 mm, mean = 3.3 mm, n = 29), oblong, brownish yellow, mottled with dark brown; pronotum usually with acute hind angles; elytra covering all but last abdominal segment.

Larvae: Length, 4.8 to 5.9 mm. Body mostly creamy white. Head golden brown. Body appears to have dorsal horizontal stripes, caused by debris trapped in the tubercles on the dorsum of each segment. Ninth abdominal segment with two pair of small, straight urogomphi and numerous tubercles from which setae (single small hairs) protrude.



The following species of *Glischrochilus* are relatively large, oblong, shiny sap beetles. Those described here are attractively marked dorsally (on top) with yellow, yellow-orange, or red. The elytra leave only the tip of the last abdominal segment exposed. *Glischrochilus* spp. are common in a variety of habitats other than oak wilt mats. Their presence in oak woodlands is not necessarily an indication of oak wilt.

***Glischrochilus fasciatus* (Olivier)**

Adults: Medium to large (4.2 to 6.6 mm, mean = 5.6 mm, n = 26), head and pronotum black. Elytra black with large, paired yellow blotches that are symmetrical relative to the median suture. The yellow blotches located anteriorly (toward the head) are tri-lobed.

Larvae: Unknown.

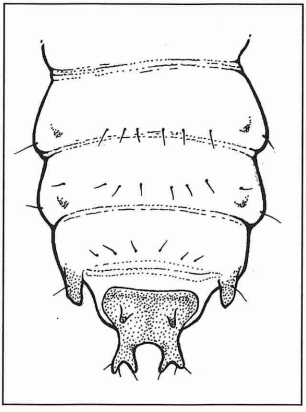
Glischrochilus quadrisignatus (Say)

Adults: Medium to large (3.9 to 6.7 mm, mean = 5.2 mm, n = 28). *Glischrochilus quadrisignatus* can be distinguished from *G. fasciatus* in two ways: the yellow elytral blotches are smaller, and the anterior blotches are farther from the median suture than the posterior (rear) blotches.

Larvae: Similar to *G. sanguinolentus* (Connell 1991).

Glischrochilus sanguinolentus **(Olivier)**

Adults: Medium to large (4.4 to 6.4 mm, mean = 5.2 mm, n = 20); elytra red with black submedian spot and black apical one-third.



Larvae: Length, based on two specimens, 7.9 to 8.4 mm. Body mostly creamy white and somewhat flattened. Head, pronotum, and ninth abdominal segment golden brown. Abdominal spiracles protrude, increasing in length down the body, and are golden brown at the tips. Ninth abdominal segment with one pair pregomphi and one pair branched urogomphi.

References

Cease, K.R.; Juzwik, J. 2001. Predominant nitidulid species (Coleoptera: Nitidulidae) associated with spring oak wilt mats in Minnesota. Canadian Journal of Forest Research. 31: 635-643.

Connell, W.A. 1991. Insect and mite pests in food, an illustrated key. Vol. 2. Sap beetles (Nitidulidae, Coleoptera). Agric. Handb. 655. Washington, DC: U.S. Department of Agriculture, Forest Service: 151-174.

Downie, N.M.; Arnett, R.H., Jr. 1996. The beetles of northeastern North America, Volume II: Polyphaga, Superfamily Bostrichoidea thru Curculionoidea. Gainesville, FL: The Sandhill Crane Press.

Himelick, E.B.; Curl, E.A. 1958. Transmission of *Ceratocystis fagacearum* by insects and mites. Plant Disease Report. 42: 538-540.

Juzwik, J. 2001. Overland transmission of *Ceratocystis fagacearum*: extending our understanding. In: Shade tree wilt diseases. St. Paul, MN: American Phytopathological Society, APS Press. Chapter 8.

Juzwik, J.; Skalbeck, T.C.; Neuman, M.F. 1999. Nitidulid species associated with fresh wounds on red oaks during spring in Minnesota. Phytopathology. 89: S38.

McMullen, L.H.; Shenefelt, R.D.; Kuntz, J.E. 1960. A study of insect transmission of oak wilt in Wisconsin. Wisconsin Academy of Sciences, Arts and Letters. 49: 73-84.

Parsons, C.T. 1943. A revision of the nearctic Nitidulidae (Coleoptera). Bulletin of the Museum of Comparative Zoology. 92: 121-278.

Glossary

Abdomen.—The third or posterior division of the insect body; consists normally of 9 or 10 apparent segments; bears no functional legs in the adult stage.

Anterior.—In front; before; having to do with the forward section of something.

Apical.—At, near, or pertaining to the apex, which is that part of any joint or segment opposite the base by which it is attached. For example, that point of a wing furthest removed from the base or at the end.

Dorsal.—Of or belonging to the upper surface.

Elytron/Elytra.—The anterior leathery or chitinous wings of beetles, serving as coverings to the hind wings, commonly meeting in a straight line down the middle of the body when the beetle is at rest and not flying.

Larva/Larvae.—A young insect that quits the egg in an early stage of development and differs fundamentally in form from the adult. The immature form of animals that undergo metamorphosis; other related terms are nymph, caterpillar, slug, maggot, and grub.

Lateral.—Relating, pertaining, or attached to the side.

Posterior.—In the rear; after; having to do with the hind section of something; opposite of anterior.

Pronotum.—The upper or dorsal surface of the prothorax.

Prothorax.—The first thoracic ring or segment; it bears the anterior legs but no wings in adult insects.

Pubescent.—Downy; clothed with soft, short, fine closely set hair.

Punctate.—Having impressed points or punctures.

Root graft.—Roots that have grown together so that a graft union is made between the conducting tissues of both roots. The oak wilt pathogen can move through grafted roots between infected and healthy trees to cause new infections.

Rufous.—Pale red.

Sclerite.—Any piece of the insect body wall bounded by sutures. Sclerotization is the hardening of the insect body wall by the deposition of sclerotizing substances.

Seta/Setae.—Hairs or bristles that are hollow structures developed as extensions of the insect epidermis.

Spiracles.—Breathing pores or openings in the sides of the insect body through which air enters the body.

Submedian.—Below a line drawn through the middle of a structure or animal.

Tergite.—A dorsal sclerite or part of a segment, especially when such part consists of a single sclerite or plate.

Tergum/Terga.—The upper or dorsal surface of any body segment of an insect, whether it consists of one or more than one sclerite or plate.

Thorax/Thoraces.—The second or intermediate region of the insect body bearing the true legs and wings, made up of three rings, named in order, pro-, meso-, and metathorax. As adjective, thoracic.

Truncate.—Cut off squarely at the tip.

Tubercles.—Little solid pimples or small buttons on the surface of the insect body, sometimes bearing setae or bristles.

Urogomphus/Urogomphi.—Fixed or mobile structure found on the terminal segments of certain larval insects. Pregomphi are related structures that are generally smaller and are located anterior to the urogomphi.

Vector.—An organism such as an insect, mite, nematode, or a higher animal such as a bird or rodent that carries a pathogenic agent to a susceptible host.

Companion Publications

Pokorny, J. 1999. **How to collect field samples and identify the oak wilt fungus in the laboratory**. NA-FR-01-99. St. Paul, MN: U.S. Department of Agriculture, Forest Service, Northeastern Area State and Private Forestry. 12 p.

O'Brien, J.G.; Mielke, M.E.; Starkey, D.; Juzwik, J. 2000. **How to identify, prevent, and control oak wilt**. NA-PR-03-00. St. Paul, MN: U.S. Department of Agriculture, Forest Service, Northeastern Area State and Private Forestry. 28 p.