

Association of *Ophiostoma novo-ulmi* with *Scolytus schevyrewi* (Scolytidae) in Colorado

W. R. Jacobi and R. D. Koski, Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins 80523; T. C. Harrington, Department of Plant Pathology, Iowa State University, Ames 50011; and J. J. Witcosky, U.S. Department of Agriculture, Forest Service, Forest Health Management, Lakewood Service Center, Lakewood, CO 80225

ABSTRACT

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The smaller European elm bark beetle, *Scolytus multistriatus*, has been the primary vector of the Dutch elm disease fungus, *Ophiostoma novo-ulmi*, in elm trees in Colorado since 1948. An exotic from Asia, the banded elm bark beetle, *Scolytus schevyrewi*, was found in Siberian elm, *Ulmus pumila*, in Colorado in April of 2003; this was the first report of *S. schevyrewi* in North America. *S. schevyrewi* is now found throughout much of Colorado and in at least 21 other states. The similarities in breeding and feeding habits between *S. schevyrewi* and *S. multistriatus* have raised concerns about the ability of *S. schevyrewi* to serve as a vector for *O. novo-ulmi*. The objective of this preliminary study was to determine if *O. novo-ulmi* could be isolated from adult *S. schevyrewi* emerging from diseased elm trees. *S. schevyrewi* and *S. multistriatus* were allowed to infest diseased stem segments of American elm, *Ulmus americana*. The infested stem segments were caged and isolations were made from the adult brood that emerged. *O. novo-ulmi* was isolated from most of the adults of both beetle species, showing that *S. schevyrewi* could acquire the pathogen as effectively as *S. multistriatus*. Future studies are needed to determine if *S. schevyrewi* can effectively transmit the pathogen to healthy trees.

Dutch elm disease (DED) is a vascular wilt disease of elms, *Ulmus* spp., incited by the exotic fungi *Ophiostoma ulmi* and *Ophiostoma novo-ulmi* (2,7,18,19). The originally introduced species *O. ulmi* has been largely replaced by the more aggressive *O. novo-ulmi* in the United States (3,4,15). The pathogens are commonly spread from tree to tree by root grafts and also by elm bark beetles (Coleoptera: Scolytidae) (17–19,22). In North America, *Hylurgopinus rufipes* Eich., the native elm bark beetle, and *Scolytus multistriatus* Marsham., the smaller European elm bark beetle, are the two known vectors of the DED fungi in eastern North America (1,19). Since the first confirmed report of DED in Colorado in 1948 (20), the only known vector of the DED pathogens is the smaller European elm bark beetle (12,20). In regions where both *S. multistriatus* and *H. rufipes* are present in eastern North America, the former is considered the more efficient vector (19). However, *S. multistriatus* does not over-

winter well in cold climates, and *H. rufipes* is the principal vector in the northern parts of eastern North America (1,19).

Scolytus schevyrewi Semenov, the banded elm bark beetle, is native to northern Chinese provinces, as well as Korea, Mongolia, Russia, Kazakhstan, Uzbekistan, Kyrgyzstan, Turkmenistan, and Tajikistan (14). The banded elm beetle was first collected in North America in April 2003 in Aurora, CO, from drought-stressed *Ulmus pumila* (Siberian elm) (14). By the spring of 2005, *S. schevyrewi* had been found in Arizona, California, Colorado, Idaho, Illinois, Indiana, Kansas, Nebraska, Nevada, New Mexico, Maryland, Michigan, Minnesota, Montana, Oklahoma, Oregon, South Dakota, Utah, and Wyoming (13). The banded elm bark beetle has not been reported from either Canada or Mexico. In its native range on the Asian continent, *S. schevyrewi* is recorded on *Ulmus*, *Prunus*, *Salix*, *Eleagnus*, and *Caragana* species. In the United States, *S. schevyrewi* has only been collected from four *Ulmus* species: *U. americana*, *U. procera*, *U. pumila*, and *U. thomasi* (14).

The role of *S. multistriatus* in the spread of DED in North America has been studied and documented since the 1940s (6,16,17). The similarities in biology between *S. schevyrewi* and *S. multistriatus* warranted studies to determine the ability and efficiency of *S. schevyrewi* as a vector. Thus, the objective of this study was to determine if *O. novo-ulmi* could be acquired by adult

S. schevyrewi. If the fungus is acquired by *S. schevyrewi*, this would warrant additional studies to determine the effectiveness and efficiency of *S. schevyrewi* as a vector.

MATERIALS AND METHODS

Stem sections of DED-symptomatic *U. americana* trees in Colorado were exposed to natural populations of elm bark beetles and placed in rearing cages. Isolation attempts were made from the emerging brood using two techniques, one employed at Iowa State University (ISU) in Ames and another at Colorado State University (CSU) in Fort Collins.

Beetle sources. Stem segments from three DED-afflicted American elm (*U. americana*) trees were removed from landscapes in Colorado Springs (two sites) and Denver in July 2004. At the time of their removal, each tree had symptoms of DED but had no evidence of infestation by bark beetles. The stem segments from the diseased trees were moved to a location at the Denver Federal Center, where *S. schevyrewi* and *S. multistriatus* were known to be present. After 2 to 4 weeks of exposure to natural bark beetle attack (5), the stem segments were cut into 0.3- to 0.6-m bolts and placed in rearing cages located at a U.S.D.A. Forest Service lab in Lakewood, CO, or at CSU. Adult beetles from the brood that emerged were periodically collected from the rearing cages, killed by crushing the beetle, identified to species by their distinguishing elytra and sternite spine shape (11), placed individually into a sterile gelatin capsule, and stored at 4°C.

Isolations from beetles at ISU. Crushed beetles collected from stem segments at the U.S.D.A. Forest Service lab were shipped on ice overnight to ISU. Five batches of beetles were shipped between 28 September and 26 October. Upon arrival at ISU, the beetles were stored at 4°C until plating, generally within 72 h after shipping. Each beetle was cut into quarters (head, prothorax, the rest of the thorax, and abdomen), and each quarter was plated in cycloheximide-streptomycin-malt agar (CSMA) (1.5% malt extract, 2.0% agar, 100 ppm streptomycin sulfate, and 500 ppm cycloheximide), which is semi-selective for species of *Ophiostoma* (8). The petri plates were incubated at room temperature (20 to 23°C) for up to 3 weeks and examined

Corresponding author: William Jacobi
E-mail: William.Jacobi@ColoState.EDU

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periodically for colonies emanating from the beetle parts.

Isolation of fungi from beetles at CSU. Beetles collected from rearing cages in Fort Collins were stored individually in gelatin capsules at 2°C and processed an average of 15 days later. The beetles were identified, crushed, and placed into amended elm sawwood agar (CESA) (3.0% agar, 2.5% elm sawdust, 100 ppm streptomycin sulfate, and 200 ppm cycloheximide). Plates were incubated at 18 to 23°C for up to 4 weeks and examined periodically with a dissecting microscope for the presence of the synnemal state of *O. novo-ulmi*.

***O. novo-ulmi* in elm stems.** To verify the presence of *O. novo-ulmi* in each of the three trees, disks (3 to 9 cm thick) were removed from each of 49 stem segments obtained from the three trees and kept in plastic bags at 2°C for 1 to 5 days. Bark was removed from each disk, and 16 wood (xylem) chips underneath the bark were removed from four locations around the disk and placed four to a plate in CSMA medium (1.5% malt extract, 2.0% agar, 100 ppm streptomycin sulfate, and 200 ppm cycloheximide). Plated wood chips were incubated at 18 to 23°C for up to 2 weeks and examined periodically for the presence of the synnema.

RESULTS

Beetle emergence from stem segments at CSU. All exposed stem segments from diseased trees contained brood of both *S. schevyrewi* and *S. multistriatus*, but the predominant insect species varied from tree to tree. Beetles were collected from caged tree stem segments for 8 to 16 weeks during August to November, depending on tree number (Fig. 1). Most

beetles emerged 10 to 13 weeks after logs were placed into rearing cages. Very few beetles emerged from segments taken from tree two (Fig. 1). Ninety-eight percent of the *Scolytus* that emerged from the caged stem segments were *S. schevyrewi* and 2% were *S. multistriatus*.

Isolation of *O. novo-ulmi* from beetles. An apparent *Ophiostoma* species was isolated from most of the plated beetles. The characteristic *Sporothrix* state was noted in isolations on CSMA medium, and a synnemal state (*Pesotum* sp.) was produced on plates containing the elm sawdust medium. At ISU, select colonies suspected to be *Ophiostoma* species were transferred to CSMA plates and observed to form white, fast-growing cultures (average radial growth rate of 3.2 mm/day) with concentric rings of heavy *Sporothrix* sporulation, which is typical for *O. novo-ulmi*. The absence of the uniform powdery phenotype also suggested that the fungus was *O. novo-ulmi* subsp. *americana* (4). All of the

subcultured colonies had the same mycelial morphology and growth rate. One such colony from each of the three trees was used for DNA sequencing (9). The internal transcribed spacer region (ITS) of the rDNA was amplified with the primers ITS1-F and ITS-4, and the product was sequenced with the same primers at the DNA Sequencing and Synthesis Facility at Iowa State University. The sequences of the three isolates were 100% identical to each other and to other sequences of *O. novo-ulmi* previously submitted to GenBank (National Center for Biotechnology Information) (9). At CSU, conidial masses from selected synnema were transferred onto CSMA and observed to form white, fast-growing cultures with concentric rings of heavy *Sporothrix* sporulation, which is typical for *O. novo-ulmi*. All of the subcultured colonies had the same mycelial morphology and growth rate.

Of the *Scolytus* adults plated at ISU, *O. novo-ulmi* was isolated from 84% of adult

Table 1. Isolation of *Ophiostoma novo-ulmi* from adult *Scolytus schevyrewi* and *S. multistriatus* insects reared from three *Ulmus americana* trees

Location ^a	Tree ^b	<i>S. schevyrewi</i>		<i>S. multistriatus</i>	
		Number	Percent positive	Number	Percent positive
ISU	One	23	8.7	0	0.0
	Two	233	91.0	71	91.5
	Three	32	84.4	19	68.4
	Totals	288	83.7	90	86.7
CSU	One	440	30.9	4	0.0
	Two	52	63.5	3	66.7
	Three	367	44.1	12	58.3
	Totals	859	46.2	19	41.7

^a Location of isolation attempts: ISU = Iowa State University, CSU = Colorado State University.

^b Three Dutch elm disease-afflicted American elm (*Ulmus americana*) trees removed from landscapes in Colorado Springs, and Denver, CO. Duplicate stem segments were placed in rearing cages in Denver, Colorado (ISU) and in Fort Collins, Colorado (CSU).

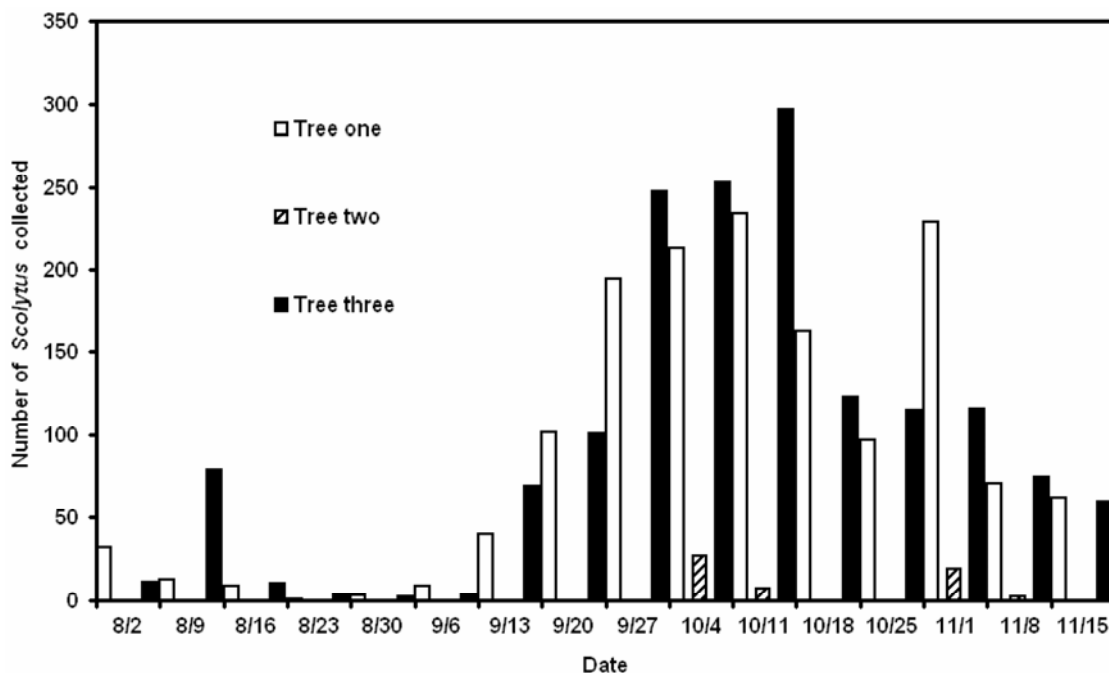


Fig. 1. Total *Scolytus multistriatus* and *S. schevyrewi* brood emergence from elm logs in rearing cages at Fort Collins, CO, 2004.

S. schevyrewi and 87% of adult *S. multistriatus* (Table 1). Only *S. schevyrewi* was reared from tree one, and *O. novo-ulmi* was isolated from only 2 of these 23 beetles. In contrast, *O. novo-ulmi* was isolated from 91 and 84% of *S. schevyrewi* adults from trees two and three, respectively (Table 1). At CSU, isolation of *O. novo-ulmi* from emerged *S. schevyrewi* adults ranged from 31 to 64% (Table 1). The greatest percent isolation of the pathogen from *S. schevyrewi* was from tree two. Of the *Scolytus* adults plated at CSU, *O. novo-ulmi* was isolated from 46% of the *S. schevyrewi* plated and from 42% of the *S. multistriatus* plated (Table 1).

Recovery of *O. novo-ulmi* from stem segments at CSU. Based on isolations from wood chips, all three of the trees utilized in this study were infected with *O. novo-ulmi*. *O. novo-ulmi* was isolated from 93, 100, and 100%, respectively, from trees one, two, and three.

DISCUSSION

While stem segments from all three trees were infested with both *S. schevyrewi* and *S. multistriatus*, the number of *S. schevyrewi* exceeded the number of *S. multistriatus* in all three trees. The variation in the number of beetles used for isolation from a particular tree at CSU and ISU was the result of collecting beetles from different stem segments caged in Lakewood or Fort Collins, CO. Most of the sampled disks from the segments were well-colonized by *O. novo-ulmi*, but there was some variation. The lower frequency of isolation of *O. novo-ulmi* from some tree segments may be due to unequal distribution of the pathogen in the trees. This likely resulted in the lower percentage isolation of *O. novo-ulmi* from the *Scolytus* adults reared from segments taken from tree one.

O. novo-ulmi was isolated from 9 to 91% of the *S. schevyrewi* adults and 0 to 92% of the *S. multistriatus* adults. The higher isolation rate at ISU versus CSU may be due to the quartering of the beetles before plating at ISU and the higher concentration of cycloheximide used in CSMA at ISU. The pathogen was recovered from *S. schevyrewi* in percentages comparable to that from the most important vector (*S. multistriatus*) in North America. The high percentage of recovery of *O. novo-ulmi* from *S. schevyrewi* suggests that it has the potential to be an important vector of the DED fungus. However, our results should be considered preliminary because the attacking bark beetles were infesting stem segments rather than standing trees, and trees from only three sites were sampled. Nonetheless, it is clear that *S. schevyrewi* is capable of acquiring *O. novo-ulmi* in high percentages from naturally diseased trees.

Pathogen recovery from plated adults of the two *Scolytus* species (41 to 84%) is in line with the earlier findings of Parker et

al. (16,17), who successfully isolated *O. ulmi* from 76% of plated *S. multistriatus* surface washings and 14% from plated *S. multistriatus* intestinal tracts. Of the *S. multistriatus* beetles that emerged from infested elm trees in Spain, 35 to 64% were contaminated with aggressive and nonaggressive strains of *O. ulmi* (now recognized as *O. novo-ulmi* and *O. ulmi*, respectively) (21). Spore loads (number of spores on beetles) were not assessed in our study, but previous work in Spain indicated a large variation in spore loads within and among *Scolytus* species in Europe. Thus, further studies are needed to compare spore loads on *S. schevyrewi* and *S. multistriatus* in the United States to compare their effectiveness as vectors of *O. novo-ulmi*.

The biology of *S. schevyrewi*, including breeding in stressed (diseased) elm trees (14), pupation in the outer bark, and maturation feeding in twigs, is very similar to that of *S. multistriatus*, suggesting that *S. schevyrewi* could serve as an effective vector of *O. novo-ulmi* (10,14). Future studies are needed to determine if *S. schevyrewi* can effectively introduce the pathogen into an infection court and establish the fungus in a living elm tree. The timing of maturation feeding by the newly emerged adults, wound location, depth of wounds, amount of inoculum transferred by beetles, and the susceptibility of trees during the season need to be assessed. If *S. schevyrewi* can effectively transmit the fungus and it continues to spread and dominate over *S. multistriatus* in North America, then it could become the principal vector of *O. novo-ulmi*. The native range of *S. schevyrewi* in Asia suggests that this species tolerates cold winter temperatures much better than does *S. multistriatus*, so the newly introduced *S. schevyrewi* could be especially important in colder climates (10,14).

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