

# Do mites phoretic on elm bark beetles contribute to the transmission of Dutch elm disease?

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**Abstract** Dutch elm disease (DED) is a destructive vascular wilt disease of elm (*Ulmus*) trees caused by the introduced Ascomycete fungus *Ophiostoma novo-ulmi*. In Europe, this DED pathogen is transmitted by elm bark beetles in the genus *Scolytus*. These insects carry phoretic mites to new, suitable habitats. The aim of this study was to record and quantify conidia and ascospores of *O. novo-ulmi* on phoretic mites of the three elm bark beetle species *Scolytus multistriatus*, *Scolytus pygmaeus*, and *Scolytus scolytus*. Spores of *O. novo-ulmi* were found on four of the ten mite species phoretic on *Scolytus* spp. These included *Elattoma fraxini*, *Proctolaelaps scolyti*, *Pseudotarsonemoides eccoptogasteri*, and *Tarsonemus crassus*. All four species had spores attached externally to their body surfaces. However, *T. crassus* carried most spores within its sporothecae, two paired pocket-like structures adapted

for fungal transmission. Individuals of *Pr. scolyti* also had *O. novo-ulmi* conidia and ascospores frequently in their digestive system, where they may remain viable. While *E. fraxini* and *P. eccoptogasteri* rarely had spores attached to their bodies, large portions of *Pr. scolyti* and *T. crassus* carried significant numbers of conidia and/or ascospores of *O. novo-ulmi*. *P. scolyti* and *T. crassus*, which likely are fungivores, may thus contribute to the transmission of *O. novo-ulmi*, by increasing the spore loads of individual *Scolytus* beetles during their maturation feeding on twigs of healthy elm trees, enhancing the chance for successful infection with the pathogen. Only *S. scolytus*, which is the most efficient vector of *O. novo-ulmi* in Europe, carried high numbers of *Pr. scolyti* and *T. crassus*, in contrast to *S. multistriatus* and *S. pygmaeus*, which are known as less efficient vectors. The high efficiency of *S. scolytus* in spreading Dutch elm disease may be partly due to its association with these two mites and the hyperphoretic spores of *O. novo-ulmi* they carry.

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#### Introduction

*Ophiostoma novo-ulmi* (Ascomycota, Ophiostomatales) is the causal agent of Dutch elm disease (DED), a lethal vascular wilt disease of elm species (*Ulmus* spp.) in Europe, parts of Asia, and North America (Brasier 1991). Since their introduction in the 1940s, into areas where they are not native, the two subspecies of this fungus, *O. novo-ulmi* ssp. *novo-ulmi* and ssp. *americana* and their hybrids have been causing a second pandemic of DED, which has led to enormous damage to

populations of susceptible elm species (Brasier 1991, 2000; Brasier and Kirk 2001; Konrad et al. 2002; Brasier et al. 2004). Although the native range of *O. novo-ulmi* is still unknown, it is suspected that the fungus originated from mountainous areas of Southern Asia, possibly somewhere in the Himalayas or adjacent regions (Brasier 2000).

*O. novo-ulmi* is one of the best known examples of a tree pathogen vectored by insects, particularly bark beetles (Coleoptera, Curculionidae, Scolytinae). In Europe and Asia, the fungus is transmitted by various native elm bark beetles in the genus *Scolytus*, while in North America *Scolytus multistriatus*, introduced from Europe, and the native elm bark beetle *Hylurgopinus rufipes* serve as vectors of the pathogen (Lanier and Peacock 1981; Webber 2000, 2004). Other than xylomycetophagous ambrosia beetles and some phloeomycetophagous bark beetles, *Scolytus* species on elm lack mycangia, specialized organs in the integument of the beetles that serve for the storage, transport, and transmission of fungal spores (Six 2003; Kirisits 2004). In contrast, elm bark beetles carry ascospores and conidia of *O. novo-ulmi* on the body surface and in the gut (Fransen 1939; Lanier and Peacock 1981; Webber and Brasier 1984; Webber and Gibbs 1989).

Transmission of *O. novo-ulmi* and wound inoculation of the pathogen onto its hosts occur during maturation feeding of recently emerged, sexually immature beetles on the bark of twigs and in twig crotches in the crown of healthy elm trees (Fransen 1939; Webber and Brasier 1984; Webber and Gibbs 1989). Trees succumbing due to DED are subsequently infested by *Scolytus* bark beetles that breed in the phloem of the stem and branches. The insects initiate characteristic breeding systems in the phloem and partly also on the sapwood surface consisting of female and larval galleries as well as pupal chambers, where the larvae pupate and turn to immature beetles (Lanier and Peacock 1981). In the pupal chambers, juvenile beetles are contaminated with ascospores and conidia of the DED pathogen prior to emergence and eventually are able to infect healthy elm trees (Fransen 1939; Webber and Brasier 1984; Webber and Gibbs 1989).

*Scolytus* species vary greatly in their effectiveness as vectors of *O. novo-ulmi*. The larger *Scolytus* species, in particular *Scolytus scolytus*, are considered to be more effective vectors than smaller species such as *S. multistriatus* and *Scolytus pygmaeus* (Webber and Brasier 1984; Webber and Gibbs 1989; Webber 1990, 2000). The efficiency of *Scolytus* spp. as vectors of *O. novo-ulmi* is largely determined by the kind of breeding material and the position of pupal chambers in the bark of elm trees (Webber and Brasier 1984; Webber and Gibbs 1989; Webber 1990, 2000). *S. scolytus* usually breeds in thick bark of elm stems and branches and commonly pupates in the moist inner bark, which is conducive for profuse sporulation of *O. novo-ulmi*. High loads of spores are therefore often

acquired by young *S. scolytus* beetles. Conditions for acquisition of fungal inoculum are less favorable for the smaller elm scolytines usually breeding in thinner bark that dries out more quickly. They also pupate more often in the drier outer bark where sporulation of *O. novo-ulmi* is sparser than in the inner bark.

Bark beetles are known to be commonly associated with phoretic mites that use the insects for transportation to new, suitable habitats (Bridges and Moser 1983; Moser and Bogenschütz 1984; Pernek et al. 2008). The feeding habits and ecological roles of mites are diverse. Mites phoretic on bark beetles include beetle parasitoids, insect and nematode predators, fungivores, and omnivores (Moser et al. 1978; Kinn 1983; Lombardero et al. 2000, 2003; Pernek et al. 2008). These arthropods are also involved in complex symbiotic interactions with bark beetles, fungi, especially including ophiostomatoid species (*Ophiostoma* spp. and *Ceratocystopsis* spp.), and their host trees (Klepzig et al. 2001; Kirisits 2004; Hofstetter et al. 2006). Interactions between the various partners range from mutualism, commensalism to antagonism (Klepzig et al. 2001; Hofstetter et al. 2006). Some mite species on conifers are involved in the transmission of tree pathogens, mycangial symbionts, and fungal antagonists of bark beetles (Moser et al. 1995; Lombardero et al. 2000, 2003; Klepzig et al. 2001; Kirisits 2004; Hofstetter et al. 2006). *Tarsonemus* species even possess specialized, paired, pocket-like structures, called sporothecae, in which spores of ophiostomatoid fungi are deposited and transported (Moser 1985; Bridges and Moser 1986; Magowski and Moser 2003).

*Scolytus* elm bark beetles also carry phoretic mites on their body surfaces. Recently, we have documented the assemblages of mites phoretic on *S. multistriatus* and *S. pygmaeus* in Austria, which consisted of nine species (Moser et al. 2005). These mites included *Chelacheles michalskii*, *Elattoma fraxini* (referred to as *Elattoma* sp. by Moser et al. 2005), nr. *Eueremaes* sp., *Proctolaelaps eccoptogasteris*, *Proctolaelaps scolyti*, *Pseudotarsonemoides eccoptogasteri*, *Pymotes scolyti*, *Tarsonemus crassus*, and *Trichouropoda bipilis*. Here, we extend the knowledge on phoretic mites of *Scolytus* spp. on elm in central Europe to *S. scolytus*. The main aim of this study was to record and quantify conidia and ascospores of *O. novo-ulmi* on the various mite species phoretic on the three elm bark beetles *S. multistriatus*, *S. pygmaeus*, and *S. scolytus*.

## Materials and methods

### Collection of *Scolytus* elm bark beetles

On May 17, 2002, five stem sections, each about 120 cm long and 20 cm in diameter, were cut from a European field

elm (*Ulmus minor*) tree, near Güssing (16°20'17"E, 47°04'27", 230 m asl), Burgenland, Austria (Moser et al. 2005). This tree had been dying due to DED and was subsequently attacked by two smaller elm bark beetles, *S. multistriatus* and *S. pygmaeus*. On May 14, 2004, pieces of bark and sapwood containing elm bark beetle breeding galleries were collected from an elm (*Ulmus* sp.) tree, infested by the larger *S. scolytus* near Břeclav (16°53'52"E, 48°44'45"N, 160 m asl) in the Czech Republic close to the border of Austria. The stem sections and bark pieces were placed in laboratory rearing cages at 20±1°C. Offspring beetles of *S. multistriatus* and *S. pygmaeus* emerged from 28 May to 30 June 2002 (Moser et al. 2005), and those of *S. scolytus* until the end of May 2004. The beetles were collected periodically and placed in vials containing 70% ethanol until further use.

#### Processing of beetles and mounting of mites

Individual beetles including mites still attached to their bodies and any mites in the ethanol sediments were transferred to lactophenol. This was done to clear the body contents of the mites in preparation for mounting them on slides. The mites were then sorted and mounted individually on slides using Berlese's medium (Krantz 1978). Mites still attached to the beetles were counted separately from those that fell off the beetles into the lactophenol and those that became separated from the beetles in the alcohol (Table 1). Mite specimens from *S. multistriatus* and *S. pygmaeus* collected in 2002 had earlier been cleared and mounted (Moser et al. 2005). Voucher specimens (slides) of all mite species detected in this study and that by Moser et al. (2005) are maintained in the collection of JC Moser (Southern Research Station, USDA Forest Service) and in those of mite taxonomists (see Moser et al. 2005).

For each species of phoretic mite from *S. multistriatus*, *S. pygmaeus*, and *S. scolytus* all individuals or a sub-sample of mounted specimens (those of *Proctolaelaps scolyti* from *S. scolytus*) were examined for the presence of fungal spores, especially conidia and ascospores of *O. novo-ulmi* (Upadhyay 1981; Brasier 1991; Table 2). On each of the examined mites, spores were tallied by number and type

(conidia or ascospores). In addition, records were made whether spores were present on the body surface and/or in the gut of the mites.

#### Survival of *O. novo-ulmi* conidia inside *Proctolaelaps scolyti*

To test the viability of ingested *O. novo-ulmi* conidia inside guts, single female *Pr. scolyti* were transferred to cultures of *O. novo-ulmi* on malt extract agar (MEA, 2% malt extract, 1.6% agar) in 9-cm plastic petri dishes. The mites fed on the fungal cultures and propagated quickly on them. After about 3 weeks, individual mites were removed from the cultures and surface-sterilized for 10 min (experiment 1) or 15 min (experiments 2 and 3) in a 1% sodium hypochlorite solution, in order to kill any spores on the surfaces of their bodies. The mites were then quickly killed in carbon tetrachloride, rinsed in sterile water, and placed on MEA containing 0.01% cycloheximide and 0.1% of the antibiotic streptomycin sulfate, a medium selective for *Ophiostoma* spp. (Harrington 1981). Within a 3-week period the isolation plates were inspected at irregular intervals for the growth of microorganisms (Table 3).

## Results

A total of 27 individuals of *S. scolytus*, consisting of 16 (59%) male and 11 (41%) female beetles were collected and examined for phoretic mites (Table 1). At least one mite individual was carried by 56.3% of the male and 72.7% of the female insects. These values considerably underestimated the presence of mites on individual *S. scolytus* beetles because large numbers of mite specimens became detached from the beetles and were thus present in the alcohol (70.6% out of the 850 recorded mites) or lactophenol sediments (18.6%; Table 1). Thus, only 10.8% of the mites were still present on the bodies of *S. scolytus* beetles. The four mite species *E. fraxini*, *Pleuronectocelaeno austriaca*, *Pr. scolyti*, and *T. crassus* were detected (Table 1). Of the 850 mite individuals recorded, 81.7% belonged to *Pr. scolyti* and another 18%

**Table 1** Number of mites phoretic on 16 male and 11 female *S. scolytus*

Mite species	Phoretic stage	Number of mite individuals			
		On beetles	Lactophenol sediments	Alcohol sediments	Totals
<i>E. fraxini</i>	Female	0	0	1	1
<i>Pleuronectocelaeno austriaca</i>	Female	0	0	1	1
<i>Proctolaelaps scolyti</i>	Female	82	137	476	695
<i>T. crassus</i>	Female	10	21	122	153
Total		92	158	600	850

**Table 2** Conidia, ascospores, and/or hyphae of *Ophiostoma novo-ulmi* on phoretic mites of *S. multistriatus*, *S. pygmaeus*, and *S. scolytus*

<i>Scolytus</i> species/mite species	Number of phoretic mites examined	Number (%) of examined mite individuals with spores or hyphae of <i>O. novo-ulmi</i>				
		Conidia on body surface	Ascospores on body surface	Hyphae on body <sup>b</sup>	Spores in gut	Total mites with spores <sup>c</sup>
<i>S. multistriatus</i> (n=144 <sup>d</sup> )						
<i>Chelacheles michalskii</i>	31	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>E. fraxini</i> <sup>e</sup>	4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
nr. <i>Eueremaes</i> sp.	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>Proctolaelaps eccoptogasteri</i>	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>Proctolaelaps scolyti</i>	4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>Pseudotarsonemoides eccoptogasteri</i>	94	2 (2.1)	2 (2.1)	0 (0.0)	0 (0.0)	4 (4.3)
<i>Pyemotes scolyti</i>	1430	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>T. crassus</i>	22	6 (27.3)	3 (13.6)	2 (9.1)	0 (0.0)	9 (40.9)
<i>Trichouropoda bipilis</i>	93	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Totals for <i>S. multistriatus</i>	1,680	8 (0.5)	5 (0.3)	2 (0.1)	0 (0.0)	13 (0.8)
<i>S. pygmaeus</i> (n=178 <sup>f</sup> )						
<i>Chelacheles michalskii</i>	10	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>E. fraxini</i> <sup>g</sup>	27	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
nr. <i>Eueremaes</i> sp.	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>Proctolaelaps eccoptogasteri</i>	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>Proctolaelaps scolyti</i>	8	1 (12.5)	1 (12.5)	0 (0.0)	0 (0.0)	2 (25.0)
<i>Pseudotarsonemoides eccoptogasteri</i>	422	0 (0.0)	1 (0.2)	0 (0.0)	0 (0.0)	1 (0.2)
<i>Pyemotes scolyti</i>	1702	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>T. crassus</i>	24	4 (16.7)	12 (50.0)	3 (12.5)	0 (0.0)	15 (62.5)
<i>Trichouropoda bipilis</i>	47	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Totals for <i>S. pygmaeus</i>	2,242	5 (0.2)	14 (0.6)	3 (0.1)	0 (0.0)	18 (0.8)
<i>S. scolytus</i> (n=27 <sup>h</sup> )						
<i>E. fraxini</i>	1	1 (100)	1 (100)	0 (0.0)	0 (0.0)	1 (100)
<i>Pleuronectocelaeno austriaca</i>	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>Proctolaelaps scolyti</i>	136 <sup>i</sup>	104 (76.5)	36 (26.5)	0 (0.0)	62 (45.6)	104 (76.5)
<i>T. crassus</i>	153	41 (16.8)	38 (27.9)	0 (0.0)	0 (0.0)	68 (44.4)
Totals for <i>S. scolytus</i>	291 <sup>j</sup>	146 (50.2)	75 (25.8)	0 (0.0)	62 (21.3)	173 (59.5)

<sup>a</sup> Percentages refer to the number of mite individuals carrying spores in relation to the number of mites examined, separate for each mite species phoretic on each of the three *Scolytus* species

<sup>b</sup> Hyphae occurring in the sporothecae of *T. crassus* could not unambiguously assigned to *O. novo-ulmi*, but as *O. novo-ulmi* spores were also present, the hyphae were suspected to belong also to this DED fungus

<sup>c</sup> Totals may not agree because conidia, ascospores, and mycelia may occur on the same individual and mites may contain spores both on their body surface and in the gut

<sup>d</sup> 56 male and 88 female *S. multistriatus* were examined, but note footnote e for *E. fraxini* (see Moser et al. 2005)

<sup>e</sup> Totals from 59 beetles sampled (see Moser et al. 2005)

<sup>f</sup> 67 male and 111 female *S. multistriatus* were examined, but note footnote g for *E. fraxini* (see Moser et al. 2005)

<sup>g</sup> Totals from 52 beetles sampled (see Moser et al. 2005)

<sup>h</sup> 16 male and 11 female *S. scolytus* were examined (see Table 1)

<sup>i</sup> 136 out of 695 (19.6%) recorded individuals of *P. scolyti* from *S. scolytus* (see Table 1) were examined for fungal spores

<sup>j</sup> 291 out of 850 (34.2%) recorded mite individuals from *S. scolytus* (see Table 1) were examined for fungal spores

to *T. crassus*, whereas *E. fraxini* and *P. austriaca* were each represented by only one individual (Table 1).

Conidia and ascospores of *O. novo-ulmi* and spores of other unidentified fungi were found on four of the ten mite

species phoretic on *S. multistriatus*, *S. pygmaeus*, and *S. scolytus* (Fig. 1). These mites included *E. fraxini* on *S. scolytus*, *Pseudotarsonemoides eccoptogasteri* on *S. multistriatus* and *S. pygmaeus*, *Pr. scolyti* (Fig. 1a–c)

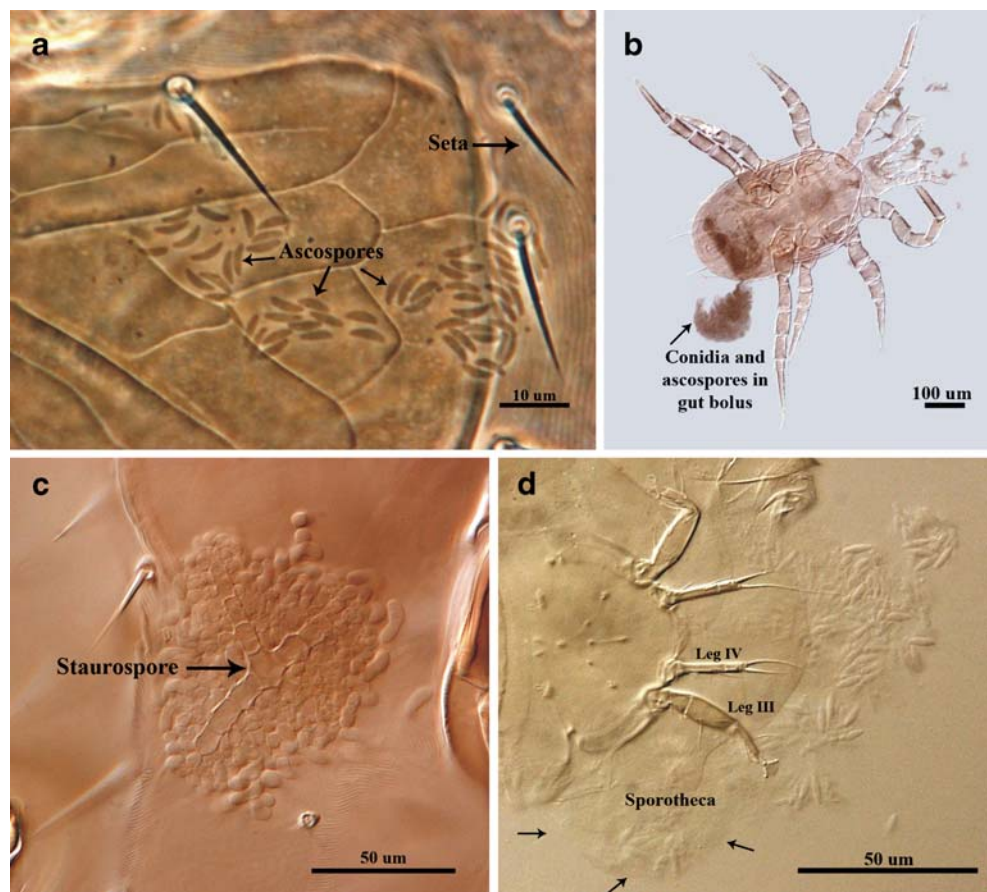
**Table 3** Percentage of successful isolations of bacteria and *O. novo-ulmi* from surface-sterilized females of *Proctolaelaps scolyti* that were reared on cultures of *O. novo-ulmi* for about 3 weeks

Growth of microorganisms	Experiment 1 (n=125)	Experiment 2 (n=30)	Experiment 3 (n=30)	All three experiments (n=185)
Bacteria only	24.0	20.0	6.7	20.5
Bacteria and <i>O. novo-ulmi</i>	39.2	26.7	13.3	33.0
<i>O. novo-ulmi</i> only	5.6	26.7	3.3	7.0
No growth of microorganisms	31.2	36.7	76.7	39.5

n Number of mites examined

on *S. pygmaeus* and *S. scolytus*, and *T. crassus* (Fig. 1d) on all three *Scolytus* species (Table 2). Thus, two out of the nine mite species phoretic on *S. multistriatus*, three out of the nine species on *S. pygmaeus*, and three out of the four species on *S. scolytus* carried spores of *O. novo-ulmi*. Considering all mite specimens investigated, the percentage of individuals with *O. novo-ulmi* spores was much higher for the mites phoretic on *S. scolytus* (59.5%) than for those phoretic on *S. multistriatus* and *S. pygmaeus* (0.8% each, Table 2).

**Fig. 1** Fungal spores on mites phoretic on *Scolytus* elm bark beetles: **a** Ascospores of *O. novo-ulmi* on opisthosomal surface of a *Proctolaelaps scolyti* female (slide no. 46396), **b** Hundreds of conidia and ascospores of *O. novo-ulmi* flowing from the squashed bolus of a *Proctolaelaps scolyti* female (slide no. 46095), **c** Conidia of *O. novo-ulmi* and a staurospore conidium in the gut of a *Proctolaelaps scolyti* individual (slide no. 46099), **d** Ascospores of *O. novo-ulmi* flowing out from a squashed sporotheca (marked with arrows) of a *T. crassus* female (slide no. 44426)



*Proctolaelaps scolyti* females, the phoretic stage of this species, are large mites with dorsal shields of specimens measuring approximately  $400 \times 320 \mu\text{m}$ . Only 12 *Pr. scolyti* females were phoretic on the 322 small-sized *S. multistriatus* and *S. pygmaeus* (Moser et al. 2005, Table 2), whereas 695 specimens of this species were recorded on only 27 of the larger-sized *S. scolytus* (Table 1). Thus, when considering also those individuals that were found in the alcohol and lactophenol sediments, each *S. scolytus* beetle would on average have carried almost 26 *Pr. scolyti*.

Only two (16.7%) of the 12 *Pr. scolyti* females phoretic on *S. multistriatus* and *S. pygmaeus* carried spores of *O. novo-ulmi* on their bodies (Table 2). These two mites contained only three ascospores and one conidium, respectively. Of the 695 *Pr. scolyti* from *S. scolytus*, a sample of 136 individuals was examined for the occurrence of fungal spores. Of these, 104 (76.5%) carried *O. novo-ulmi* conidia, and 36 out of those 104 mites with conidia additionally had *O. novo-ulmi* ascospores attached to their bodies (Table 2). Of the 104 mites with conidia present, nine had one to nine conidia, 56 had ten to 29 conidia, and 39 had 30 to 100+ conidia on their body surfaces. Of the 36 *Pr. scolyti* specimens with ascospores, 35 had ten or fewer ascospores, and only one carried more than ten ascospores on the surface of its opisthosoma (Fig. 1a). Of the 136 *Pr. scolyti*

examined, 62 (45.6%) were estimated to contain each 1,000 or more conidia and ascospores in their guts (Fig. 1b–c). A few *Pr. scolyti* also contained one or more staurospore conidia (Alexopoulos et al. 1996) in their digestive systems (Fig. 1c).

*T. crassus* females, which are phoretic on *Scolytus* spp., are small mites, the length and width of specimens averaging approximately 160×100 µm. Only 46 individuals of this species were phoretic on the 322 adult *S. multistriatus* and *S. pygmaeus* (Moser et al. 2005, Table 2), whereas 153 specimens occurred on the 27 adults of *S. scolytus* (Tables 1 and 2). As certain other species of *Tarsonemus*, *T. crassus* carried most spores within its paired sporothecae, two pocket-like structures under tergite C (Magowski and Moser 2003; Fig. 1d), inside which fungal spores are placed, preserved, and transported (Moser 1985). While ascospores, conidia, and likely, also mycelia were present in sporothecae of *T. crassus* individuals phoretic on *S. multistriatus* and *S. pygmaeus*, only conidia and ascospores were present on those *T. crassus* taken from *S. scolytus* (Table 2). Of the 46 females of *T. crassus* phoretic on *S. multistriatus* and *S. pygmaeus*, 24 (52.2%) carried spores of *O. novo-ulmi*; 15 specimens carried ascospores and ten had conidia on their bodies (Table 2). Most of the *T. crassus* specimens with spores carried more than 50 *O. novo-ulmi* propagules. Of the 153 *T. crassus* females phoretic on *S. scolytus*, 68 (44.4%) contained conidia and/or ascospores of *O. novo-ulmi* (Table 2). Unlike *T. crassus* individuals phoretic on the smaller-sized *S. multistriatus* and *S. pygmaeus*, virtually all specimens of this mite species on *S. scolytus* carried less than five spores and none carried numbers greater than 20.

The length and width of the dorsal shields of *Ps. eccoptogastri* females averaged approximately 200×100 µm. This mite species occurred in large numbers on *S. multistriatus* and *S. pygmaeus*, but it was totally absent from *S. scolytus*. Of the 516 specimens of *Ps. eccoptogastri* phoretic on *S. multistriatus* and *S. pygmaeus* only five (1%) carried ascospores or conidia of *O. novo-ulmi* (Table 2). Up to 100 *O. novo-ulmi* ascospores were seen on specimens of *Ps. eccoptogastri*, but the spores tended to float from the mite bodies making accurate counts impossible.

*E. fraxini* was the smallest of the four mite species carrying spores of *O. novo-ulmi*. The length and width of the dorsal shields of females of this species averaged approximately 122×80 µm. No *O. novo-ulmi* spores were seen on the 31 *E. fraxini* taken from *S. multistriatus* and *S. pygmaeus*, but the single female of *E. fraxini* from *S. scolytus* harbored about ten each of *O. novo-ulmi* conidia and ascospores (Table 2).

Bacteria and/or *O. novo-ulmi* were isolated from a portion of surface-sterilized *Pr. scolyti* females that had

been reared for about 3 weeks on cultures of *O. novo-ulmi* (Table 3). When combining the results of the three experiments, the DED fungus was isolated from 40% of the *Pr. scolyti* individuals, bacteria were isolated from 53.5% of the specimens, and 39.5% of the mites did not yield growth of any microorganisms (Table 3). The isolations indicated that at least a portion of conidia of *O. novo-ulmi* ingested by *Pr. scolyti* can remain viable.

## Discussion

In this study and our previous one (Moser et al. 2005), a total of ten species of mites were recorded as phoretic associates of the three elm bark beetles *S. multistriatus*, *S. pygmaeus*, and *S. scolytus* in central Europe (Tables 1 and 2). Nine of these mite taxa had already been reported previously from *S. multistriatus* and *S. pygmaeus* (Moser et al. 2005). Here, we collected a tenth phoretic species, *P. austriaca*, represented by a single specimen on *S. scolytus* (Table 1). *S. multistriatus* and *S. pygmaeus* were associated with the same nine phoretic mite species (Moser et al. 2005, Table 2). Likewise, the relative abundance of mites was very similar for both of these elm scolytines, with *Py. scolyti* occurring most frequently and *Ps. eccoptogasteri* as well as *T. bipilis* being relatively common (Moser et al. 2005; Table 2). In contrast, only four mite species were found phoretic on *S. scolytus* in the present study (Table 1). Of these, *Pr. scolyti* was the dominant species and *T. crassus* occurred in relatively large numbers (Table 2). These two mites occurred only rarely on the two smaller *Scolytus* species (Moser et al. 2005; Table 2). The two other phoretic mites of *S. scolytus*, *E. fraxini*, and *P. austriaca* were extremely rare as they were each represented by one individual only (Table 1). While *E. fraxini* was previously recorded as phoretic on *S. multistriatus* and *S. pygmaeus* (Moser et al. 2005; Table 2), *P. austriaca* is a newly reported mite associate of *S. scolytus*.

Four of the ten mite species phoretic on *Scolytus* spp. were found to carry spores of the DED fungus *O. novo-ulmi*. Of these, only 3.1% of the individuals of the generally rare *E. fraxini* and 1% of the specimens of the more common *Ps. eccoptogasteri* had *O. novo-ulmi* spores on their body surfaces (Table 2). In contrast, large portions of the individuals of *Pr. scolyti* (71.6%) and *T. crassus* (46.2%) carried conidia and/or ascospores of *O. novo-ulmi* (Table 2), often in high numbers.

Females of *E. fraxini*, *P. eccoptogasteri*, and *T. crassus* have movable cheliceral digits modified for piercing, permitting only ingestion of liquids (Lindquist 1969). Thus, these mites can carry fungal spores only on their body surfaces and, in the case of *T. crassus* individuals, in their sporothecae. Sporothecae of *T. crassus* contained conidia,

ascospores, and likely also hyphae of *O. novo-ulmi* (Table 2), in contrast to *Tarsonemus krantzi*, which only carries ascospores, but not conidia or hyphae of *Ophiostoma minus* and *Ceratocystiopsis ranaculosus* in its sporothecae (Klepzig et al. 2001). The mouthparts of the much larger *Pr. scolyti* females include a multidenticulate fixed digit and a tridentate movable digit (Evans 1958), enabling this mite to ingest large numbers of entire fungal spores. As at least a portion of *O. novo-ulmi* conidia ingested by *Pr. scolyti* can remain viable (Table 3), this mite transports the DED fungus both externally and in its digestive system. Actually, the numbers of ascospores and conidia seen in guts were far greater than those adhering on the bodies of *Pr. scolyti*, which suggests that, for this mite species, internal transport of fungal spores may be more important than adherence of spores to the body surface.

The feeding habits of phoretic mites of bark beetles are diverse but often poorly known, and this is also the case for the species associated with *Scolytus* spp. on elm (Moser et al. 2005). Our observations of *O. novo-ulmi* spores on the body surface and in the gut of *Pr. scolyti* indicate that this mite species is a fungivore. *T. crassus* most likely also feeds on *O. novo-ulmi*, which would agree well with the biology of other related *Tarsonemus* species phoretic on bark beetles (Magowski and Moser 2003). In laboratory experiments *T. fusari*, *T. ips*, and *T. krantzi* associated with the southern pine beetle, *Dendroctonus frontalis*, had positive growth rates when feeding on cultures of *O. minus* and *C. ranaculosus*, spores of which they transport in their sporothecae (Lombardero et al. 2000, 2003). As *T. crassus* transports only *O. novo-ulmi* spores in its sporothecae, this DED fungus may be of similar nutritional importance for this mite species. As a very low percentage of *Pseudotarsonemoides eccoptogasteri* and *E. fraxini* carried *O. novo-ulmi* spores, their dependence on fungi for nutrition is questionable.

The occurrence of conidia and ascospores of *O. novo-ulmi* on phoretic mites of *Scolytus* spp. suggests that these minute arthropods, particularly *Pr. scolyti* and *T. crassus*, may play a significant role in the processes involved in the transmission of DED. As other mites occurring in the galleries of elm bark beetles (Jacot 1934, 1936; Fransen 1939; Brasier 1978), *Pr. scolyti* and *T. crassus* likely spread the fungus within the gallery system and play a role in fertilization of *O. novo-ulmi* protoperithecia, thereby enhancing sexual reproduction. Likewise, these mites most probably spread different fungal genotypes, especially by dissemination of ascospores and may therefore be very important for intermixing of *O. novo-ulmi* genets in the breeding systems of *Scolytus* spp.

Besides their ecological role in the breeding galleries of elm bark beetles, *Pr. scolyti* and *T. crassus* may be directly involved in the transmission of *O. novo-ulmi*, during

maturation feeding of *Scolytus* beetles on twigs and in twig crotches of healthy elm trees. In the feeding grooves initiated by the insects, mite individuals could become detached from the beetles, deposit spores in the phloem, and on the xylem surface and thereby infect *Ulmus* trees with *O. novo-ulmi*. Beetles can remain for hours, days, and sometimes more than a week in a single feeding groove (Webber and Brasier 1984; Webber 2000, 2004), a time span giving multiple opportunities for mites to become temporarily or permanently separated from the beetles and come in contact with phloem and xylem tissues susceptible to fungal infection.

In contrast to *Pr. scolyti* and *T. crassus*, other mites occurring in elm bark beetle galleries (Jacot 1934, 1936; Fransen 1939; Brasier 1978; Doberski 1980) cannot transmit *O. novo-ulmi* spores to healthy elm trees, as they are not phoretic on *Scolytus* spp. For example, *Tyrophagus putrescentiae*, occurring in elm bark beetle galleries (Brasier 1978; Doberski 1980), is a ubiquitous acarid tramp species in laboratories throughout the world, a common pest of stored food, and once established, reproduces in large numbers and transmits fungal spores (Griffiths et al. 1959; Hughes 1961). But this mite is likely incapable of transmitting *O. novo-ulmi* from tree to tree due to the absence of an effective phoretic mechanism, such as the deutonymph phoretic stage of most other acarid mites (O'Connor 1982). On very rare occasions, it may have been transported under the elytra of the cerambycid *Neacanthocinus obsoletus* and the clerid *Thanasimus dubius* which are common on North American southern pine species (Moser and Roton 1971).

A number of studies have shown that a certain quantity of spores brought by a vectoring elm bark beetle to a feeding groove is required to lead to infection of an elm tree by *O. novo-ulmi*. In inoculation experiments in England, at least 500 to 1,000 conidia were necessary to incite infections on *Ulmus procera* (Webber 1987; Webber and Gibbs 1989; Sutherland and Brasier 1997), but lower and higher spore loads resulting in infections were reported in other experiments (Webber and Brasier 1994; Faccoli and Battisti 1997; Webber 2000, 2004). Our observations suggest that the phoretic mites *Pr. scolyti* and *T. crassus* may greatly increase the spore loads of individual beetles in feeding grooves on healthy elm trees which will enhance the chance for infection by *O. novo-ulmi* to occur.

Among the three *Scolytus* species examined for phoretic mites, *S. scolytus* is considered as the most important vector of *O. novo-ulmi*, being much more efficient than *S. multistriatus* and other, smaller *Scolytus* species (Webber and Brasier 1984; Webber and Gibbs 1989; Webber 1990, 2000). This is because a large portion of *S. scolytus* individuals carry spores of *O. novo-ulmi* and many of these are contaminated with a critical load of at least 500 to 1,000

spores to incite infections of elm trees (Webber and Brasier 1984; Webber and Gibbs 1989; Webber 1990, 2000). Intriguingly, *Pr. scolyti* and *T. crassus*, the only phoretic mites carrying spores of *O. novo-ulmi* consistently and often in high numbers (Table 2), were common mite associates of *S. scolytus*, while they rarely occurred on *S. multistriatus* and *S. pygmaeus* (Table 1). In addition, none of the individuals of *Pr. scolyti* from *S. multistriatus* and only a few from *S. pygmaeus* carried *O. novo-ulmi* spores (Table 2) and if spores were present, they occurred in very low numbers. In contrast, about the same proportion of *T. crassus* from *S. multistriatus* and *S. pygmaeus* (52.2%) as well as from *S. scolytus* (44.4%) had *O. novo-ulmi* spores on their bodies and those from the two smaller *Scolytus* species carried higher spore loads than those from *S. scolytus* (Table 2). The main difference between the three elm bark beetles was that *T. crassus* was much more abundant on *S. scolytus* than on *S. multistriatus* and *S. pygmaeus* (Table 2; Moser et al. 2005).

The high efficiency of *S. scolytus* in spreading DED may be partly because of its close association with *Pr. scolyti* and *T. crassus* and the considerable quantities of hyperphoretic spores of *O. novo-ulmi* they carry. Based on our data, each *S. scolytus* beetle would have carried on an average 25 *Pr. scolyti* and five *T. crassus* individuals. Assuming conservative and low average numbers of 20 *O. novo-ulmi* spores carried internally and/or externally by each *Pr. scolyti* individual and five spores per *T. crassus* specimen, the mites phoretic on just a single *S. scolytus* beetle would alone have carried 525 spores, a load that can be sufficient to incite *O. novo-ulmi* infections (Webber 1987; Webber and Gibbs 1989; Faccoli and Battisti 1997; Sutherland and Brasier 1997; Webber 2004).

Our findings raise questions regarding the symbiotic interactions between *Scolytus* beetles, phoretic mites, and *O. novo-ulmi* during the phase when the insects breed in the phloem of elm trees. There is evidence that *O. novo-ulmi* is detrimental to larval development, if the fungus and *Scolytus* larvae come in close physical contact with each other (Webber and Gibbs 1989). In contrast, *O. novo-ulmi* may be essential for the nutrition of both *Pr. scolyti* and *T. crassus*, and it may thus be beneficial for these mites to transmit the fungus. This situation probably resembles the complex relationships between the southern pine beetle, *D. frontalis*, the phoretic mite *T. krantzi*, and the blue-stain fungus *O. minus*, where the fungus is a mutualist of the mite, but an antagonist of the insect (Barras 1970; Bridges and Moser 1986; Klepzig et al. 2001; Lombardero et al. 2000; Hofstetter et al. 2006).

While the role of mites in DED has been examined and discussed in previous investigations (Jacot 1934, 1936; Fransen 1939; Brasier 1978; Webber and Brasier 1984), this study is the first one to consider the importance of

mites phoretic on *Scolytus* elm bark beetles for pathogen dispersal. By contributing to the transmission of *O. novo-ulmi* to elm trees, *Pr. scolyti* and *T. crassus* may be important biotic factors for the epidemiology of this destructive vascular wilt disease.

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