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Observations on the relative effectiveness of *Scolytus multistriatus* (MARSHAM) and *Scolytus pygmaeus* (FABRICIUS) (Coleoptera: Scolytidae) as vectors of the Dutch elm disease

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Some aspects of the relative effectiveness of *Scolytus multistriatus* and *S. pygmaeus* as vectors of the Dutch elm disease were studied in north-eastern Italy. About 58% of beetles collected at emergence were contaminated with the pathogen *Ophiostoma ulmi,* with no interspecific difference being observed. Under controlled conditions (beetles saturated with spores, high R.H.), successful transmission of the pathogen during maturation feeding of beetles on elm saplings was achieved in 11% of the cases. Again, no significant interaction of the species was observed, although sample size was low. These data suggest that the specific size of beetles and concomitant subordination to specific breeding habitats is unimportant in affecting the proportion of beetles contaminated with the pathogen in humid conditions. With reference to the effective transmission of the disease in the field, the influence of these factors is less clear. If the trends reported above are verified and observed in natural conditions, it would be imperative to destroy also small elm branches during sanitation felling programes, at least when these are performed in humid areas.

Keywords: Bark beetles, *Scolytus* spp., Dutch Elm Disease

INTRODUCTION

LEACH (1940) suggested that insects may be considered as vectors of plant diseases if (a) they are regularly associated with diseased hosts; (b) they visit healthy hosts under conditions suitable for infection; (c) they carry viable inoculum of the pathogen in the field; and (d) they can transmit successfully the pathogen to the host under laboratory conditions. With reference to the transmission and spread of the pathogen *Ophiostoma ulmi* (SCHWARZ) NANF. [= *Ceratocystis ulmi* (BUISM.) MOR.] on *Ulmus* spp. (the "Dutch elm disease", DED) in Europe, most, but not all, of these conditions have been demonstrated for several scolytid species belonging to the genera *Scolytus* GEOFFROY and *Pteleobius* BEDEL (e.g., PFEFFER, 1979; LANIER & PEACOCK, 1981; SENGONÇA & LEISSE, 1984; WEBBER & GIBBS, 1989). Usually, the pathogen is transmitted to healthy trees during twig crotch-feeding, which may aid in sexual maturation (e.g., WEBBER & BRASIER, 1984). *Scolytus multistriatus* (MARSHAM) and *Scolytus pygmaeus* (FABRICIUS) are among these harmful species which perform maturation feeding.

However, the relative effectiveness of different scolytid species as vectors of DED is known poorly. It is not certain whether each species is equally important in

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transmitting and spreading the pathogen or if some are more harmful than others. If the former is correct, then the entire scolytid community associated with *Ulmus* spp. may represent a synergic agent which, in turn, may complicate any attempt to control the disease, as suggested by ZANTA & BATTISTI (1990). If the latter is correct, applied research should concentrate on the more harmful species. The effectiveness of the scolytid vector is likely to depend on several factors such as: (a) species abundance, voltinism and the number of maternal galleries bored by each female (OWEN & LOWNSBERY, 1989); (b) the proportion of beetles carrying viable spores of the fungi (WEBBER, 1990; WEBBER & BRASIER, 1984); (c) the spore load (i.e., the number of spores) carried by individual beetles (WEBBER, 1990; WEBBER & BRASIER, 1984); (d) beetle flight-occurrence and activity, which may affect the viability of spores; (e) beetle flight range and speed, which may influence the spread of the pathogen; (f) the proportion of beetles involved in maturation feeding (WEBBER & BRASIER, 1984); (g) the effective transmission rate of DED by beetles during maturation feeding (WEBBER & BRASIER, 1984).

Two simple experiments were performed in order to compare some aspects of the relative effectiveness as vectors of DED of the two most common scolytid species in north-eastern Italy, *S. multistriatus* and *S. pygmaeus* (ZANTA & BATTISTI, 1990). We investigated whether there were interspecific differences in the proportion of beetles contaminated with *Ophiostoma ulmi* at emergence and in the effective transmission of DED (or at least in its initiation) during maturation feeding by beetles.

MATERIAL AND METHODS

Elm logs infested with scolytids were collected from several sites in Veneto, Italy, during April-May 1991 (Tab.1). They were cut from elm trees (*Ulmus minor* Miller) which exhibited obvious symptoms of DED (such as dark streaks or spots in the xylem of the upper branches, presence of "shepherd's crooks"). Logs and branches were cut into small pieces (about 30 cm long; diameter ranging from 3 to 30 cm) and stored vertically in black nylon bags, at room temperature. Newly emerging beetles were collected daily from clear plastic vials placed at the top of the bags and were stored in sterilised glass vials. After identification, beetles were killed by crushing the head with sterilised forceps and plated into Petri dishes containing a selective medium for *Ophiostoma ulmi*. This consisted of carrot agar (500 g boiled carrots and 20 g agar x l^{-1}) amended with cycloheximide (1 g x l^{-1}) and streptomycin sulphate (300 mg x l^{-1}) (BRASIER, 1981; MITTEMPERGHER, 1981). In these media, *O. ulmi* grew well, contrary to most other fungi and bacteria. Our experimental conditions ensured that most of the beetles were plated within 24 hours after

Tab. 1. Origin of logs and scolytid beetles used in the first experiment.

Site	Location (1)	Site aspect	No. trees	No. logs	No. beetles
Favaro Veneto (VE)	33TTL 883/452	hedgerow	1	3	7
Lison (VE), Bosco del Merlo	33TUL 243/685	wood	3	51	144
Cà Noghera (VE)	33TTL 948/455	wood	2	8	8
Muzzana del Turgnano (UD), Bosco Bando	33TUL 561/740	forest	2	6	12
Muzzana del Turgnano (UD), Bosco Baredi	33TUL 538/727	forest	1	1	1

(1) UTM coordinates

their apparent emergence into plastic vials. The media were incubated in the dark, at about 25°C and inspected after two weeks. As far as possible the colonies were identified as belonging to: (a) *O. ulmi*; (b) unidentified fungi; and (c) unidentified bacteria. In dubious cases, isolates suspected to be *O. ulmi* were sub-cultured. At the end of the experiment, the bark of logs from which beetles had been collected was stripped off, checked visually for the presence of *O. ulmi* and fragments of wood and bark were plated into the selective media, in order to check that all logs were infected with the pathogen.

Fifty healthy elm saplings (*U. minor* X *U. pumila* LINNAEUS; 1.5 - 3 m in height) were planted and grown in March-July 1991 within the campus of the Facoltà di Agraria, Padova. High numbers of synnemata of the isolate H351 of *O. ulmi* were obtained by rearing the fungi within an elm agar medium (15 g agar, 500 ml distilled water and 50 g fresh elm saw; media incubated in dark at 25°C; L. MITTEMPERGHER, pers. comm.). Preliminary observations showed that after allowing beetles to crawl for 90 minutes around Petri dishes coated with elm agar and plating them into the selective medium, the proportion of beetles from which *O. ulmi* could be reared, and therefore the proportion of beetles carrying viable spores/hyphae of this species, was 100% (n = 22). Scolytids emerging from the logs of the first experiment were allowed to crawl for 90 minutes over the elm agar medium. Then, they were identified and caged individually into plastic vials affixed using Parafilm to potential sites of maturation feeding on elm saplings, with several vials attached to some saplings. After 3 days, vials and beetles were removed and any feeding groove recorded. A month later, fragments of woody tissues were excised, with a sterile scalpel, from two locations for each sample (3 cm and 6 cm below the feeding groove), transferred into selective media and incubated. Two weeks later, agar plates were inspected for colonies of *O. ulmi* and the proportion of infected locations was determined for the two scolytid species. Results were analysed by table contigency analysis, using χ^2-tests and, when frequencies were low, G-tests (ZAR, 1984).

RESULTS

The proportion of *S. multistriatus* and *S. pygmaeus* from which *O. ulmi* could be reared at emergence amounted to 58% (Tab.2). We also isolated *O. ulmi* from *Scolytus sulcifrons* REY and *Pteleobius vittatus* (FABRICIUS), but the small number of beetles sampled and tested precluded any statistical analysis. The proportion of beetles contaminated with the fungus was identical in *S. multistriatus* and *S. pygmaeus* and there was no significant influence of beetle species upon the isolation of the pathogen (table contigency analysis, $\chi^2 = 0.06$, $p = 0.82$). Similarly, the proportion of beetles contaminated with other fungi was identical in both species (Tab. 2) and not influenced by beetle species ($\chi^2 = 0.02$, $p = 0.89$). Most of these fungi appeared to belong to the genus *Penicillium* (R. CAUSIN, pers. comm.). Furthermore,

Tab. 2. Numbers and percentages of scolytids contaminated with *O. ulmi*, with other fungi and with heavy loads of bacteria (categories not mutually exclusive).

Species	*O. ulmi*			Other fungi			Bacteria		
	+	-	%	+	-	%	+	-	%
S. multistriatus	72	53	57.6	81	44	64.8	32	93	25.6
S. pygmaeus	28	19	59.6	31	16	66.0	10	37	21.3

on specimens contaminated with fungi, there was no influence of beetle species on the type of fungus carried (e.g., *Ophiostoma* or others) ($\chi^2 = 0.03$, $p = 0.96$). 32.0% of *S. multistriatus* specimens were infected with both types of fungi, whereas this proportion amounted to 29.8% of *S. pygmaeus* specimens. Again, there was no influence of beetle species on these results ($\chi^2 = 0.27$, $p = 0.61$). The proportion of scolytids infested heavily with bacteria was similar and not influenced by beetle species ($\chi^2 = 0.35$, $p = 0.56$; Tab.2).

Stripping bark from elm logs and plating wood fragments from the logs indicated that most were infected with the pathogen, which was recovered from 61 out of 69 logs. Of the 103 beetles found contaminated, 91 emerged from infected logs and 12 from those, from which, presumably, the pathogen was not recovered. A single uncontaminated beetle emerged from an apparently uninfected log and 68 uncontaminated beetles emerged from infested logs. Since our samples were largely dominated by scolytids obtained from one location (Tab. 1), we checked that these samples were not significantly different from others (comparison between Bosco del Merlo and other locations for the presence or absence of *O. ulmi* on all beetles: $\chi^2 = 0.10$, $p = 0.75$). When we considered logs from which at least 5 beetles were collected and plated, there was no influence of individual logs on the contamination of beetles by *O. ulmi* (7 logs; G-test 7 x 2, $G = 5.24$, $p = 0.51$). This trend persisted when we performed the analysis for both scolytid species and for *S. pygmaeus* alone with the following 3 classes of mean log diameter: ≤ 5 cm, 5 - 15 cm, ≥ 15 cm ($\chi^2 = 0.51$, $p = 0.77$ and $G = 3.25$, $p = 0.20$, respectively).

Tab. 3. Numbers and percentages of successful infestation of young elm saplings by scolytids saturated with *O. ulmi* spores.

Species	*3 cm below feeding groove*			6 cm below feeding groove		
	+	-	%	+	-	%
S. multistriatus	4	30	11.8	4	30	11.8
S. pygmaeus	2	20	9.1	2	20	9.1

The effective rate of transmission of the disease in our experimental conditions amounted to 10.7% (Tab.3). It was similar for both scolytid species and there was no significant interaction of the species (G-test = 0.10, $p = 0.75$). Results of isolation 6 cm below the feeding groove were identical to those 3 cm above. Most of *O. ulmi* isolates were found on different saplings.

DISCUSSION

Whilst few comparative data are available for *S. pygmaeus*, the proportion of *S. multistriatus* contaminated at emergence with *O. ulmi* (57.6%) was within the upper range of previously reported values: 5.7-7.7% (COLLINS, 1941); 3-20% (GIBBS & SMITH, 1978), 1.3-38% (RANKIN *et al.*, 1941), 76% (PARKER *et al.*, 1941). The presumably constant and high humidity under the nylon bags may have represented optimal conditions for the growth of *O. ulmi*. This may also be true for other fungi resistant to cycloheximide, such as *Penicillium* spp. (R. CAUSIN, pers. comm.). These are associated regularly with scolytids, particularly with ambrosia beetles (BEAVER,

1989) and recovered regularly from elm bark samples (WEBBER & HEDGER, 1986). Since *Penicillium* spp. are moderately to strongly antagonistic to *O. ulmi* and compete with it in vitro (WEBBER & HEDGER, 1986), our percentage recovery of the pathogen may be underestimated.

From the results of our first experiment, we conclude that there is no interspecific difference at emergence between *S. multistriatus* and *S. pygmaeus* with reference to the proportions of beetles contaminated with *O. ulmi*. Since these considerations also applied to the proportion of beetles contaminated with other fungi, with heavy loads of bacteria and with both *O. ulmi* and other fungi, any possible difference in the relative effectiveness as vector between the two scolytid species is unlikely to be due to a difference in the proportion of beetles contaminated with the pathogen at emergence.

These findings are in contrast with those of WEBBER (1990) who found consistent differences between the proportion of *S. multistriatus* and *S. kirschi* SKALITZKI (a smaller species than *S. multistriatus*, very similar in size and in breeding habits to *S. pygmaeus*; see ZANTA & BATTISTI, 1990) contaminated with *O. ulmi* at emergence. In two outbreak areas in Spain, she found that the pathogen could be isolated from 6/8%, 35/64% and 100/95% of *S. kirschi*, *S. multistriatus* and *S. scolytus* FABRICIUS, respectively. WEBBER (1990) suggested that beetles pupating in habitat less favourable for the sporulation of *O. ulmi*, such as the outer or thinner bark which is more likely to dessicate quickly, may be less consistently contaminated. Thus, the apparent difference in the proportion of beetles contaminated between *S. kirschi* and *S. pygmaeus* may give some support to the contention that spore load is not totally dependent on beetle size, but may also depend on other factors, such as beetle pupation-behaviour (WEBBER, 1990). Therefore, a study upon the comparative biology of *S. kirschi* and *S. pygmaeus* appears essential. Alternatively, the dissimilar degree of contamination reported for these two small species may be attributed to climatic differences, particularly in relative humidity and rainfall, between Spain and northern Italy. Changes in humidity may expose logs and branches of small diameter, which represent the breeding habitats of small *Scolytus* species (e.g., ZANTA & BATTISTI, 1990), to the effects of desiccation. In turn, this may affect more consistently the inoculum load of these species than those of the larger species of *Scolytus,* which breed in thicker bark. In addition, *S. kirschi* seems to be almost absent in the humid areas of northern Italy whereas it is very frequent in the dry regions of central and southern Italy (e.g., ZANTA & BATTISTI, 1990). Therefore, independently on their pupation behavior, the climatic conditions more favourable to the development of the fungus are found in the areas where *S. pygmaeus* prevails.

Despite using hybrids between *U. minor* and *U. pumila*, which are more resistant to DED than *U. minor* (L. MITTEMPERGHER, pers. comm.), the successful rate of infection was as high in our experimental conditions as in natural or sub-natural conditions with susceptible elm species (< 5%: WEBBER & BRASIER, 1984; 13%: PARKER *et al.*, 1941). Our conditions were probably nearly optimal for pathogen infection: beetles saturated with spores; high relative humidity inside plastic vials; reduced period of time between beetle emergence and maturation feeding; etc. The experiment proved that contact between beetle and elm xylem during a period of 72 h was sufficient for successful transmission of the disease. This event might even have been more frequent if beetles would have been left for longer in feeding grooves.

We failed to find interspecific differences in the transmission rate of DED by *S. multistriatus* and *S. pygmaeus*, but this may have been due to small sample size. Possible differences may result from different specific beetle size (since larger spe-

cies are more likely to carry more inoculum), different competition rates of micro-organisms in feeding grooves (WEBBER & BRASIER, 1984) and, possibly, the time and energy devoted to feeding activities, which may result in different exposure of xylem areas. However, our first experiment did not suggest that there are important qualitative differences in the load of micro-organisms between the two scolytid species.

Overall, our data do not suggest that important differences exist in the relative effectiveness of *S. multistriatus* and *S. pygmaeus* as vectors of DED, at least with reference to our experimental conditions and to the aspects of vector effectiveness investigated. Beetle size and the concomitant subordination to certain breeding habitats does not appear to affect the proportion of beetles contaminated with the pathogen, at least in conditions of optimal relative humidity. However, these factors may be important in natural conditions, particularly in the driest parts of Europe, and may affect the transmission rates of the disease by smaller *Scolytus* species. If not, then it is imperative to destroy habitats suitable for these species (small branches, logs of small diameter) during sanitation felling programmes, particularly when performed in humid regions. Furthermore, some species such as *S. pygmaeus* may require further study, since they may be responsible for the evolution or hybridisation of aggressive strains of *Ophiostoma,* through host-switching and their oligophagous habits (BRASIER, 1990). To summarise, future research upon vector effectiveness should intend to investigate whether it is possible to disregard smaller *Scolytus* species as effective vectors of DED.

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RÉSUMÉ

Certains aspects de l'efficacité relative de *Scolytus multistriatus* et *S. pygmaeus* comme vecteurs de la graphiose de l'orme ont été étudiés au nord-est de l'Italie. Environ 58% des scolytes observés à l'émergence étaient contaminés avec le pathogène *Ophiostoma ulmi,* sans aucune différence interspécifique décelable. Sous conditions controlées (scolytes saturés de spores, humidité relative optimale), la transmission effective du pathogène lors de l'alimentation de maturation des scolytes sur de jeunes ormes a été réalisée dans 11% des cas, sans aucune interaction significative des espèces en présence, bien que les analyses soient peu concluantes en raison du faible nombre d'échantillons. Ces résultats suggèrent que la taille spécifique des scolytes et leur subordination respective à certains habitats pour la reproduction ne représentent pas des facteurs suceptibles d'influencer la proportion des insectes contaminés avec le pathogène dans des conditions humides. L'influence de ces facteurs sur la transmission effective de la maladie sur le terrain est moins claire et si les tendances reportées ci-dessus sont vérifiées et observées en conditions naturelles, il est impératif de détruire également les branches d'ormes de petit diamètre durant les programmes d'assainissement par abattage entrepris dans les régions humides.

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