Experimental studies on factors influencing the transmission of Dutch elm disease

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Abstract

Although various species of insects and arthropods have been considered as potential vectors of Dutch elm disease, there is little doubt that the current pandemic of the disease has been driven by the activities of just a few, highly effective bark beetle vectors mainly within the genus *Scolytus*. The disease transmission process, however, is highly complex. The behaviour of each beetle species, and even the behaviour of individual beetles, influences the transmission of *Ophiostoma novo-ulmi* and *O. ulmi* at many points in the disease cycle. Beetle behaviour during breeding in the bark of diseased trees affects the quantity and quality of pathogen spores carried by newly emerged beetle vectors. The behaviour of beetles during dissemination and host seeking affects how successful the transfer of the pathogen from vector to feeding groove will be and, ultimately, the potential for colonisation of a tree's vascular system. The resistance mechanisms of elms themselves also play a part disease transmission including different resistance mechanisms operating in the feeding groove compared with the resistance mechanism in the vascular system. Experiments assessing the significance of these processes are presented in this paper.

Key words: Scolytus, feeding groove, spore inoculum.

Resumen

Estudios experimentales sobre los factores que influyen en la transmisión de la grafiosis del olmo holandés

Si bien han sido varias las especies de insectos y otros artrópodos que han sido consideradas como potenciales vectores de la grafiosis del olmo, hay pocas dudas de que la actual pandemia ha sido propiciada por la actividad de unas pocas especies de barrenillos muy efectivos, pertenecientes en su mayoría al género *Scolitus*. El proceso de transmisión de la enfermedad, sin embargo, es muy complejo. El comportamiento de cada especie de barrenillo, e incluso de cada insecto individual, influye en la transmisión de *Ophiostoma novo-ulmi* y *O. ulmi* en muchos momentos del ciclo de la enfermedad. El comportamiento del insecto durante su alimentación en la corteza de los olmos enfermos afecta a la cantidad y calidad de las esporas del patógeno transportadas por los insectos vectores recién emergidos. El comportamiento de los barrenillos durante la diseminación y la búsqueda de hospedante afecta al éxito de la transferencia del patógeno desde el vector hasta la mordedura de alimentación y, finalmente, al potencial de colonización del sistema vascular del árbol. Los mecanismos de resistencia de los olmos juegan también un papel en la transmisión de la enfermedad, incluidos los mecanismos que operan en la mordedura de alimentación y en el sistema vascular. En este artículo se presentan diversos ensayos realizados con el fin de evaluar la significación de estos procesos.

Palabras clave: Scolytus, mordedura de alimentación, inoculación de esporas.

Components of Dutch elm disease

Dutch elm disease (DED) is the complex blending of fungal pathogen and insect vector, acting on populations of elm species which range from the highly susceptible to completely resistant. We now know that the pathogen, once regarded as single species, consists of two ancient, clearly delineated but closely related sibling species – *Ophiostoma ulmi* and *O. novo-ulmi* (Brasier, 1991; Brasier, 2004 this volume). *O. ulmi* has been responsible for the epidemics of DED seen throughout Europe and North America in the first half of the 20th century, while *O. novo-ulmi* is the cause of the current pandemics of the disease seen throughout much of the Northern Hemisphere.

A wide range of beetles species, mainly in the genus *Scolytus*, have been identified as potential vectors of the disease because of the frequency with which they have been found associated with elms, and parti-

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Elm associated Beetle bettles (mm) Beetle		Geographical distribution	Typical breeding habitat
Scolytus scolytus	2-6	Most of Europe, south western Asia	Main trunk and larger branches
S. multistriatus	2-4	Southern Europe into Russia, introduced to USA, Canada, Australia and New Zealand	Smaller branches, trunk of sapling elms
S. pygmaeus	1.5-3	Central, southern, eastern Europe, Ukraine	Smaller branches
S. laevis	3-4.5	Northern Europe, Scandinavia, introduced to USA	Main trunk and larger branches
S. kirschi	1.5-3	Central/southern Europe, parts of Asia, North Africa	Smaller branches down to 1 cm diameter
S. mali	3-5 Central and eastern Europe, Scandinavia, introduced to eastern USA		Larger branches
S. orientalis	2-4	Central Europe, Crimea and central Asia	Smaller branches
S. ensifer	1.5-3.5	Central and southern Europe, Ukraine, Crimea	Branches
S. sulcifrons	2-5	Central and southern Europe	Main trunk and larger branches
S. zaitzevi	1.5-3	Crimea, Russia	Smaller branches
S. schevyrewi	3-3.8	Asia, China	Main trunk and larger branches
S. semonovi	1.6-2.6	Asia, China	Small branches and twigs
S. jacobsoni	3-3.5	Asia, China, Japan	Smaller branches
Pteleobius vittanus	1.8-2.5	Central, southern and eastern Europe	Larger branches
P. kraatzi	1.8-2.2	Central, southern and eastern Europe	Larger branches
S. kashmirensis*	2-4	Himalayas	Smaller branches
S. chelogastera*	2-4	Himalayas	Probably smaller branches
S. himalulmia*	2-4	Himalayas	Probably smaller branches
Hylurgopinus rufipes	2-3	3 North America Main trunk	

Table 1. Potential vectors of Dutch Elm Disease (adapted from Grüne, 1979; Maslov, 1970)

* Act as vectors of *Ophiostoma himul-ulmi*, another Dutch elm disease pathogen only present in the Himalayas (Brasier and Mehrotra, 1995).

cularly diseased elms (Table 1). However, there are still some areas such as China where several *Scolytus* species are associated with elms but where the disease is still absent (Brasier, 1990). This demonstrates that the link between the DED pathogens and the insect vectors is not an obligatory part of the vector's life cycle, but it can be a highly effective mutualistic relationship which has resulted in the destruction of many millions of elms over the last century. These dead and dying elms have provided an abundance of breeding material for the beetles and this, combined with changes in the global climate, are probably facilitating the spread of some vector species beyond their established range (Heal, 2003).

Early work on DED

Much of the seminal early work on DED was undertaken in The Netherlands in the 1920's and 1930's. It continues to underpin much of our knowledge of the disease today. The early focus was on *Scolytus scoly*- tus and S. multistriatus and as the disease cycle was studied, the way in which the behaviour of the beetle vectors could alter the likelihood of disease transmission became apparent. Marchal (1927) was the first to suggest that elm bark beetles transmitted DED, and Wollenweber and Stapp (1928) underlined this link when they isolated O. ulmi from scolytid beetle frass. Perhaps most significantly, Betram (1929) realised the activity of beetle maturation feeding in twig crotches led to the transmission of disease from diseased to healthy elms. However, it was the research carried out by J.J. Fransen in the 1930's for his doctoral thesis at the University in Wageningen that elucidated much of the disease cycle.

Fransen's work gave many remarkable insights into the behaviour of the European beetle vectors of DED (eg Fransen, 1931a,b; Fransen and Buisman, 1935; Fransen, 1939a). He showed that *S. scolytus* carried the elm pathogen *O. ulmi* not only externally on the exoskeleton but also internally in the gut. Beetles became contaminated with spores of the pathogen as they lay in pupal chambers prior to emergence, and the po-

sition of the pupal chamber in the elm bark could have marked influence on the amount of inoculum available to contaminate the insects. Fransen also investigated the process of disease transmission in feeding grooves produced during maturation feeding and found that beetles made different types of feeding grooves, which varied in their size and the amount of exposed xylem tissue. He was convinced that the structure of feeding grooves affected the likelihood of disease transmission during beetle maturation feeding. To study disease transmission he caged beetles caught in the open field on elm trees and observed disease symptoms develop in these previously healthy trees. He also artificially contaminated some beetles with spores of O. ulmi and caged these on elms as well. The results of the experiments showed that the beetles transmitted DED to previously healthy trees during maturation feeding, but the beetles with artificially augmented pathogen loads were much more likely to transmit the disease - an early indication that the amount of inoculum carried by beetles can be critical to the process of infection (Table 2).

Despite the relevance of Fransen's research to our current understanding of DED, much of his work was overlooked during the early stages of the new pandemics now known to be caused by *O. nov-ulmi*. As researchers sought to understand the changes that had occurred in the pathogen and grappled with the new and even more destructive disease, the importance of the beetle vectors and how their behaviour could alter the disease cycle seemed less important. However, there are many points in the disease cycle when the scolytid beetle vectors can interact with the pathogen, often in very subtle ways. These can have a very significant impact on the process of disease transmission.

Influences on disease transmission

One of the most important factors in the success of disease transmission is the number of beetles which

carry spores of the elm disease pathogen following their emergence from brood trees. Research undertaken in several countries (including during the first disease epidemics), has shown that sometimes only a small proportion of beetles carry the pathogen when they emerge from their breeding galleries in elm bark (Fransen, 1939a; Parker, 1939; Parker *et al.*, 1947; Maslov, 1970; Webber and Brasier, 1984; Webber, 1990; Faccoli and Battisti, 1997; Pajares *et al.*, 2003). This is most noticeable with the smaller species of vector beetles such as *S. multistriatus*, *S. pygmaeus* and *S. kirschi* (Table 1). Climatic variables and beetle behaviour during development from larva to adult are also likely to significantly influence whether individual beetles become vectors of DED.

As the scolytid vectors develop through a series of larval stages, then pupa and imago, the brood galleries they produce are usually extensively colonised by the elm pathogen. Despite this, acquisition of spore inoculum of *O. ulmi* and *O. novo-ulmi* by beetles occurs during a very distinct period of the life cycle of the vector – between pupation and emergence as the developing adults lie in pupal chambers cut in elm bark. The amount of inoculum acquired by each beetle depends on the extent to which the elm pathogen colonises and sporulates on the walls of each pupal chamber (Webber *et al.*, 1987). This, in turn, is influenced by the position of the chamber within the bark, the length of time the beetle remains within the chamber and environmental conditions.

Faccoli and Battisti (1997) noted that more than half of the individuals of *S. multistriatus* and *S. pygmaeus* carried the elm pathogen if they emerged in spring when environmental conditions were likely to favour the development of the elm pathogen in pupal chambers. In contrast, less than 10% of the beetles emerging in the summer carried the pathogen. They suggested hot summer temperatures inhibited sporulation in pupal chambers, a strong possibility in the case of *O. novo-ulmi* which has a growth temperature optimum of 20-22°C and an upper limit for growth of 33°C (Bra-

Table 2. Effectiveness of Scolytus scolytus in transmitting Ophiostoma ulmi to healthy elms (after Fransen, 1939b)

Number of trees	Number of beetles –	Artificially contaminated beetles		Beetles collected in open field	
		Infected trees	Uninfected trees	Infected trees	Uninfected trees
24	100	9	3	3	9
24	50	8	4	2	10
20	50	9	1	3	7

	Type of fruiting structures in pupal chamber (%)					
-	Ase	exual	Sexual			
-	Mycelium	Synnemata	Perithecia	Perithecia+Synnemata		
Tree 1						
Inner bark (phloem)	0	25	15	40		
Outer bark	0	0	0	0		
Tree 2						
Inner bark (phloem)	5	26	21	17		
Outer bark	0	0	0	0		

Table 3. Percentage of pupal chambers in elm bark (Ulmus procera) with visible fruiting structures of Ophiostoma novo-ulmi

sier *et al.*, 1981). However, summer emerging adults may be the second or third generation of elm bark beetles produced within a single year, so the time spent in pupal chambers may be brief with little time for sporulation by *O. novo-ulmi* or for the developing adult to come into contact with any spores.

O. novo-ulmi sporulates most profusely in pupal chambers located in the inner bark (phloem tissue), but only sparsely or not at all in chambers found in the outer bark (Table 3). Thus beetles which pupate in the outer bark are the least likely to come into contact with spores and therefore much less likely to emerge as vectors of DED. The various species of scolytid which act as vectors of O. novo-ulmi can have distinct behaviours at pupation. For example, S. laevis and S. ensifer usually pupate in the sapwood, whereas S. scolytus is usually found pupating in the inner bark (phloem). In areas where S. scolytus is abundant (this would have been the case in most pre-epidemic locations in Europe), only the smaller diameter stems and branches of elms tend to be available as breeding material for the smaller species of scolytid beetles such as S. multistriatus and S. kirschi. This means these beetles frequently pupate in the outer bark or thin-barked material which dries out quickly - thereby inhibiting the process of sporulation. Inevitably, this not only has an effect on the number of beetles that vector the DED pathogen but also on the number of spores carried by individual beetles (Webber, 1990; Webber, 2000).

Host seeking and maturation feeding

Following emergence, the time taken for dispersal by scolytid beetles depends on climatic factors and the availability of elms for maturation feeding and breeding. Arriving at a healthy elm may be a chance event, but there is evidence that beetles are more likely to alight and feed on certain types of elms. The height and silhouette presented by an elm tree may present a strong visual signal which attracts flying beetles and encourages them to land. Sengonca and Leisse (1984) found that the older, taller elms were preferred for feeding by bark beetles and therefore much more likely to become diseased compared with younger trees. Even with small sapling trees of 1-2.5 m in height, there is a significant positive correlation between tree height and the number of beetles that alight and settle to maturation feed (Fig. 1).

Fransen (1939b) was the first to show that *Scolytus* species can have distinct feeding preferences for particular elm species and these findings have been extended more recently (Colin, 2004 this volume; Sacchetti *et al.*, 1990; Webber, 2000; Webber and Kirby, 1983). Presented with a choice of elms for maturation



Figure 1. Correlation between the amount of feeding by *Scolytus scolytus* and the tree height of *Ulmus procera* showing the tendency of the beetles to make more feeding grooves on the taller trees.

feeding, S. scolytus and S. multistriatus feed significantly more frequently on species such as Ulmus procera (English elm), U. carpinifolia (smooth-leaved elm) and U. pumila (Siberian elm) (Fig. 2). In contrast, U. glabra (wych or mountain elm) and U. laevis (European white elm) are much less favoured for feeding (Fig. 2). Although the field performance of U. laevis and U. glabra tends to indicate they are moderately resistant to DED, when inoculated with O. novo-ulmi they are extremely susceptible to the pathogen. Their survival in the field is therefore largely due to their unattractiveness to the vectors of DED and not because of their intrinsic resistance to the fungus.

If the maturation feeding behaviour of beetles on a single tree is observed closely over a period of days or weeks, a pattern soon emerges. My studies have shown that as individuals of S. scolytus and S. multistriatus start to feed, they disperse throughout the crown of the tree and are randomly distributed (Webber, unpublished data). As more beetles arrive to feed, they aggregate together and the feeding grooves are no longer made at random but are clustered, sometimes very closely together. Some beetles also mate while feeding, and the sex pheromones they produce will attract even more beetles to the tree (Svihra, 1982, Blight et al., 1983). Certain trees appear to be highly attractive and act as 'sinks' for large numbers of beetles which alight and then remain to feed. Such trees may have high levels of chemical feeding stimulants in the bark, and a bark texture that also encourages feeding activity. Some feeding grooves seem to be so attractive to beetles that they remain feeding for several days, and the same



Figure 2. Feeding preferences of *Scolytus* bark beetles given a choice of different elm species. a) Preference of *S. scolytus* exposed to *Ulmus procera* and *U. glabra*. b) *S. scolytus* exposed to *U. carpinifolia* and *U. glabra*. c) *S. multistriatus* exposed to *Ulmus procera* and *U. glabra*. d) *S. scolytus* exposed to *U. pumila* and *U. laevis*.



Figure 3. Amount of time spent in the same feeding grooves by individuals of *Scolytus scolytus* feeding on *Ulmus procera* and *U. glabra*.

grooves may be revisited by other feeding beetles and extended and made much larger (Fig. 3). Interestingly, revisiting of feeding grooves happens in a similar proportion of the grooves regardless of whether they are on a preferred and less preferred elm species for feeding. This suggests that it is the physical rather than chemical properties of elm bark that encourages (or discourages) a beetle to start feeding, and that once a feeding groove has been established it may be a more attractive feeding place to other beetles compared to the intact bark in another twig crotch. Similarly, Fransen (1939b) also noticed that beetles preferred to feed in positions that had surface irregularities such as old feeding scars or other roughened patches of bark.

Environmental conditions and host susceptibility

Just as environmental conditions influence the sporulation process in pupal chambers, the environment is also likely to have a profound impact on the process of infection which takes place within the feeding groove. At this point in the disease cycle, the vector, pathogen and host tree can all be affected by changes in temperature, humidity and season.

Perhaps the easiest feature to observe is the influence of temperature on the activity of the vector beetles. It is well documented that certain external temperature thresholds have to be reached before *Scolytus* beetles will emerge and fly (eg Fairhurst and King, 1983), but



Figure 4. Effect of temperature on the feeding activity of *Scolytus scolytus* on *Ulmus procera*.

their feeding activity is also modified by temperature. Between 20-30 C°, increases in temperature are strongly correlated with increased feeding (Fig. 4). As the temperature increases to 25 C° and above, beetles become more active and move energetically, cutting feeding grooves rapidly and often mining deeply into the bark and underlying wood in twig crotches. This vigorous activity is likely to increase the opportunity for spores of the elm pathogen on the exoskeleton of the beetles to be scraped onto the surface of feeding wounds. Moreover, if several beetles all visit the same twig crotch and feed, this will potentially increase the number of spores in a feeding groove and so further enhance the chances of infection taking place.

Climate can also have an impact on a micro-scale within and around the feeding groove, although the quantity of spores delivered by a beetle to a feeding groove is probably the most critical factor in whether or not infection of the xylem, and ultimately the entire tree, takes place. The number of spores that are required for infection probably depends, at least in part, on the species of elm, its intrinsic level of resistance and also its seasonal susceptibility. The difficulty of controlling all these variables when assessing infection in feeding grooves is illustrated by the results obtained in different studies. In many experiments carried out over a decade in southern England, Webber (1987 and unpublished) and Sutherland and Brasier (1997) have reported that at least 500 to 1,000 conidia of O. novo-ulmi are required for infection to occur via feeding grooves in U. procera. In contrast, Faccoli and Battisti (1997) working in northern Italy have found

that infection can occur in *U. glabra* and *U. minor* x *U. pumila* hybrids with an average inoculum load of 130 spores, although the overall level of infection could sometimes as low as 3.3%.

Undoubtedly, high levels of humidity around a feeding groove will enhance the probability of infection and even reduce the number of spores needed for infection. This is illustrated by an experiment with *U. procera*: known quantities of *O. novo-ulmi* conidia were applied to artificially made 'feeding grooves' in twig crotches. Half of these were then individually enclosed for two days in small chambers which maintained an environment of 100% relative humidity; the remainder were subject to the ambient temperature and humidity in the field. The outcome of the experiment 30-35 days later was striking (Fig. 5). The rates of infection via the feeding grooves maintained at 100% humidity were usually at least double those at ambient humidity and fewer spores were needed for infection.

However, despite the emphasis in testing and breeding elms for resistance to DED carried out over many years, there has been little consideration of the way in which the critical threshold of spores required for natural infection in feeding grooves may differ between elm species. There is some evidence from studies on *U. procera* that more spores of the less aggressive elm pathogen *O. ulmi* are required to cause infection via



Figure 5. Infection success following the inoculation of feeding grooves on *Ulmus porcera* with known numbers of conidia of *Ophiostoma novo-ulmi*. Feeding were either maintained at 100% relative humidity (RH) or exposed to ambient RH.

feeding grooves compared with O. novo-ulmi. Thus, for successful xylem infection in 20% of inoculated feeding grooves, at least 10,000 conidia of O. ulmi are required in contrast to only 1,000 conidia of O. novoulmi (Webber, unpublished). It also appears that as many as 5,000 spores of O.novo-ulmi are required for successful infection via feeding grooves in moderately resistant elms such as Commelin (U. x hollandica cross) compared with the 500-1,000 required to infect more susceptible elms such as U. procera (Webber and Brasier, 1994). This could be due to the resistance mechanisms that operate throughout the entire vascular system in elms with resistance to DED. There may also be non-specific antifungal substances such as phenolics that are produced in the bark of some elms in response to feeding damage beetles, and which in this instance inhibit germination of O. novo-ulmi spores.

Finally, into this complex equation of the quantity of spores required for infection, levels of host resistance and pathogenicity of the fungus, must be added yet another variable – the increasing then decreasing susceptibility of the elm as season progresses from early to late summer.

Conclusions

Evident from the research described above, starting with the work of Fransen in the first epidemic and then with subsequent research undertaken more recently, we now have a growing awareness of how beetle feeding preferences can alter the likelihood of a tree becoming infected. The survival in the field of elm species such as *U. glabra* and *U. leavis* which we know are highly susceptible to *O. novo-ulmi*, demonstrates that beetle feeding preferences do operate in natural systems. So far, however, we have failed to exploit this phenomenon, either in conventional elm breeding programmes or in the way we could deploy elms in mixtures in the landscape to counter the spread of DED.

We have also gradually gained an understanding of how the number of *O. novo-ulmi* spores carried by vectors can vary according to beetle species and modifies their effectiveness as vectors. Many aspects of beetle behaviour, interacting with the environment, exert an influence on how individual scolytids acquire spores of the elm pathogen. This makes it difficult to predict how effective some widely geographically distributed species of scolytids can be as vectors. *S. multistriatus*, for example, is found throughout central and southern Europe extending into parts of Russia, and has also been introduced into North America, Australia and New Zealand. In these different regions it encounters a wide range of elm species of different susceptibilities, is exposed to different climates, and competes with many other species of *Scolytus* for breeding material. It is not surprising therefore that in some regions it operates as a very effective vector of DED, but elsewhere it plays a much less significant part. Clearly, understanding the conditions that make a *Scolytus* species an effective vector, and realising when these conditions have changed, is a major part of learning how to control DED.

The part of the DED cycle where, arguably, we have made the fewest gains in understanding is at the point of the feeding groove. Certain threshold quantities of spores in feeding grooves appear to be critical to the likelihood of infection taking place, although this can be modified by the environment. But how infection takes place and whether resistance mechanisms in feeding grooves can impede the process is still largely a matter of speculation. Considered from a simplistic viewpoint, it would seem that several beetles visiting the same feeding groove should enhance the likelihood of infection if it increases the amount of inoculum. It may, however, bring complications that are likely to hamper the infection process. If a series of beetles feed in the same twig crotch, they may all contribute spores of a different genetic composition leading to genotype competition that could inhibit successful colonisation and infection. Alternatively, if the beetles are vectoring O. novo-ulmi at an epidemic front where the pathogen exists as a clone (see Brasier, 1988, Brasier, 2004 this volume), genotype competition will not be a problem but spread of viruses that debilitate the pathogen will exert a negative effect instead (Webber, 1987).

Various questions about the transmission of DED are still unresolved as we enter the 21st century. The legacy of more than 80 years of research on this disease has given us many insights, but the dynamic interaction between multiple species of host and vector, and the two species of pathogen make DED difficult to predict and control. A much deeper understanding is needed if we are to manage this disease more effectively and see a return of the mature elm to our landscape.

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