Elm breeding for resistance against bark beetles

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Abstract

Dutch Elm Disease (DED) forms a complex system of three elements closely interacting: a host, a pathogen and an insect vector. Elm breeding programmes for resistance to DED have been exclusively based on selecting or obtaining pathogen-resistant trees, thus stressing only the host–pathogen side of the interaction and missing the vector component. Resistance against the bark beetle vectors would involve mechanisms other than those implicated in fungus resistance. As a consequence the search for resistance to the vectors would offer more than just a different way of obtaining resistant elms as it would allow incorporation of a new type of resistance into pathogen-resistant trees. Suppressing twig crotch feeding by elm bark beetles would greatly reduce infection on healthy elms. Evidence of preferences by elm bark beetles among different elm species has been documented in the field and in controlled experiments, demonstrating that *U. minor* is preferred over *U. laevis* and *U. glabra*, whereas intraspecific preferences among individual elms or clones remain largely unexplored. The selection of an individual plant by an insect is a two-step process that involves finding and accepting the host. Plant chemicals can play a key role in both of these steps, acting either as long-range stimuli, such as attractants or repellents, or as close-range contact cues, such as feeding stimulants and deterrents, operating after landing on the plant surface. Research on the chemical aspects of elm selection by bark beetles is here reviewed, and prospects for the future are discussed.

Key words: Dutch elm disease, *Scolytus* spp*.,* host selection, elm breeding.

Resumen

Mejora del olmo para su resistencia contra los escolítidos

La enfermedad de la grafiosis de los olmos constituye un sistema formado por tres elementos estrechamente relacionados: un hospedante, un patógeno y un insecto vector. Los programas de mejora de olmos frente a la grafiosis se han basado en seleccionar u obtener árboles resistentes al patógeno, poniendo el énfasis sólo en la parte patógeno-hospedante de la interacción y olvidándose del componente vector. La resistencia frente a los escolítidos vectores supondría mecanismos distintos de aquéllos implicados en la resistencia frente al hongo. Como consecuencia, la búsqueda de resistencia frente a los vectores ofrecería no sólo una via diferente de obtener olmos resistentes, sino además la posibilidad de incorporar un nuevo tipo de resistencia a los árboles resistentes al patógeno. La supresión de la alimentación en las ramillas por los escolítidos del olmo reduciría notablemente la infección en los olmos sanos. La evidencia de preferencias en los escolítidos del olmo entre especies diferentes de olmos ha sido documentada en el campo y en experimentos controlados, demostrándose que *U. minor* es más preferido que *U. laevis* o que *U. glabra*, si bien, la existencia de preferencias intraespecíficas entre árboles o clones permanece inexplorada. La selección de una determinada planta por un insecto es un proceso doble que supone encontrar y aceptar al hospedante. Los compuestos químicos vegetales pueden representar un papel clave en ambas fases, actuando bien como estímulos a larga distancia, tales como atrayentes o repelentes, o bien como señales a corta distancia, tales como fagoestimulantes o fagodisuasores, que operan después del aterrizaje sobre la superficie vegetal. Se revisa aquí la investigación sobre los aspectos químicos en la selección de los olmos por los escolítidos y se discuten las perspectivas futuras.

Palabras clave: grafiosis, *Scolytus* spp*.,* selección del hospedante, mejora de olmos.

Introduction

Dutch Elm Disease, a three element system

Since the discovery in the mid 1930's that elm bark beetles are crucially involved in the transmission of the Dutch Elm Disease (DED) pathogens, *Ophiostoma ulmi* and *O. novo-ulmi*, it has become clear that DED forms a complex system of three elements closely interacting: a host, a pathogen and an insect vector. Prior to the advent of the fungal pathogen, the bark beetle interaction with elms was typical of a secondary, non-aggressive species: bark beetles colonized decaying, moribund elms and their dynamics were governed by a negative density-dependent feedback operating via food competition (Fig. 1, left side). Additionally, their feeding on healthy elms was of no consequence. The introduction of a new element to this scenario, the pathogen, drastically changed the interaction to one of positive density-dependence, propelling local populations of bark beetles to outbreak levels once the disease had appeared in an area.

Although the association between the pathogen and the vector was initially accidental, its efficiency was such that both elements have since become closely linked: bark beetles provide the fungus the only means of entry into distant, healthy elms, and are thus a necessary element for the pathogenic disease cycle to develop. In the return, the fungus provides the bark beetles with increased amounts of breeding material, the diseased elms, an interaction which results in the explosive rise of the beetle population levels and, con-

sequently, of the infection rate (Fig. 1). The failure of any one of these three elements to operate, will break the infection cycle and prevent new infections.

Breeding for resistance to Dutch Elm Disease

Soon after the discovery of DED in Europe, an elm breeding program was started in the Netherlands (1928), much later followed by similar initiatives in other countries such as Italy (1978) and Spain (1987). In North America, diverse elm breeding programs against DED have been developed since 1933. In all of these programs, resistance against the disease was approached in a classical way, focusing on the search for elms resistant to the pathogen, and thus stressing only the host-pathogen side of the interaction.

Elm breeding programs have been strongly based on the incorporation of known sources of resistance to the fungus, by crossing some resistant Asiatic elm species such as *U. pumila*, *U. wallichiana*, *U. parvifolia* and *U. japonica*, with the native European elm species. The aim was thus produce resistant hybrids displaying desired growth and shape features. Among the remarkable achievements of these programs we should mention the development of several Dutch hybrid cultivars, the selection of Asiatic cultivars and the production of new, potentially resistant *U. minor* clones currently being tested (See chapters 14 to 16 in this volume for the history and current developments in elm breeding).

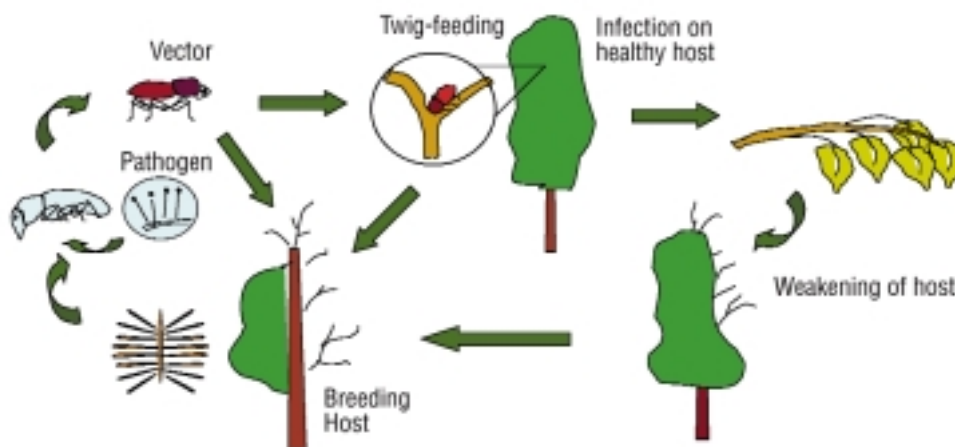Encouraging as they may be, these programs have almost never focused on the third component, the in-

Figure 1. Dynamics of the interaction between host, vector and pathogen in the Dutch elm disease system. Introduction of the pathogen in the interaction between host and insect creates a positive feedback (right side) resulting in more infections.

sect vectors. Those who pioneered the Dutch breeding program were aware of it and included the search for resistance against the bark beetles in their breeding. Unfortunately, this strategy «had to be dropped as being too complicated» (Heybroek, 1998). Prospective work on resistance against bark beetles will then emphasize the overlooked host-vector side of the interaction. This means that a new type of disease control will be sought, involving completely different mechanisms as compared to those known to act against the pathogen. Furthermore, it must be stressed that this kind of endeavor should not be narrowly focused on the search for tree resistance against the insects, but aimed at the incorporation of such resistance into pathogen-resistant trees.

Resistance against bark beetles

In a search for resistance against elm bark beetles in elm trees, one must first determine which point of the host colonization process must be targeted. Unlike many other well known bark beetles (i.e. conifer bark beetles), elm bark beetles feature a two-phase host-contact interaction: a transient, very short phase of twig crotch feeding in the crowns of healthy elms («feeding attacks»), and a final, definitive phase of reproductive mass attack where the beetles colonize the phloem in stems and branches of stressed, diseased or dead elms to breed («reproductive attacks»).

Searching for mechanisms preventing reproductive attack will pose serious difficulties. Even though several host defense responses to boring attempts are quite well documented in conifer bark beetles, such as primary resin flow, hypersensitive or wound reaction (Lieutier, 2002), no defense mechanisms have been identified so far against bark beetles colonizing elms or other hardwoods. Besides, and more importantly, this type of resistance would appear too late in the disease cycle, since mass attack by elm bark beetles is only carried out in already diseased trees.

Thus, the option of seeking prevention against twig-crotch feeding seems the right direction to follow. Twig- crotch feeding is a crucial event in the disease cycle since it is the only means by which the fungus gains access to remote healthy elms. Consequently, avoiding or minimizing this step will stop or greatly reduce infection rates of healthy trees. Since no induced defense responses in the feeding grooves are expected, deterrence of this behavior would represent a

constitutive defense, resulting in the tree being unattractive or unsuitable for the beetles to feed upon.

The host-vector and host-pathogen interaction being of a different nature, it is reasonable to assume that resistance against insect feeding will be compatible with resistance against the pathogen, such that both types of resistance could be incorporated in the same tree. This widens the prospect for obtaining resistant individuals, since partial beetle resistance coupled with partial fungus resistance could lead to trees highly resistant to DED. Thus, the selection of non-attractive elm trees for bark beetle feeding should be addressed to fill the current gap in elm breeding.

Feeding Preferences

Twig-crotch feeding behavior

As already mentioned, feeding in twig crotches and at the base of leaf petioles is a key process in DED transmission. However, the role of this behavior in bark beetle biology is not well understood. Contrary to earlier thinking, it was demonstrated for several elm bark beetle species that beetle feeding on healthy hosts is not a prerequisite for sexual maturation [Von Keyserling (1975) for *S. scolytus*, Choudury (1979) for *S. multistriatus* and Pajares (1987) for *S. kirschii*] and that adults can successfully complete their life cycle emerging from brood trees and colonizing new brood trees without the involvement of this feeding.

Twig-crotch feeding behavior has since been explained as a type of «refueling» process for in-flight adults while in search for breeding material. Thus, twig-crotch feeding would be a means by which beetles gain moisture and energy reserves for survival when breeding space is not available (Baker and Norris, 1968; Rabaglia and Lanier, 1983). In addition to securing food reserves, the beetles may use feeding scars as mating ground, although most beetles tend to mate at the entrance of the breeding galleries.

Twig-crotch feeding by *S. mulstistriatus* in healthy elms is also influenced by the colonization of nearby diseased trees. In a study by Rabaglia and Lanier (1983) where elm trees were baited with different combinations of the beetle pheromonal components α-multistriatin and 4-methyl-3-heptanol, it was found that the former compound released alone or in excess of its natural ratio (1:1) to methyl-heptanol induced twig-crotch feeding. The following scenario was then pro-

posed: when a tree is initially colonized, virgin females release both compounds in equal amounts, resulting in the attraction of many individuals from the local beetle population. As the established females are mated, the release of methyl-heptanol ceases and the ratio α-multistriatin to methyl-heptanol increases; beetles are still being attracted to the vicinity of the attacked tree, but the biased M:H ratio inhibits their landing in the already saturated bark and instead, the incoming beetles are deflected to the crowns of near healthy elms where they feed in the twigs (Fig. 2). This observation further stresses the importance of sanitation of diseased trees in integrated DED control programs.

Beetle preferences among elms

If we were to select non-attractive elm trees, then we must first consider the available evidence for the existence of such differential preference. At this point we may ask whether elm bark beetles are only specific to elms or whether they can use other host trees. According to records in the taxonomic literature (i.e. the review of genus *Scolytus* by Michalski, 1973), the answer would be that many tree species from more than 10 genera can serve as hosts to *Scolytus* elm bark beetles. However, in a simple experiment, Dixon (1964) confined *S. multistriatus* adults to bolts of each of 18 tree species, including species of *Malus, Tilia, Fagus, Prunus, Ulmus, Vitis, Celtis, Carpinus, Gleditsia, Acer,*

Morus, Maclura, Ostrya, Sassafras, Platanus and *Pyrus*, and obtained reproductive galleries and offspring only from elm and pear trees. Similarly, in other trials assessing twig feeding responses in confinement with 11 different hosts, only elm twigs were fed upon during the first day; however, other host species such as common apple, hawthorn, white oak, silver maple, eastern poplar and common pear showed feeding grooves when confinement was extended to a week. These results, combined with our own observations, suggest that elm bark beetles can be considered to be as fairly elm-specific, although under unnatural experimental conditions they can be forced to accept feeding on tree species that would otherwise be rejected.

Once host specificity in elm bark beetles has been established, the question of preferences among different elm trees must then be addressed. The following summarizes the current evidence on preferences exhibited by *Scolytus* elm bark beetles when choosing elm trees:

Interespecific preferences

One remarkable observation made during the last DED pandemic in Europe was the delayed occurrence of infection in *U. laevis* and in *U. glabra* elm populations. Contrary to early expectations, the superior initial performance of these species was not related to resistance against the pathogen, both elm species appearing very susceptible to *Ophiostoma novo-ulmi*. Ins-
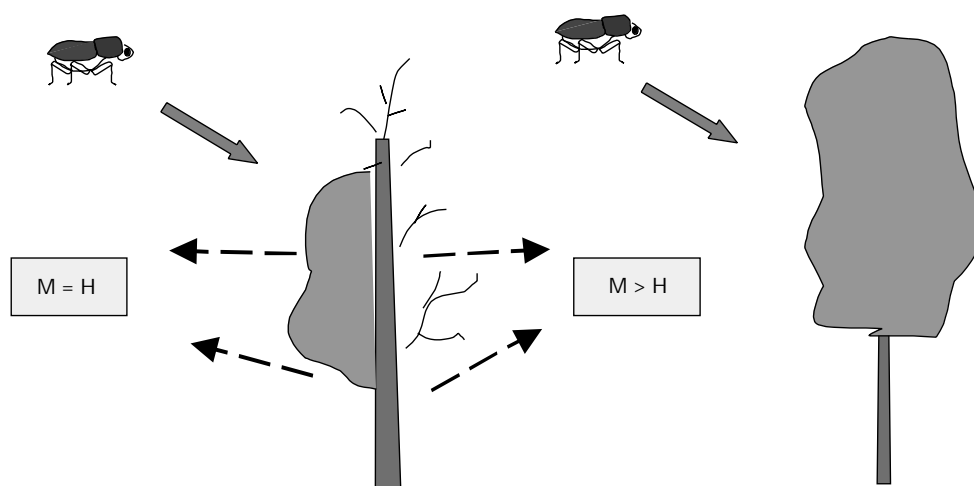

Figure 2. Virgin females release equal amounts of α-multistriatin (M) and methyl-heptanol (H) and attract beetles to the tree under attack (left); beetles are deflected to the crowns of nearby trees by mated females (M > H, right). Adapted from Rabaglia and Lanier (1983).

tead, the lower infection rates were the result of a reduced attractiveness to bark beetles in search of feeding hosts, as demonstrated by some simple experiments in which bark beetles were exposed to young potted plants of different species in enclosures.

In these studies the following observations were made: (1) There was greater degree of twig-crotch feeding by *S. scolytus* on *U. procera* (*U. minor*) than on *U. glabra* or *U. laevis*. Similarly, though less marked, *S. multistriatus* displayed a preference for *U. procera* relative to *U. glabra* (Webber and Kirby, 1983; Webber, 2000). (2) There was a greater feeding response by *S. multistriatus* on *U. carpinifolia* (*U. minor*) than on *U. laevis* (Sachetti *et al.*, 1990). (3) *S. scolytus* and *S. multistriatus* showed a strong preference for *U .minor* when offered together with *U. glabra* or *U. laevis* (Piou, 2002). Thus, it has become clear that white elm (*U. laevis*) and wych elm (*U. glabra*) are not preferred or even disliked by elm bark beetles, whereas, common elm, *U. minor* (sensu lato), and Siberian elm (*U. pumila*) appear very attractive for beetle twig feeding. These results have been recently confirmed in laboratory feeding bioassays comparing elm twig extracts (Pajares *et al.,* this volume).

Intraspecific preferences

Although there is evidence that elm bark beetles prefer some elm species over others for feeding, the possible existence of intraspecific preferences among individual trees or clones has remained mostly unexplored. However, we can predict that such differences exist given the high intraspecific genetic variability in elms. Apparently, no two plants are chemically identical, with plant compounds varying both qualitatively and quantitatively, so «the importance of intraspecific variation in host plant acceptability is recognized by plant breeders when they produce insect-resistant cultivars» (Bernays and Chapman, 1994). In fact, intraspecific genetic variation for individual chemicals has been found wherever it has been investigated. Not surprisingly, Webber and Kirby (1983) observed in their trials that some *U. procera* individuals were clearly fed upon to a greater extent than others. Moreover, in recent tests comparing feeding responses by *S. scolytus* to elm twig extracts from different *U. minor* trees in two-choice bioassays, one tree was less attractive than the other four. (Pajares *et al.*, this volume).

Evidence for intraspecific differences in feeding attacks is likely to be obtained from the study of surviving trees in DED epidemic areas, focusing on trees that do not show resistance to the pathogen in inoculation trials. If neither fungal resistance nor spatial escape can account for the survival of these trees, then we may postulate that they have been avoided by elm bark beetles. Thus, a search for such cases holds potential for the detection of intraspecific differences and for the identification of the host factors involved.

Tree selection by elm bark beetles

Host plant selection

All phytophagous insects display some level of selectivity in the plants they consume. The concept of host plant selection extends beyond the choice of the habitual plant species, as the insect must also select, within that species, a particular plant that is suitable for feeding or development. The selection of an individual host plant by an insect is a two-step: (i) finding and (ii) accepting the host plant. These steps may then be divided into several sequential steps or behavioral events, each governed by signals or stimuli of a varying nature (Miller and Strickler, 1984): (1) Movement at random (not influenced by plant cues). (2) Movement oriented to the host (non-contact cues). (3) Arrestment on or at the plant (non-contact and contact cues). (4) Inspection via sensory apparatus (contact and non-contact). (5) Trying or «nibbling» (contact and non-contact). (6) Sustained ingestion or consumption (contact and non-contact). Host finding would include the first three steps in the sequence, whereas host acceptance would require the last three.

Besides visual and physical factors, chemical stimuli from the plant play a key role in host selection. Such stimuli include plant odors acting at long range, such as attractants and repellents that may be involved in finding the host, or plant constituents perceived by the insect at short range, either by olfaction or gustation, such as feeding stimulants and deterrents that are crucial in the acceptance of the plant. Feeding behaviors are also greatly influenced by the internal condition of the insect, so the final decision whether to accept the plant or not relies on the balance between the external and internal (reserves, fatigue level) stimulatory and inhibitory factors, as exemplified in Fig. 3.
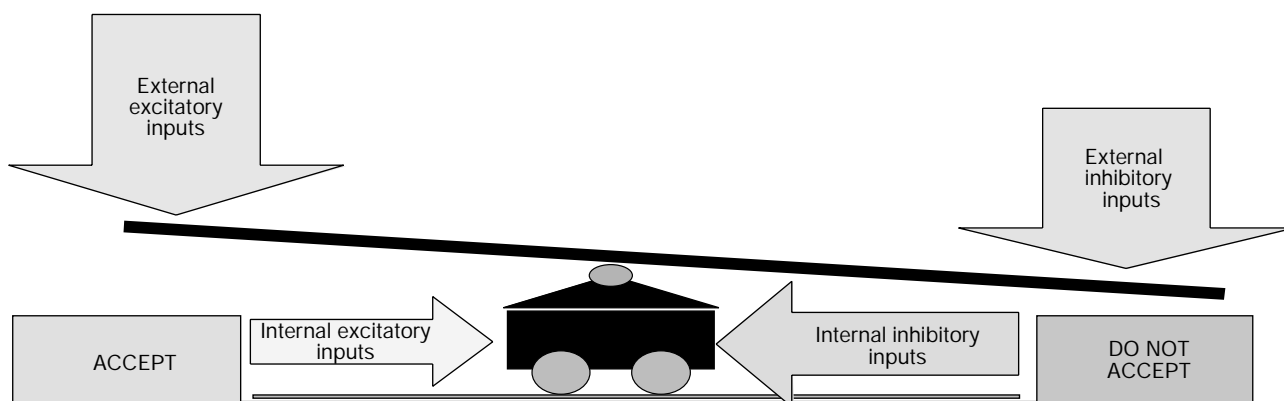
Figure 3. Mechanical model of host acceptance where external excitatory and inhibitory factors are balanced, and the position of the rolling fulcrum is dependent on the insect internal status. Adapted from Dethier (1982).

Host finding by elm bark beetles

Distance orientation to suitable hosts mediated by plant volatiles has been profusely studied in conifer bark beetles, particularly in *Dendroctonus* and *Ips* species. Currently, two theories for locating hosts, each with documented examples, have been put forward (Byers, 1995): in the first, host finding is achieved by orientation to volatile chemicals emanating from suitable hosts (primary attraction) and this would be the case for secondary bark beetles; in the second, assumed to work in more aggressive species, hosts are found at random by the beetles which, after landing, test their suitability.

Much less is known, however, on host finding by other bark beetles living on hardwoods. Within the genus *Scolytus*, *S. quadrispinosus* was caught in similar numbers on traps disposed around the crown of *Carya ovata* host trees and in nearby non host *Quercus alba* trees, thus suggesting a random finding of the host by this species (Goeden and Norris, 1965).

Several studies on host finding have been devoted to elm bark beetles, but in these, elm volatiles have always been considered within the context of secondary attraction, as synergists of the pheromonal bouquet released by the beetles. Below is a brief summary of the main results obtained for *S. scolytus* and *S. multistriatus*.

— The elm sesquiterpene α-cubebene synergized attraction to the methyl-heptanol pheromone released by *S. scolytus* (Blight *et al*., 1980). However, in a later study, even though olfactory sensilla in *S. scolytus* were found to be tuned to the elm monoterpenes (-)-β-pinene, (-)-limonene and α-cubebene, none of them, alone or combined, showed activity in field trials (Grove, 1983).

— Again, in several field tests, α-cubebene was found to synergize attraction of *S. multistriatus* to its pheromonal components (Pearce *et al*., 1975; Peacock *et al*., 1975; Blight *et al*., 1983).

— Out of the 14 sexquiterpenes identified from the phloem of *U. americana* by Millar *et al*., (1986), δ-cadinene, γ-cadinene, α-cubebene, γ-muurolene and β-elemene showed some attractiveness to *S. multistriatus* in laboratory olfactometer, but none of them, alone or in a range of combinations, were more attractive to this species than empty blanks, nor did they improve the response to pheromone baits in field trials.

— Finally, an increased attraction of *S multistriatus* to pheromone baits was obtained when «green leaf» volatiles common to many green plants (hexanal and hexen-1-ol) were added (Dickens *et al*., 1990).

Some conclusions might be drawn from the results just mentioned: these elm bark beetle species are able to perceive elm volatiles, but in the absence of pheromonal signals, remote location of host trees (i.e. healthy elms) mediated by elm volatiles remains questionable. Instead, it seems likely that elm bark beetles can find their hosts flying at random, being guided by a general attraction to vegetation and to tree silhouettes; after landing into a particular tree, host acceptance would be determined by close-range olfaction and gustation. In any case, detailed studies, specifically designed, would be required to ascertain these provisional conclusions.

Host acceptance by elm bark beetles

Once the beetle has landed on or approached a tree, it has to decide whether to accept it or not. The

process of host acceptance by elm bark beetles may be influenced by several factors of varying nature, including visual and tactile stimuli, but surely chemicals would be the most important signals. These chemicals may be plant volatiles, which may be present at high concentrations in the thin boundary layer surrounding the bark and other plant surfaces, and can be perceived through close-range olfaction by the antennae, or they may be plant constituents which may be present in the bark and tested by contact chemoreception (gustation); the bark cells contain a great diversity of chemicals, some of which are able to activate the contact chemoreceptors in the mouthparts, whether inducing phagostimulatory effect or feeding deterrence.

Most of the limited knowledge on the chemical factors involved in elm bark beetle feeding comes from the pioneering work carried out by Norris and coworkers at the University of Wisconsin during the late 60's and early 70's. They could demonstrate the phagostimulatory effect of the following elm bark compounds, though none of them were specific to elms:

— Vanillin and syringaldehyde, two lignin derivatives, were observed to be attractive at short-range in olfactometer assays and also induced feeding on the discs (Meyer and Norris, 1967).

— Feeding stimulant activity was also reported for an unidentified pentacyclic triterpene (Baker and Norris, 1967), p-hydroxybenzaldehyde (Baker *et al*., 1968), p-hydroquinone (Norris, 1970), pyrocatechin (Borg and Norris, 1971) and some lignin intermediates (benzaldehydes and hydroxybenzenes) (Meyer and Norris, 1974).

Several assays in the context of a study of deterrence from non-hosts were also conducted, and some antifeedants for *S. multistriatus* were identified in the extracts from several trees:

— Juglone (5-hydroxy-1,4-naphtoquinone) from *Carya ovata*, *C. cordiformis* and *Juglans regia*, demonstrated to be a potent feeding deterrent (Gilbert *et al*., 1967).

— Several compounds from different chemical groups reduced feeding when they were added to elm extracts, such as the flavonoids phloretin (from *Malus pumila*), kaempferol (*Robinia pseudoacacia*) and quercetin (*Quercus macrocarpa*), the coumarins aesculetin (*Aesculus octandra*) and fraxetin (*Fraxinus americana*), and the alkaloids gramine (*Acer negundo*) and magnoline (*Magnolia acuminata*) (Norris, 1977).

These results led Norris and coworkers to conclude that the high specificity in host selection by *S.multistriatus*, once the beetle has landed on the tree, was ex-

plained by the combined effect of host feeding stimulants together with the absence of feeding deterrents, repellents or inhibitors as those present in non-host trees. Since the feeding stimulants identified were not specific to elms, Norris stressed the importance of deterrents in host selection, supported by several results. In an experiment, discs treated with elm extracts were avoided by *S. multistriatus* when the deterrent juglone was added to them, and, most notably, the insects fed on discs treated with extracts from *C. ovata* that were deprived of juglone (Gilbert and Norris, 1968), suggesting that the absence of a single deterrent may render acceptable a non host tree.

Only a few other investigations have been devoted to the study of host selection by elm bark beetles. A glycoside, catechin xylopiranoside, and the wax lupeyl-cerotate were reported by Doskotch *et al*. (1970) as having significant phagostimulatory activity for *S. multistriatus* in bioassays. Several metabolites were isolated from the elm-colonizing fungus *Phomopsis oblonga* that reduced feeding by *S. scolytus* when applied to elm bark pieces. These compounds were identified as: a nonsesquiterpene γ-lactone, two tiglic esters of 5,6-dihydro-5-hydroxy-2-pyrones, nectriapyrone, 4-hydroxyphenylethanol, 5-methylmellein, and the acids 2-furoic, orsellinic, 3-nitropropanoic and mellein-5-carboxilic (Claydon *et al*., 1985). Since these are fungal metabolites and *P. oblonga* only colonizes the phloem of stressed, diseased elms, their implication in the process of host acceptance by *S. scolytus* for twig feeding on healthy elms is not likely to be significant.

Thus, it can be concluded with confidence that the whole process of host selection by elm bark beetles is still far from being well understood. In this context, studies have been recently carried out to acquire more detailed information on the bark chemical components of several elm species and hybrids and their significance in beetle feeding, leading to the identification of many interesting compounds that may be involved in host acceptance (see Dario *et al*., this volume). Laboratory two-choice feeding bioassays (Fig. 4) comparing beetle responses to bark extracts and compounds are currently underway to further characterize the host selection process (see Pajares *et al*., this volume).

Searching for non attractive elms

Twig-crotch feeding in a particular elm tree may be avoided if any of the steps in the finding and accep-
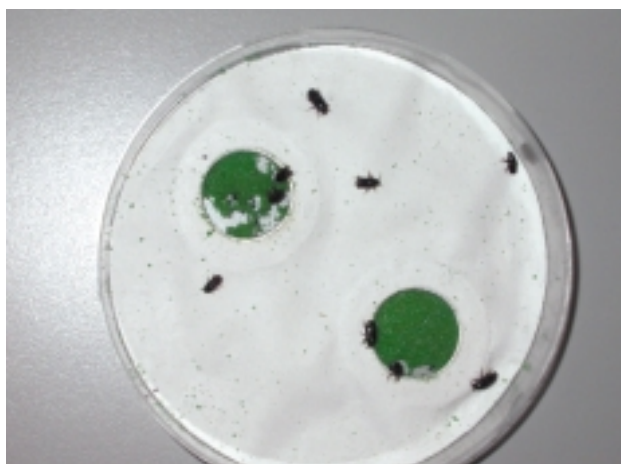
Figure 4. Two-choice feeding bioassay to test beetle feeding responses to elm bark components.

ting sequence fails to occur. The decisive factors for the bark beetles to feed or not on a given visited tree will depend on contact stimuli. Through short-range olfaction and, specially, contact chemoreception, elm bark beetles would detect feeding stimulants and feeding deterrents/repellents. Thus, a by-passed elm tree may lack enough stimulants, and/or contain deterrents. Unless elm-specific feeding stimulants are found, tree rejection due to the absence of stimulants appears unlikely, since many compounds apt to induce feeding by the elm bark beetles may occur, all of which would not probably be altogether required for this action. The presence of deterrents, albeit rare, unusual compounds or common elm compounds present at unusually high rates, seems a more promising possibility for tree selection. In any case, a deeper understanding of the chemical factors operating in host selection for twig-feeding will be necessary to undertake a search for non attractive elms.

Testing non-attractiveness or non-preference of elm trees by elm bark beetles will require different experimental procedures depending on the step of the host selection process examined. The study of the activity of volatile chemicals involved in host finding by the beetles would require electroantennographic detection (GC-EAD) for identifying biologically active compounds; however, this method only detects sensory activity and does not provide information on the behavior evoked, so behavioral tests by olfactometer bioassays would also be necessary. Activity of contact chemicals, stimulants and deterrents, influencing host acceptance could be determined by electrophysiological recording of chemoreceptors (taste recep-

tors in the mouth parts) and by effective feeding bioassays in choice experiments with extracts of known chemical composition and compounds applied to neutral substrates. Experimental settings with plants in enclosures or in the field, should later confirm the results obtained.

Besides serving as a guide for screening potentially non attractive elms, feeding deterrents or repellents might also be used in biotechnology. Genetic engineering for the insertion of genes encoding enzymes involved in the synthesis of feeding inhibitors would render the trees less liable to become infected with DED. Genetically modified elm plants expressing deterrents from other elm species or even from other tree genera would likewise enhance the prospects of obtaining elms resistant to DED.

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