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Feeding responses by *Scolytus scolytus* to twig bark extracts from elms

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Abstract

Feeding responses by *Scolytus scolytus* were tested using elm twig bark extracts in a laboratory bioassay. One to 4-years-old elm twigs or small branches were sampled in spring and their bark extracted separately with methanol and with a mixture of petroleum ether and diethyl ether (1:1) as solvents. Bark extracts were tested in a two choice feeding bioassay consisting of two polyurethane discs placed in a 10 cm diameter Petri dish. Extracts were applied onto the discs and the amount of disc eaten by ten freshly emerged *S. scolytus* adults was recorded after 24 hours. Ten *U. minor*, two *U. laevis*, six *U. glabra*, three Dutch hybrids (European x Asiatic) and one *U. pumila* trees were tested in several comparisons. Discs with extracts from both *U. laevis* trees were significantly less eaten than those from *U. pumila* or from *U. minor* trees in two choice tests. Similarly, extracts from all *U. glabra* trees received less feeding than those from *U. minor*. On the contrary, *S. scolytus* showed no difference in feeding between *U. pumila* and *U. minor* extracts, and similarly for Dutch hybrids in comparison with two *U. minor* clones. Again, beetles preferred to feed on Dutch hybrid extracts better than in those from *U. laevis*. Significant intraspecific differences in feeding were obtained in *U. minor*. One of the *U. minor* clones resulted less chosen when compared to other four trees. Extracts from a dying *U. minor* tree received more feeding than those from a healthy tree. Comparisons were also made between bark extracts from 2-to 4-year-old vs. current-year twigs within the same trees. In one of the four *U. minor* tested, a significant preference for the older twig extracts was recorded.

Key words: feeding preferences, *Ulmus* spp., *Scolytus* spp., bark extracts.

Resumen

Respuestas de alimentación de *Scolytus scolytus* a extractos del floema de ramillas de olmo

Se estudió la respuesta de alimentación de *S. scolytus* a extractos del floema de ramillas de olmo en bioensayos de laboratorio. Se muestrearon en primavera ramillas de olmo de uno a cuatro años de edad y su floema fue extraído independientemente con metanol o con una mezcla de eter de petróleo y eter dietílico (1:1). Los extractos del floema se evaluaron en un bioensayo de doble elección consistente en dos discos de poliuretano dispuestos en una placa Petri de 10 cm de diámetro. Se aplicaron los extractos a los discos y se midió la superficie de disco comida por diez adultos recién emergidos de *S. scolytus* durante 24 h. Se ensayaron diez *U. minor*, dos *U. laevis*, seis *U. glabra*, tres híbridos holandeses (europeo x asiático) y un *U. pumila* en diversas comparaciones. Los discos con extractos de ambos *U. laevis* fueron significativamente menos comidos que aquéllos con los de *U. pumila* o de *U. minor*. Igualmente, los extractos de todos los *U. glabra* recibieron menor alimentación que aquéllos de *U. minor*. Por el contrario, *S. scolytus* no mostró preferencias entre los extractos de *U. minor* y de *U. pumila*, e igualmente, entre los de *U. minor* y los de híbridos holandeses. Nuevamente, los escolítidos prefirieron alimentarse menos de los extractos de *U. laevis* que de los híbridos holandeses. Se encontraron diferencias intraespecíficas significativas en *U. minor*. Uno de los clones de *U. minor* resultó menos preferido cuando se le comparó con otros cuatro árboles. Los extractos de un *U. minor* moribundo recibieron mayor alimentación que los de un árbol sano. Se realizaron comparaciones entre los extractos del floema de ramillas de 2 a 4 años de edad y de ramillas del año en curso de un mismo árbol. En uno de cuatro *U. minor* ensayados se observó una preferencia significativa por los extractos de las ramillas más viejas.

Palabras clave: preferencias en la alimentación, *Ulmus* spp., *Scolytus* spp., extractos del floema.

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Introduction

Dutch elm disease is the result of a complex feedback system requiring the concourse of three elements: elms, fungus and elm bark beetles. The participation of all three elements is so necessary that the disengagement of any will stop the disease cycle and the occurrence of new infections. Elm breeding programs for resistance to the disease, since the pioneer Dutch breeding program to the current Spanish program, have only been focused in obtaining resistant individuals to the pathogen, missing the fact that the insect component, the bark beetles, is also necessary for the disease to develop. Thus, breeding for resistance against elm bark beetles would fill a gap in current breeding programs, searching for a different type of protection from infection that might be incorporated to pathogen resistant trees.

Twig-crotch feeding is a vital event in the disease process, since it is the only way for the fungus to make contact and infect distant healthy elms. Then, it becomes clear that non-attractive or unsuitable trees for beetle twig feeding would escape to the disease, even if they are susceptible to the pathogen. There is evidence that elm bark beetles prefer feeding on some elm species better than on others. The better performance of *Ulmus glabra* and *U. laevis* to the *Ophiostoma novo-ulmi* epidemic in Europe suggested that these species were less attractive for beetle feeding. It was demonstrated that *S. scolytus* and S. *multistriatus* clearly preferred to feed on *U. minor* plants than on *U. glabra* or on to *U. laevis* (Sachetti *et al*., 1990; Webber and Kirby, 1983; Webber, 2000; Piou, 2002). However, differences in beetle attractiveness among individuals of the same species have not yet been studied, though these are expected to appear considering the high intraspecific variability in elms. For example, Webber and Kirby (1983) noted that some of the *U. minor* plants received clearly more feeding than others.

The selection of an individual tree by a beetle is a sequential process that first involves host finding, and then acceptance. These steps, from distance movement to the host to finally consuming it, are influenced by different signals from the host. It is well known that chemical stimuli play a key role in host plant selection by feeding insects, and these could include plant odors acting in long range detection, such as attractants and repellents, and plant chemicals involved in short range host acceptance, such as feeding stimulants and deterrents (Bernays and Chapman, 1994).

Almost forty years ago, Norris and coworkers developed a two-choice feeding bioassay to study the chemical factors governing feeding in *S. multistriatus*. Using elderberry pith discs with elm bark extracts and pure chemicals, they demonstrated the phagostimulatory effect of several elm compounds, such as vanillin, syringaldehyde (Meyer and Norris, 1967), a pentacyclic triterpene (Baker and Norris, 1967), p-hydroxybenzaldehyde (Baker *et al*., 1968), p-hydroquinone (Norris, 1970), pyrocatechin (Borg and Norris, 1971) and other lignin-related compounds (Meyer and Norris, 1974). Several feeding deterrents were identified from the extracts of different trees, particularly juglone from *Carya ovata* and *Juglans regia* (Gilbert *et al*., 1967), but also the flavonoids phloretin, kaempferol and quercetin, the coumarins aesculetin and fraxetin, and the alkaloids gramine and magnoline (Norris, 1977). As none of the feeding stimulants were elm specific, the role of deterrents was outlined, and it was hypothesised that the high host selectivity by the elm bark beetles, once they have arrived to a particular tree, could be explained by the presence of feeding stimulants in the host trees, combined with the absence of feeding deterrents or inhibitors occurring in non-host trees.

Since Norris time, no further advances have been obtained in the knowledge of the chemical factors involved in the host acceptance process by the elm bark beetles. However, a deeper understanding of this process will be required if we were to envisage the selection of elm trees unsuitable for beetle feeding. The present work reports on the feeding responses by *Scolytus scolytus* to elm twig bark extracts from several species and clones, aimed to detect feeding stimulants and deterrents that might be useful in breeding elms resistant to Dutch elm disease through insect avoidance.

Material and Methods

Sampling

Two to 4-year-old twigs and small branches were collected in spring (May/June) from several elm trees of the species *U. minor*, *U. laevis*, *U. pumila* and Dutch hybrids [(*U.glabra* x *U.wallichiana*)x], located at the elm clone collection of Puerta de Hierro (DGCN, Madrid), and at the Rivas-Vaciamadrid elm stand (Madrid). Also, samples from *U. glabra* trees were collected in several valleys located in the Central moun-

Table 1. Plant material specifications

Site	Clon/tree	Species[c]	Location	Sampling
Elm clone collection	CA-AL3	*U. pumila*	Cádiz	11/06/02
	LE-BL1	*U. laevis*	León	11/06/02
	M-QM2	*U. laevis*	Madrid	25/05/01
	M-DV1[a]	*U. minor*	Madrid	11/06/02
	SG-CC1	*U. minor*	Segovia	11/06/02
	TO-PB1	*U. minor*	Toledo	11/06/02
	V-JR1	*U. minor*	Valencia	11/06/02
	M-DV5	*U. minor*	Madrid	11/06/03
	H-454 (Lobel)	A x B[c]	The Netherlands	25/05/01
	H-826	[A x (o.p.)] x [o.p.][c]	The Netherlands	25/05/01
	H-1020	[A x C] x [D][c]	The Netherlands	25/05/01
Rivas-Vaciamadrid	MRV-104	*U. minor*	Madrid	27/05/02
	MRV-122	*U. minor*	Madrid	27/05/02
	MRV-172[a]	*U. minor*	Madrid	27/05/02
	MRV-175[a]	*U. minor*	Madrid	27/05/02
	MRV-221[b]	*U. minor*	Madrid	09/07/02
	MRV-295[a]	*U. minor*	Madrid	27/05/02
	MRV-308[a]	*U. minor*	Madrid	27/05/02
Central range	AV-IR1	*U. glabra*	Ávila	23/05/03
	AV-IR2	*U. glabra*	Ávila	23/05/03
	AV-CA2	*U. glabra*	Ávila	23/05/03
	AV-CA3	*U. glabra*	Ávila	23/05/03
	AV-CA5	*U. glabra*	Ávila	23/05/03
	M-RO3	*U. glabra*	Madrid	23/05/03

[a] Also current year twigs sampled. [b] Dying tree. [c] *U. glabra* «Exoniensis» x *U. wallichiana* P39 (A); *U. hollandica* «Bea Schwarz» o.p. (B); *U. minor* var. *minor* 1 x *U. minor* var. *minor* 28 (C); *U. hollandica* «Vegeta» x *U. minor* var. *minor* 1 (D)

tain range (Ávila, Madrid). In some trees, current-year twigs were also sampled. Trees were 5 to 12-year-old in the first site, around 60 years of age in the second site, and estimated between 30 to 80 years in the third site (Table 1).

Extracts

Within the same day of sampling, the bark of twigs was peeled off and cut in small pieces. Twenty grams of the bark pieces per sample were added to 200 ml methanol for 48 h at room temperature in the dark for extraction of the polar compounds. Similarly, 30 g. of bark were introduced in 200 ml of petroleum ether : diethyl ether (1:1) for extraction of the non polar compounds. Ether extracts were decanted, filtered and dried under Nitrogen flow, whereas for the methanol extracts solvent was first removed by a rotavapor, then 20 ml water added and the solution frozen and lyophilized. Dried extracts were stored at –45ºC until used.

Bioassay

Feeding responses were tested in a two-choice bioassay arena inspired in Norris and Baker (1967). Discs made of extruded polyurethane (floral foam), 0.9 mm thick and 17 mm in diameter, were used as feeding substrate. Treatments were applied to the discs and after solvent evaporation for 5 h, two discs were placed separatedly on the bottom of a 10 cm diameter plastic Petri dish. Each disk was fixed on place by gently pressing on it an aluminium washer, 1 mm thick and 30 mm/16 mm external and internal diameters. The metallic washer, wich provided a slight thigmotactic stimulus, was partially covered with parafilm to facilitate fixation on the plastic bottom. A filter paper with two circular holes 16 mm in diameter was affixed on top covering the washer and providing a walking surface for the beetles (Fig. 1); a water soaked filter paper was also fitted to the inside of the Petri lid for providing a moist environment. Treatments consisted of 1 mg. of the dried methanol (M) extracts or 0.2 mg of the ether : ether (E) extracts. Extracts were previously

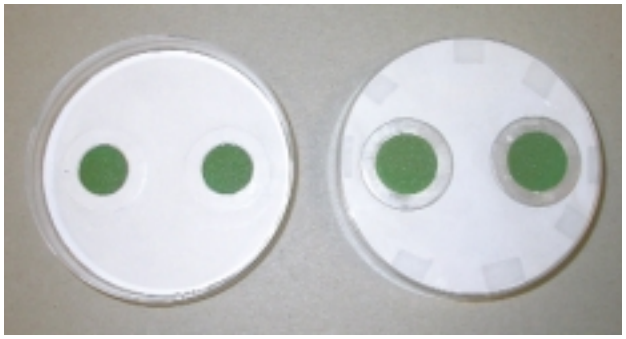

Figure 1. Two choice bioassay arena: bottom of the Petri dish from above (left) and below (right).

re-dissolved in 200 µl of the same solvents and applied to each disc. Responses to extracts from two different trees in the same arena were compared. Tested treatments were methanol plus ether extracts or methanol alone extracts. Also, since direct comparison of ether extracts was not possible due to the low feeding induced by these extracts when presented alone (at the doses used), to test their influence in feeding preferences they were compared in discs having previously received a common treatment of methanol extract from one of the trees tested. No comparisons between extracts and blanks were made.

Test insects used were freshly emerged, un-fed *S. scolytus* adults obtained from wild populations or laboratory reared. Wild adults emerged in laboratory cages from elm bark pieces collected in the field containing overwintering larvae; once assayed, these adults were used as breeding parents for laboratory rearing of *S. scolytus* in elm bolts. Ten adults were introduced in each of the Petris and 5 dishes (replications) were assayed per test. Beetles were allowed to feed on the discs for 24 h under dark at 25ºC, and the area of disc eaten was then measured using an image analyzer (WINDIAS, Delta T Devices Ltd, Cambridge, U.K.). Data on area removed fulfilled normality assumptions and was analyzed untransformed as feeding percentage by paired samples Student's t test.

Results

Interspecific choices

U. laevis vs. *U. minor*. Extracts from two *U. laevis* trees were compared against three *U. minor*. Results of the feeding response by *S. scolytus* are shown in Table 2. Methanol + ether extracts from *U. laevis* LE-BL1 were significantly less preferred by *S. scolytus* than extracts from *U. minor* SG-CC1. The same result was obtained when only methanol extracts were compared. Similar responses were recorded when methanol + ether or methanol extracts from LE-BL1 were compared to those from *U. minor* MRV-122. Comparisons of the ether extracts (offered in discs treated with methanol extracts from MRV-122) showed that discs having received the *U. laevis* ether extracts were less preferred. Similarly, *S. scolytus* much preferred discs containing

Table 2. Interespecific comparisons among *Ulmus laevis* and *U. minor* on feeding responses by *Scolytus scolytus*

U. laevis* vs. *U. minor						
Tree			**Treatment**	**n**	**Feeding (%)**	
A	***vs***	**B**			**A**	**B**
LE-BL1		SG-CC1	M+E	4	0,0	21,6	*[c]
LE-BL1		SG-CC1	M	4	0,0	14,5	**
LE-BL1		MRV-122	M+E	5	0,9	25,4	***
LE-BL1		MRV-122	M	5	6,6	21,9	*
LE-BL1		MRV-122	E[a]	5	7,4	21,9	**
LE-BL1		TO-PB1	M+E	5	6,6	13,8	**
LE-BL1		TO-PB1	M+E	5	3,4	17,2	**
LE-BL1		TO-PB1	M	5	17,9	10,7	*
LE-BL1		TO-PB1	M	5	16,6	9,8	*
M-QM2		MRV-122	E[a]	5	8,9	31,0	***
M-QM2		SG-CC1	E[b]	5	2,9	25,8	***

[a] Methanol extracts from MRV-122 common to both treatments. [b] Methanol extracts from SG-CC1 common to both treatments. [c] Significance levels: $P < 0.10$ (*); $P < 0.05$ (**); $P < 0.01$ (***).

Table 3. Interespecific comparisons among *Ulmus glabra* and *U. minor* on feeding responses by *Scolytus scolytus*

U. glabra* vs. *U. minor						
Tree			**Treatment**	**n**	**Feeding (%)**	
A	***vs***	**B**			**A**	**B**
AV-IR2		MRV-122	M+E	5	0,0	7,4 ***[c]
M-RO3		MRV-122	M+E	5	4,5	14,6 **
AV-IR2		M-DV5	M+E	5	2,4	17,1 **
M-RO3		M-DV5	M+E	5	3,5	18,8 *
AV-IR1		M-DV5	M+E	5	0,3	13,6 ***
AV-CA2		M-DV5	M+E	5	1,3	25,2 **
AV-CA2		MRV-122	M+E	5	0,0	9,6 **
AV-CA2		MRV-122	M	5	0,0	16,8 ***
AV-CA2		MRV-122	E[a]	5	2,4	13,8 **
AV-CA3		M-DV5	M+E	5	2,6	15,2 *
AV-CA3		M-DV5	E[b]	5	2,4	20,6 *
AV-CA5		M-DV5	M+E	5	0,0	14,7 *
AV-CA5		M-DV5	E[b]	5	2,7	13,4 **

[a] Methanol extracts from MRV-122 common to both treatments. [b] Methanol extracts from M-DV5 common to both treatments. [c] Significance levels: $P < 0.10$ (*); $P < 0.05$ (**); $P < 0.01$ (***).

U. minor MRV-122 ether extracts than those containing *U. laevis* M-QM2 extracts. When *U. minor* TO-PB1 was tested against LE-BL1, results were partially different. Again, with complete (M+E) extracts, significantly greater feeding was recorded from this *U. minor* clone than from *U. laevis*. Since the differences seemed not as high as with the other trees tested, the experiment was repeated yielding a similar result. Surprisingly, when only the methanol extracts from both trees were compared, in a replicated test, the opposite response was observed, being *U laevis* LE-BL1 significantly more preferred than *U. minor* TO-PB1.

U. glabra vs. *U. minor*. Comparisons were made between extracts from six *U. glabra* trees and from two *U. minor* trees (Table 3). *S. scolytus* much preferred to fed on discs with methanol + ether extracts from *U. minor* MRV-122 than on those from *U. glabra* AV-IR2, M-RO3 or AV-CA2. Similar response was observed when complete extracts from a second *U. minor* tree, M-DV5, were compared to those from the trees before and also from *U. glabra* AV-IR1, AV-CA3 and AV-CA5 trees (Table 3). A significant greater feeding on extracts from *U. minor* MRV-122 than on those from *U. glabra* AV-CA2 was recorded when only the methanol extracts were compared (Table 3). Comparisons between the ether extracts from both species were tested on discs previously treated with the methanol extracts from the *U. minor* tree. Again, *S. scolytus* chose better extracts from *U. minor* MRV-122 than those from *U.glabra* AV-CA2, and the same result was obtained when tree M-DV5 was compared to AV-CA3 and to AV-CA5 (Table 3).

U. laevis vs. *U. pumila*. Direct comparison of methanol + ether or methanol alone extracts between clones *U. laevis* LE-BL1 and *U. pumila* CA-AL3 showed a significant preference for *U. pumila* in both cases (Table 4).

U. pumila vs. *U. minor*. Comparisons between *U. pumila* clone CA-AL3 and *U. minor* clones SG-CC1 and MRV-122, whether testing methanol extracts or mixed methanol plus ether extracts, showed no differences in feeding (Table 4).

U. minor vs. Dutch Hybrids. The ether extracts from *U. minor* MRV-122 were compared to those from Dutch hybrids H-826 and H-454, extracts of each being added to a common treatment of methanol extracts from tree MRV-122, and no differences in feeding were observed in any case. The same results were obtained in a similar test between *U. minor* SG-CC1 and H-826. Also, no differences in response were found between the ether extracts from hybrids H-826 and H-1020 (Table 4).

U. laevis vs. Dutch Hybrid. Ether extracts from *U. laevis* M-QM2 were compared to those from Dutch hybrid H-826, tested with these extracts added to a common treatment of methanol extracts from MRV-122, as above. Beetles clearly preferred the discs con-

Table 4. Interespecific comparisons among different elm species and hybrids on feeding responses by *Scolytus scolytus*

Other interspecific comparisons						
Tree		Treatment	n	Feeding (%)		
A	*vs* B			A	B	
U. laevis	*U. pumila*					
LE-BL1	CA-AL3	M+E	4	2,4	16,1	*[c]
LE-BL1	CA-AL3	M	4	0,0	15,0	***
U. pumila	*U. minor*					
CA-AL3	SG-CC1	M+E	4	7,1	1,9	N.S.
CA-AL3	SG-CC1	M	4	10,7	7,5	N.S.
CA-AL3	MRV-122	M+E	5	7,4	8,7	N.S.
U. minor	Hybrids					
MRV-122	H-826	E[a]	5	20,3	18,9	N.S.
MRV-122	H-454	E[a]	5	17,9	13,6	N.S.
SG-CC1	H-826	E[b]	5	12,7	15,7	N.S.
U. laevis	Hybrid					
M-QM2	H-826	E[a]	5	8,5	31,7	**
U. laevis	*U. laevis*					
M-QM2	LE-BL1	E[a]	5	9,0	12,1	N.S.
Hybrid	Hybrid					
H-1020	H-826	E[a]	5	27,2	23,24	N.S.

[a] Methanol extracts from MRV-122 common to both treatments. [b] Methanol extracts from SG-CC1 common to both treatments. [c] Significance levels: non significant (N.S.); $P < 0.10$ (*); $P < 0.05$ (**); $P < 0.01$ (***).

taining the ether extracts from the hybrid than those from *U. laevis* (Table 4). Low feeding and no differences in response were found when ether extracts from both *U. laevis* clones, LE-BL1 and M-QM2, were compared to each other in a similar manner (Table 4).

Ulmus minor intraspecific choices

Clone TO-PB1 vs. others. As comparatively more feeding was received in the methanol extracts from *U. laevis* clone LE-BL1 than from *U. minor* clone TO-PB1, a series of choices between extracts from this clone and from other *U. minor* trees were carried out to test the potential lesser suitability of clone TO-PB1 to *S. scolytus*. Repeatedly, TO-PB1 extracts, whether (methanol + ether) or methanol alone, appeared to be significantly less attractive to *S. scolytus* when offered paired with extracts from MRV-122, SG-CC1, MRV-104 and V-JR1 (Table 5). These results suggest intraspecific differences in *U. minor* related to feeding suitability or acceptability to elm bark beetles.

Clone V-JR1 vs. Clone SG-CC1. Methanol extracts from both clones did not differ in response, but when the ether extracts were added, feeding was significantly higher in SG-CC1 than in V-JR1 (Table 5), pointing out to possible presence of additional attractive compounds in the former.

Tree MRV-308 vs. tree MRV-221 (dying). When ether extracts from the stem bark of a dying tree, MRV-221, were tested vs. those from the twigs of the healthy elm MRV-308, *S. scolytus* greatly preferred to feed on the former than on the latter. The same result was obtained when the experiment was repeated (Table 5). Thus, it appears that chemicals present in the ether extracts are also involved in *U. minor* intraspecific differences on beetle response. However, these suspected individual differences in bark constituents might be also associated to phenological changes, as samples

Table 5. Intraspecific comparisons among *Ulmus minor* trees on feeding responses by *Scolytus scolytus*

U. minor* vs *U. minor						
Tree			**Treatment**	**n**	**Feeding (%)**	
A	***vs***	**B**			**A**	**B**	
TO-PB1		MRV-122	M+E	5	3,5	9,8	**[b]
TO-PB1		MRV-122	M+E	5	4,	29,0	***
TO-PB1		MRV-122	M	5	12,2	34,0	***
TO-PB1		SG-CC1	M+E	5	5,4	20,5	***
TO-PB1		SG-CC1	M	5	12,1	37,5	***
TO-PB1		MRV-104	M+E	5	3,2	10,2	*
TO-PB1		MRV-104	M	5	8,6	17,3	*
TO-PB1		V-JR1	M+E	5	1,9	8,7	**
TO-PB1		V-JR1	M	5	11,9	20,8	**
V-JR1		SG-CC1	M+E	5	6,6	17,1	**
V-JR1		SG-CC1	M	5	13,8	11,5	N.S.
MRV-221		MRV-308	E[a]	5	17,1	1,8	***
MRV-221		MRV-308	E[a]	5	24,8	2,4	***

[a] Methanol extracts from MRV-308 common to both treatments. [b] Significance levels: non significant (N.S.); $P < 0.10$ (*); $P < 0.05$ (**); $P < 0.01$ (***).

from the dying tree were harvested 6 weeks later than those from the healthy tree.

Old *vs.* current-year twig choices

To find out if there could be a lesser preference of very young twigs by *S. scolytus*, several comparisons were made between extracts from 2 to 4-year-old twigs vs. current-year twigs collected in spring from the same trees. Results of these tests are presented in Table 6.

Tree MRV-175. When extracts from twigs of both ages sampled from *U. minor* tree MRV-175 were tested, no differences in preference were observed, neither for the complete (methanol + ether) choice, that was repeated twice, nor for the methanol extracts alone (Table 6).

Tree MRV-172 and clone M-DV1. In two other tests, ether extracts from twigs of both ages from tree MRV-172 and clone M-DV1 were assayed. Direct comparison of the these extracts was not possible and they were compared following a common treatment of methanol extracts from the old twigs. Again, no significant feeding differences were found between treatments for any of both trees (Table 6).

Tree MRV-295. Surprisingly, when the responses to (methanol + ether) extracts from tree MRV-295 were tested in two trials, a highly significant preference for older twig extracts was observed. Comparisons of the methanol extracts alone showed also a clear preference for the old twig extracts (Table 6). In the light of these results, it was decided to compare also the responses to the ether extracts, both added to a common treatment of methanol old-twig extracts. Again, fee-

Table 6. Comparisons among 2 to 4-years old and current-year twigs on feeding responses by *Scolytus scolytus*

2-4 years vs. current year					
Tree	**Treatment**	**n**	**Feeding (%)**		
			2-4 years	**Current**	
MRV-175	M+E	4	18,53	6,43	N.S.[d]
MRV-175	M+E	5	22,82	21,73	N.S.
MRV-175	M	4	8,96	6,22	N.S.
MRV-172	Ea	5	23,41	18,88	N.S.
M-DV1	Ea	5	12,49	9,75	N.S.
MRV-295	M+E	5	40,92	2,47	**
MRV-295	M+E	7	31,69	11,16	*
MRV-295	M	5	44,11	16,96	**
MRV-295	E[a]	5	30,67	13,53	**
MRV-295	S vs E[b]	5	41,07	24,47	N.S.
MRV-295	S vs 2E[c]	5	22,90	29,20	N.S.

[a] Methanol from old twig extracts common to both treatments. [b] S vs E: solvent vs. current year ether extracts; complete (M+E) extracts from old twigs common. [c] S vs 2E: solvent vs. double current year ether extracts; same as above. [d] Significance levels: non significant (N.S.); $P < 0.10$ (*); $P < 0.05$ (**); $P < 0.01$ (***).

ding was significantly lower in the discs with the extracts of the current-year twigs. To test if such result could be attributed to whether the lacking of enough feeding stimulants or contrarily to the presence of deterrent compounds, ether extracts from current-year twigs were applied to discs already treated with complete (methanol + ether) extracts from old-twigs and compared with discs having received only the latter treatment. The feeding response was similar in both treatments. The same result was obtained when the experiment was repeated doubling the dosage of the current-year ether extracts, thus suggesting that a deterrent effect in these extracts was unlikely (Table 6).

Discussion

Results from the interspecific tests clearly showed that extracts from *U. laevis* and from *U. glabra* were less preferred for feeding by *S. scolytus* than those from *U. minor*, confirming earlier results with plants in enclosures (Sachetti *et al*., 1990; Webber and Kirby, 1983; Webber, 2000; Piou, 2002). It is demonstrated for the first time that this lesser attractiveness of *U. laevis* and of *U. glabra* is likely due to the chemical constituents of the bark. In this sense, it seems that compounds occurring in both the methanol and the ether extracts are involved in inducing a lower feeding response by the elm bark beetles. These differences found are likely attributable to specific differences in the chemical composition of the bark among *U. minor*, *U. laevis* and *U. glabra*. However, caution is necessary, since most comparisons were made between extracts from trees of different sites and of different age and they may reflect site and/or age effects, so obtained results may apply only to these specific sites. Only in the interespecific comparisons between trees from the elm clonal bank these effects can be dismissed, and in these, both *U. laevis* clones resulted less preferred than the two *U. minor* clones tested and than one *U. pumila* and one Dutch hybrid clones. This lesser preference of *U. laevis* and of *U. glabra* by elm bark beetles could have practical implications, as both species might be used as standard of comparison in screening for less attractive *U. minor* elm trees. In one of the tests, *S. scolytus* chose better methanol extracts from *U. laevis* LE-BL1 than from *U. minor* TO-PB1, but when the ether extracts were added, the preference was somewhat reversed, pointing out to that chemicals in the ether extracts, whether stimulants or deterrents, were involved in the reversal of response and that clone TO-PB1 could be less attractive for feeding than other *U. minor* trees.

Interspecific choice tests between *U. minor* and *U. pumila* indicated that there were no differences, and both species seemed of comparable acceptability to *S. scolytus* for feeding; however, only one Siberian elm clone was assayed and comparisons with more clones would be required. Similarly, responses to *U. minor* and to some Dutch hybrids were not different, at least when the ether extracts were compared.

Differences in beetle feeding response to bark extracts from *U. minor* trees were also observed. Thus, methanol extracts from one of the trees, TO-PB1, was consistently less preferred by the beetles when they were compared with extracts from other four trees of the same species. These results suggest intraspecific differences in *U. minor* related to feeding suitability or acceptability to elm bark beetles and that chemical composition of the bark (i.e. the methanol extracts) is involved in these differences. Here, the particular influence of compounds contained in the ether extracts was not examined. It is not known whether such differences would be maintained if not just extracts but whole plants or trees were confronted, but this possibility deserves to be further explored. As already mentioned, in two of these comparisons trees were of quite different age and from different sites, so the possibility that differences were associated to these factors must be taken in account.

Elm bark beetles usually feed in the crotches of 2-year-old and older twigs or at the junctions of old and new twigs. Norris and Baker (1967) found in their bioassays that *S. multistriatus* did not feed on elderberry pith discs treated with elm bark extracts from current-year twigs collected at the beginning of the growing season, but they did when these twigs were aged in the season. Our comparisons between extracts from old and current twigs indicate that *S. scolytus* might prefer the former to the latter, related to the chemicals in the bark, similar to that suggested for *S. multistriatus*. However, this seems not to be a common situation, since such a preference was observed only in one tree out of four tested. In this case, compounds in both the methanol and the ether extracts appeared responsible for the observed differences; the lesser preference obtained by the ether extracts from the current growth seemed more related to a lower level of stimulants than to the presence of deterrents.

The results presented here, though preliminary, are quite promising. The observed differences in feeding

preference of elm bark beetles for some elm species or for some individuals within the same species, due to chemicals in the bark of twigs, were shown for the first time. So far, the study has been limited to testing extracts, but a comparative analysis of feeding responses to these extracts and of their chemical composition (see Martín *et al.*, this volume) would lead to the identification of bioactive compounds, whether inducing or deterring beetle feeding, as a first step in the selection elm trees that could escape the disease.

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