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Feeding preference of *Tomicus destruens* progeny adults on shoots of five pine species

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Abstract

This paper presents the results of a two-year investigation on the preferences of *Tomicus destruens* (Wollaston) (Coleoptera Curculionidae) progeny adults for shoots of different pine species in three distinct trials. In the first one, cut shoots of four pine species (*Pinus pinaster* Aiton, *P. pinea* L., *P. halepensis* Mill. and *P. nigra* Arnold) were offered in cages to progeny adults that emerged from logs from two coastal pine forests (Tuscany and Basilicata), and one hilly pine forest (Tuscany). In the second trial, progeny adults from the Tuscan coastal pine forest were allowed to emerge in a mixed young plantation of *P. pinea*, *P. nigra*, *P. pinaster* and *P. radiata* D. Don. In the third trial, using cages, progeny adults that had emerged from logs taken from the Tuscan coastal pine forest were offered four-year-old potted trees of *P. nigra* and *P. halepensis*. In the first trial, progeny adults from the coastal pine forests preferred the pine species on which the larvae had developed, i.e., *P. pinea* and *P. halepensis*, while adults from the hilly pine forest favoured *P. nigra*. In the second one, this same trend occurred with progeny adults shoot-feeding heavily on both *P. pinea* (the pine species from which they emerged) and *P. nigra*. In the last trial with potted trees, no significant preferences were noted. In addition, in the tests with cut shoots, a high number of empty tunnels were recorded, particularly on *P. halepensis*.

Key words: *Pinus*, progeny adults, bark beetle, maturation feeding, Scolytinae.

Introduction

Tomicus destruens (Wollaston) (Coleoptera Curculionidae Scolytinae) is a common bark beetle along the coastal areas of the Mediterranean Sea and the Atlantic Ocean of Portugal, Spain, France and Morocco. In Italy damage caused by this bark beetle has been recorded in the coastal pine forests of northern Adriatic, central and southern regions (Masutti, 1969; Triggiani and Santini, 1989; Nanni and Tiberi, 1997; Sabbatini Peverieri *et al.*, 2008). In more recent years, *T. destruens* has also been recorded in many pine forests in the hilly internal areas of Italy (Roversi *et al.*, 2004; Faccoli *et al.*, 2005). Despite the damage caused by *T. destruens*, little is known about the shoot-feeding selection behaviour of progeny adults.

The damage done to shoots negatively impacts the physiological state of the pine tree as shown in studies on *Tomicus piniperda* (L.) and *Tomicus minor* (Hartig) in both European and non-European countries. As a matter of fact, shoot feeding can reduce tree growth, by 20-45% (Michalski and Witkowski, 1962; Andersson, 1974; Nilsson, 1974), and up to 50%, as observed in North American pine forests (Czokajlo *et al.*, 1997).

In regards to selection of host trees for maturation feeding, in the case of *T. piniperda* and *T. minor* progeny adults it is clear that they feed primarily on those pine trees closest to where they had developed as larvae (Sauvard *et al.*, 1987; Långström and Hellqvist, 1990; Borkowski, 2001). The results of studies carried out by Siegert and McCullough (2001) in laboratory trials with current-year pine shoots show that in the selection of shoots one important factor is their diameter. As a matter of fact, these authors observed that the highest rates of *T. piniperda* attack occurred on shoots whose diameter ranged from 4 to 5 mm. The influence of shoot thickness had already been pointed out by Långström (1980) for *T. piniperda* and *T. minor* progeny adults when feeding on *Pinus sylvestris* L. (Scots pine). This author also observed that shoot attacks by these two *Tomicus* species were higher in the upper whorls of the crown where there was a higher number of suitable shoots. This preference was again confirmed by Kauffman *et al.* (1998) and Haack *et al.* (2001) in North America.

There are few studies, however, on the preference of *Tomicus* progeny adults for shoots of different pine species. The most interesting experiments on this topic were the ones carried out in North America by Siegert and McCullough (2001), who, in cage studies, offered *T. piniperda* progeny adults shoots of different pine species, and by Eager *et al.* (2004), who tested *T. piniperda* progeny adults on shoots of potted trees of different pine species. With regards to studies in Europe, one should mention the work of Fernández Fernández *et al.* (1999) in Spanish pine forests (*P. sylvestris* and *Pinus nigra* Arnold - Austrian pine) infested by *T. minor*. What emerges from these experiments is that the progeny adults of the two *Tomicus* species appear to demonstrate a definite selection process which results in some pine species being more frequently attacked compared to others. Since there were no specific studies regarding the preference of *T. destruens* progeny adults for pine hosts in their native range we considered this aspect of their ecology worth exploring, using trials in both confined environments and in a young pine plantation.

Materials and methods

In 2004 - 2005, we carried out three series of investigations on shoot feeding behaviour of *T. destruens* in Italy. In the first trial, in a confined environment, *T. destruens* progeny adults were offered cut pine shoots. The second trial was carried out in a young mixed pine plantation where progeny adults were allowed to emerge and select hosts for feeding. In the third trial, again in a confined environment, progeny adults were offered small potted pine trees for shoot feeding.

Trial 1. Cut pine shoots

In this trial, we collected *T. destruens* in three pine forests in Italy. The first one, a 30-40-year old *Pinus pinea* L. (stone pine) forest, is on the Tyrrhenian coast near Alberese (Grosseto, Tuscany) (11°2'40"E, 42°39'39"N). The second, a 30-50-year *Pinus halepensis* Mill. (Aleppo pine) forest, is situated on the Ionian coast at Ginosa Marina (Matera, Basilicata) (16°45'27"E, 40°34'41"N). The third, an approximately 50-year-old *Pinus pinaster* Aiton (maritime pine) forest, is a hilly pine forest situated at Poggio Valicaia (Florence, Tuscany) at an altitude of about 350 m a.s.l. (11°10'27"E, 43°43'4'"N).

The methodology used was similar to that described by Siegert and McCullough (2001) in North America. Aluminium framed cages with aluminium screening were used (50 x 50 x 100 cm), which were kept outdoors, under a roof, at Poggio Valicaia. Within each cage, two pine logs were placed, each around 15 cm in diameter and 70 cm in length (which contained *T. destruens* pupae and teneral adults about to emerge), coming from one of the three pine forests chosen for the trial. Therefore the logs were of *P. pinea*, *P. halepensis*, and *P. pinaster*, according with the forest of origin (Alberese, Ginosa Marina and Poggio Valicaia, respectively). In the cages a number of one-year old shoots were placed, which were collected from the upper-middle part of the crowns of four different pine species (*P. halepensis*, *P. pinea*, *P. pinaster*, and *P. nigra*) aged 30-35 years, in the surroundings of Poggio Valicaia. *P. pinea, P. pinaster* and *P. halepensis* are typical Mediterranean species, while *P. nigra* is a mountain species. *P. pinea, P. pinaster* and *P. halepensis* have similar shoot phenology, as well. As regards *P. nigra,* when it grows at low altitudes, as in our study area, its shoot phenology is similar to that of the previous species All four pine species have two needles per fascile. *P. nigra* needles are 8-14 cm long, 5-10 cm those of *P. halepensis*, 8-20 cm those of *P. pinea* and 12-20 cm those of *P. pinaster*. The shoots, with a 4-6 mm diameter, were collected and then immediately placed into glass containers filled with water and offered to the progeny adults for the maturation feeding. A number of 15 shoots per pine species were placed in each cage at the beginning of each test and after three days they were replaced with others of the same species. In consideration of the high number of emerged adults coming from Alberese and Ginosa Marina, the number of replaced shoots was 51 (for a total of 66 shoots) and 31 (for a total of 46), respectively for each pine species, whereas in the tests with adults from Poggio Valicaia the number of replaced shoots was 15 for a total of 30 shoots per pine species per cage during the whole trial. The four pine species were alternated in a series of tests so as to try out all possible combinations (6 for each origin of the adults). The 6 tests for each origin of the adults were carried out at the same time and lasted six days, only one replicate per combination was made.

Progeny adults from Alberese were tested in March 2004, those from Ginosa Marina between the end of March and the beginning of April 2004 and finally, tests with *T. destruens* individuals from Poggio Valicaia were carried out in April 2004. Obviously, this time shift in the tests was due to the difference in the emergence pattern of the bark beetle according to the geographical location of the pine forest of origin.

Removed shoots were immediately and carefully examined, the number of those attacked for each pine species and the number of tunnels were counted, making a distinction between those which contained adults and the empty ones. The latter were not taken into consideration in the statistical analysis following the indications of Siegert and McCullough (2001), and also because cut shoot vitality decreases more rapidly in comparison with non-cut shoots and consequently adults may leave them more rapidly. Moreover, the *T. destruens* adults which emerged in the cages were taken and counted, making a distinction between the live and the dead ones.

Trial 2. Young pine plantation

The trial was carried out in 2005 in a mixed pine plantation at Montepaldi, near Florence, consisting of 120 5-year-old trees of similar height (1.6-1.8 m), of which there were 30 *P. nigra*, 30 *P. pinaster*, 30 *P. pinea* and 30 *P. radiata* D. Don (Monterey pine). The trees, planted at 2.5 x 2.5 m spacing, were randomly distributed according to a block pattern.

On 14 May 2005, 60 logs of *P. pinea* (15 cm diameter, 70 cm length, and infested with pupae and teneral adults of *T. destruens*) were taken from Alberese to the young pine plantation of Montepaldi. These logs were arranged in pairs (in all 30 groups) and homogeneously placed in the plantation. We checked for attacked shoots on a weekly basis, from 22 May to 3 July. During each check, we counted the number of attacked shoots by tree species as well as the number of entrance holes per shoot in both the one-year-old and current-year growth separately. We also estimated the number of progeny adults that emerged in the plantation based on the number of emergence holes in the logs. Overall, we counted 13123 shoots in the plantation, counting both one-year-old and current-year shoots separately. Of these, 4680 shoots were on *P. nigra*, 5208 on *P. pinea*, 1707 on *P. pinaster*, and 1528 on *P. radiata*.

Trial 3. Small potted pine trees

In spring 2005, between 19 May and 16 June, another trial was carried out at Cascine del Riccio (Florence, Tuscany). A number of 5 logs of *P. pinea* from the Alberese forest (1 m in length, 15-20 cm in diameter, which contained pupae and teneral adults of *T. destruens*) were placed at one end of a plastic screen cage (size: 1.8 x 2 x 4.5 m) located outdoors. At the other end

of the cage, four potted *P. nigra* and four potted *P. halepensis* (four years of age, about 1.3 m in height) were placed. In order to provide the progeny adults with a constant supply of vigorous pines, we replaced each tree as soon as the percentage of attacked shoots was over 50% of the total. We inspected the trees every seven days, and recorded the number of attacked pine shoots and the number of entrance holes. Two replicates were carried out at the same time.

The trial lasted 29 days, during this period, the progeny adults were offered 24 trees of *P. nigra* and 24 of *P. halepensis*, for a total of 1260 previous and current year-shoots, of which 543 were on *P. nigra* and 717 on *P. halepensis*.

Statistical Analysis

The data (number of attacked shoots relative to available ones) from trials 1 and 3 were analysed by means of the χ^2 test, while those (number of attacked shoots per tree) from trial 2 were processed by analysis of variance (ANOVA). Multiple comparison Tukey test was used to determine significant differences among the means. We used an alpha level of 0.05 in all analyses.

Results

Trial 1. Cut pine shoots

During this trial a total of 825 *T. destruens* adults emerged from the pine logs, 539 of which were found alive: 361 from *P. pinea* (Alberese), 91 from *P. halepensis* (Ginosa Marina) and finally 87 from *P. pinaster* (Poggio Valicaia). The highest percentage of dead adults (66.2%) was recorded in the tests with Alberese individuals while mortality rates in the tests with Ginosa Marina and Poggio Valicaia individuals were lower (20.9% and 32.2% respectively).

About 69% of the emerged adults coming from Ginosa Marina and from Poggio Valicaia were found inside shoots, while in the case of those from Alberese they were only 24% of the total. However, considering the available shoots (independently to the pine species), the percentages of those attacked by progeny adults from all three pine forests were rather modest: 24.6%, 12.8% and 20.4% as regards Alberese, Ginosa Marina and Poggio Valicaia, respectively.

Multiple attacks (more than one entrance hole per shoot) were sometimes observed and their incidence was higher on *P. pinea* and *P. nigra* (1.26 holes on average per attacked shoot) than on *P. halepensis* and *P. pinaster* (1.17 and 1.14 holes on average per attacked shoot, respectively). In any case the percentage of shoots with more than one hole was limited (14.71%).

Overall we recorded 380 tunnels in the attacked shoots, of which 79 were empty (i.e. they lacked adults): 62 in the tests with Alberese adults, 5 with Ginosa Marina adults, and 12 with Poggio Valicaia adults. As already mentioned in the statistical analysis they were excluded; in any case, even adding them the results are not modified.

From the statistical analysis it emerged that adults from Alberese preferred *P. halepensis* ($\chi^2 = 13.44$, df = 1, $p < 0.001$) and *P. pinea* ($\chi^2 = 28.17$, df = 1, $p < 0.01$) shoots to those of *P. pinaster*.

Adults from Ginosa Marina showed a significant preference for *P. halepensis* shoots compared not only to those of *P. pinaster* ($\chi^2 = 11.27$, df =1, $p < 0.01$) but even to those of *P. pinea* ($\chi^2 = 9.00$, df = 1, $p < 0.01$). The adults from Poggio Valicaia, instead, preferred *P. nigra* shoots to those of *P. halepensis* ($\chi^2 = 11.64$, df = 1, $p < 0.01$) (figure 1).

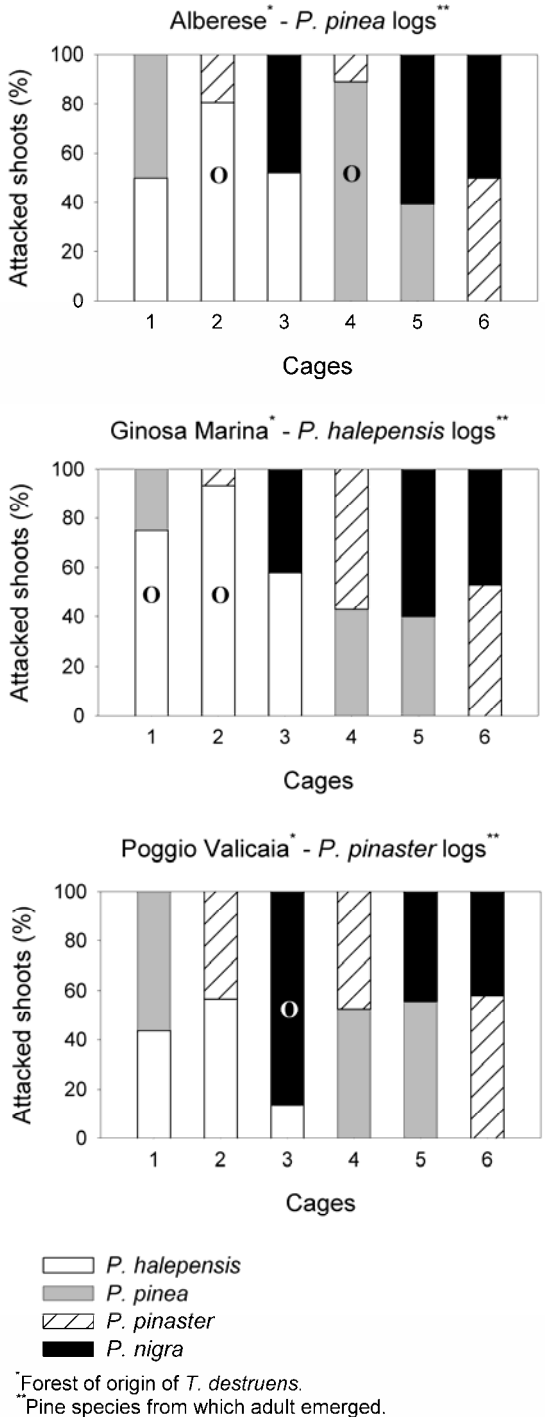

Figure 1. Percentage of attacked shoots of each pine species relative to the total attacked shoots in each cage recorded in the trial with cut shoots (trial 1). Circle symbols indicate statistical differences (χ^2 test, $p < 0.01$).

Trial 2. Young pine plantation

Attacked shoots were first observed on 22 May, one week after logs were placed in the plantation. In later checks, in relation to the increase in the emergence of adults, the percentage of attacked shoots also increased.

Adult tunnels were found more frequently in one-year shoots than in those of the current year; as a matter of fact the percentages of attacked one-year shoots of *P. nigra*, *P. pinaster* and *P. radiata* were over 78% of the total attacked shoots of each species, while regarding *P. pinea* they were slightly lower, about 64% (table 1).

We recorded a limited number of multiple attacks: 1.09 and 1.02 holes on average per attacked shoot on *P. nigra* and on *P. pinea* respectively, while on *P. pinaster* and *P. radiata* no multiple attacks were observed.

Attacked *P. nigra* and *P. pinea* shoots were homogeneously distributed on the trees belonging to these species (approximately 90% of the trees for both species), while low percentages of *P. radiata* and *P. pinaster* trees were attacked by progeny adults (26.7% and 20.0%, respectively) (table 2). However, despite the high number of emerged adults (an average of 270 ± 29 per group of logs for a total of 8089 in the whole trial) also in the case of *P. nigra* and *P. pinea* the percentages of attacked shoots relative to the available shoots were rather low (2.8% and 1.6%, respectively), even if they were higher than those of the other two species (table 2).

The four available pine species in the plantation had significantly different infestation levels ($F = 13.39$, $df = 3$, $p < 0.001$). As a matter of fact *T. destruens* progeny adults preferred shoots of both *P. nigra* and *P. pinea* (4.6 and 3.9 attacked shoots per tree, respectively), in comparison with those of *P. pinaster* and *P. radiata*, which had only 0.5 and 0.6 attacked shoots per tree, respectively (table 2).

Table 1. Percentages of attacked one-year and current-year shoots relative to the total attacked shoots for each pine species recorded in the trial carried out in the young pine plantation (trial 2).

Tested pine species	Attacked one-year shoots (%)	Attacked current-year shoots (%)
P. nigra	88.5	11.5
P. pinea	64.1	35.9
P. pinaster	82.3	17.7
P. radiata	78.6	21.4

Trial 3. Small potted pine trees

During this trial a total of 1090 (with an average number of 130 ± 15 per log) *T. destruens* adults emerged, attacked shoots were first observed on 30 May and up to the last check (16 June), when a small number of them was recorded.

Despite the high number of emerged adults in each cage (605 and 485) low percentages of attacked shoots (independently from the pine species) were recorded: 10.4% in one cage and 11.3% in the other one. However, in one cage shoot-feeding was observed on about 42% of the trees for both pine species, while in the other one both pine species had attacked shoots on 75% of the available trees (table 3). Recorded incidence of multiple attacks was higher on *P. nigra* (1.29 holes on average per attacked shoot) than on *P. halepensis* (1.17).

In both replicates percentages of *P. nigra* attacked shoots relative to available ones were slightly higher than those recorded on *P. halepensis*: 9.5% against 4.8% in the first replicate and 12.8% against 9.9% in the second one (table 3). However, statistical analysis did not show any significant difference.

Table 2. Infestation rates of each pine species in the young plantation (trial 2). Means followed by the same letter (within the column) are not significantly different at the p = 0.01 level (ANOVA).

Origin of *T. destruens*	Log species*	Tested pine species	Average No. of available shoots per adult	Attacked trees		Attacked shoots		Average No. of attacked shoots per tree ± SD
				n	%	n	%**	
Alberese	*P. pinea*	*P. nigra*	0.58	28	93.3	130	2.8	4.6 ± 4.2 A
"	"	*P. pinea*	0.64	27	90.0	81	1.6	3.9 ± 3.4 A
"	"	*P. pinaster*	0.21	6	20.0	12	0.7	0.5 ± 1.3 B
"	"	*P. radiata*	0.19	8	26.7	10	0.7	0.6 ± 1.5 B

* Pine species from which adults emerged; ** Percentages are relative to the available shoots of each species.

Table 3. Infestation rates of each pine species recorded in the trial with potted trees (trial 3) for each replicate.

Replicates	Origin of *T. destruens*	Log species*	Tested pine species	Available shoots	Average No. of available shoots per adult	Attacked trees		Attacked shoots	
						n	%	n	%**
1	Alberese	*P. pinea*	*P. nigra*	231	0.48	5	41.7	22	9.5
1	"	"	*P. halepensis*	354	0.73	5	41.7	17	4.8
2	"	"	*P. nigra*	312	0.52	9	75.0	40	12.8
2	"	"	*P. halepensis*	363	0.60	9	75.0	37	10.2

* Pine species from which adults emerged; ** Percentages are relative to the available shoots of each species.

Discussion and conclusions

The results of these studies suggest that *T. destruens* progeny adults select shoots for maturation feeding also according to pine species and that the host preference may be affected by the pine species on which they developed as larvae. As a matter of fact, in trial 1 adults from Ginosa Marina were observed shoot-feeding heavily on the pine species from which they emerged, i.e. *P. halepensis*. Regarding adults from Alberese, they preferred *P. pinea* (the species from which they emerged) and *P. halepensis* shoots in trial 1, and the preferred shoots were again those of *P. pinea* (as well as those of *P. nigra*) in trial 2. These results are in agreement with the investigations carried out in North America by Siegert and McCullough (2001), in which observed *T. piniperda* progeny adults, emerged from *Pinus resinosa* Aiton, were more likely to accept *P. resinosa* shoots than adults that developed in *P. sylvestris*. On the contrary, in our experiment, the adults from Poggio Valicaia (trial 1) showed a significant preference for *P. nigra*, which is quite sporadic in the study area. However, this pine species is doubtlessly one of the most common in the hilly and sub-mountainous areas of Tuscany.

Despite the high number of emerged adults the percentages of attacked shoots relative to the available ones were modest in all three trials: from a minimum of 4.8% to a maximum of 24.6% in the trials carried out in cages and, as expected, they were even lower in the young plantation where the adults could fly away (from 0.7% to 2.8%). However, in the latter case (trial 2) infestation levels on the preferred pine species, i.e. *P. nigra* and *P. pinea* (4.6 and 3.9 attacked shoots per tree, respectively) were similar to what other authors observed during natural infestations of *T. piniperda* in young *P. sylvestris* stands in both Canada (Ryall and Smith, 2000) and the Midwestern United States (Haack *et al.*, 2000): 5.3 and 4.6 attacked shoots per tree, respectively.

In all three trials, multiple attacks were observed: higher average values of holes per shoot were recorded in cage experiments and more precisely on *P. nigra* and *P. pinea* in trial 1 (1.26 for both pine species), and on *P. nigra* in trial 3 (1.29). Instead in the young plantation (trial 2), values were quite modest with the highest one, in this case, recorded on *P. nigra* (1.09). However, this latter value was similar to the one observed by Fernández Fernández *et al.* (1999) in a pine reforested area: 1.07 *T. minor* tunnels per *P. nigra* shoot. This behavioural aspect had already been pointed out by Långström (1980) on *P. sylvestris*, who recorded 1.4 *T. piniperda* attacks on average per damaged shoot.

Regarding the trial carried out with cut shoots (trial 1), the lower percentage (about 24%) of Alberese adults observed in the shoots, compared to those from the other two pine forests (both about 69%), could be related mainly to the high number of emerged adults from the Tuscan coastal pine forest. The number of shoots available for each adult was, consequently, modest and intraspecific competition could have occurred. This may also justify the highest mortality rate recorded in Alberese adults; as a matter of fact, Siegert and McCullough (2001) observed, in a similar experiment in North America, that percentages of *T. piniperda* dead individuals decreased by increasing the number of available shoots.

Again in trial 1, a number of shoots with empty tunnels was recorded, as already observed by Långström (1983) in trials carried out on *T. piniperda* and *T. minor*, both in confined environment and pine forest. The author suggested that the adults leave the tunnels because of decreased shoot vitality. In our experiment cut shoots were kept in cages for only three days; however, physiological modifications could have occurred all the same. Probably, empty tunnels were found almost exclusively in tests with Alberese adults because these tests took place earlier (March) than those carried out with adults from Ginosa Marina and Poggio Valicaia (end of March - April), and therefore in the first case tested shoots were in their early phases of phenological development.

Regarding the trial carried out in the young pine plantation (trial 2) the lower number of available shoots of *P. radiata* and *P. pinaster* trees, about 2/3 less than those of *P. nigra* and *P. pinea*, is due to the lower crown density of the former two pine species.

The modest percentages of attacked *P. radiata* and *P. pinaster* trees (table 2) are unlikely to be linked to their distance from the infested logs, in contrast with what other authors observed in pine forests in the case of *T. piniperda* (Sauvard *et al.,* 1987; Borkowski, 2001) and *T. minor* (Långström and Hellqvist, 1990). As a matter of fact, in our experiment the infested logs were homogeneously placed in the plantation. Also, the height of the pine trees could not have played a relevant role either, as it was similar for all four pine species. Anyway, it is worth noticing that among *P. radiata* and *P. pinaster* trees some were less vigorous, and therefore the progeny adults might have focused their shoot-feeding on the more appetizing specimens.

Regarding the behaviour of *T. destruens* adults from the Tuscan coastal pine forest, which also preferred *P. nigra* shoots, the features of the shoots themselves could have been relevant, as it was shown for *T. piniperda* in trials carried out in European and Non-European countries (Långström, 1983; Siegert and McCullough, 2001). Thus, the numerous attacks also on *P. nigra* shoots may be related to the size of these shoots themselves, which in this experiment was more suitable to the bark beetle's needs, compared to that of *P. pinaster* and *P. radiata*.

The results of the trial carried out with potted pine trees (trial 3) are in agreement with those of trial 1. More precisely, when *T. destruens* adults from Alberese were allowed to choose between *P. nigra* and *P. halepensis* shoots no significant preference emerged.

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