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Use of attractant and repellent substances to control *Tomicus destruens* (Coleoptera: Scolytidae) in *Pinus pinea* and *P. pinaster* pine forests of Tuscany[1]

ABSTRACT

We report the results of a two-year study conducted in maritime pine and stone pine forests in Tuscany to test the efficacy of various substances in attracting or repelling adults of *Tomicus destruens* in the reproductive stage, i.e. while seeking trees or parts of trees suitable for reproduction. The attractants tested were: Tomowit, (-)- and (+)-α-pinene, racemic α–pinene, Mt-mix, trans-verbenole "Destruens" and "Ipm". The repellents used were: Green leaf volatiles (Glv's, jans-2-hexen-1-ol), octanol (octanol-1-octanol), verbenone, alcohols C_6 and C_8, benzyl alcohol, (-)-β-pinene and racemic limonene. The most effective attractant was (-)-α–pinene, although bark-beetles were also caught with Tomowit and Ipm. As regards the repellents, logs protected with verbenone had the lowest level of attack, but this repellent lost its activity when combined with other substances.

Key words: terpenes, monitoring, protection, *Pinus* spp., *Scolytidae*.

INTRODUCTION

Various forest insects influence the fate of trees chronically weakened by biotic or abiotic stress factors. However, xylophagous insects are the most important in terms of survival of the host tree and, in the long run, the maintenance and functioning of the entire forest. This rather disturbing picture of ecological destabilisation affects various Italian forests, but studies carried out over various decades in several regions have shown that pine forests (particularly those along the coasts) are most at risk (MASUTTI, 1969; TRIGGIANI and SANTINI, 1989; MORIONDO *et al.*, 1995; BATTISTI and TIBERI, 1998; ROVERSI *et al.*, 2002). Among xylophagous insects associated with pine trees, those of the genus *Tomicus* Latreille undoubtedly play a highly significant role in the decay of Italian pine forests.

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[1] Work carried out with a contribution from Progetto Miur 40% 2001: "I coleotteri scolitidi del genere *Tomicus* nel quadro del deperimento delle pinete italiane".
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Infestations of these bark beetles are mainly controlled by forestry methods, which are difficult to apply because of their high costs and a lack of knowledge about the bio-ecology of these xylophagous insects. Thus, control measures are difficult to calibrate in terms of time and space, especially in view of the various species present and the progression of the infestations supported by them.

Therefore, it appears that the "*Tomicus*" problem must be tackled with new control methods. In this regard, the practical potential of interventions using attractants to monitor the adult insects (and thus the infestation breeding grounds) or to capture individuals during their reproductive stage must be assessed. At the same time, special attention should be given to the identification of substances able to prevent *Tomicus* adults from finding a suitable host for colonisation.

Many studies have attempted to identify the volatile substances (mostly monoterpenes) emitted by the host tree that play an essential role in orienting *Tomicus* toward it (CHARARAS, 1962). BYERS *et al.* (1985) identified α-pinene, terpinolene and (+)-3-carene as effective attractants of *T. piniperda* (L.). Other authors confirmed the attractive effect of these monoterpenes with respect to *T. piniperda*, using them in trials to capture adult insects (KLIMETZEK *et al.*, 1986; VITÉ *et al.*, 1986; VOLTZ, 1987; SCHROEDER, 1988; ZUMR, 1989; BYERS, 1992).

In parallel research, FRANKE and HEEMAN (1976) extracted various substances of natural origin from the hindgut of *T. piniperda* and identified them as terpenes and pheromones, including verbenone. On various occasions, verbenone has been found to be an effective repellent of *Ips typographus* (L.), *Dendroctonus ponderosae* (Hopkins) and *D. adjunctus* (Blandford) on bait logs or trees (BAKKE, 1981; LIVINGSTON *et al.*, 1983; RYKER and YANDEL, 1983). In recent decades, several studies have tested the repellent effect of verbenone against *T. piniperda* adults in the reproductive stage (BYERS *et al.*, 1989; KOHNLE *et al.*, 1992; MCCULLOUGH *et al.*, 1998). In parallel research, other authors have tested volatile substances, e.g. the alcohols C_6 and C_8, extracted from the leaves or bark of trees belonging to the genera *Betula*, *Populus*, *Acer* and *Eucalyptus* (GUERRERO *et al.*, 1997; POLAND and HAACK, 2000; SCHLYTER *et al.*, 2000).

The presence or absence of specific pheromones of aggregation or disaggregation in bark-beetles belonging to the genus *Tomicus* is still actively debated. SCHÖNHERR (1972) and CARLE (1974) concluded that secondary attraction occurs in *T. piniperda* and *T. destruens* (Woll.), respectively. In contrast, BYERS *et al.* (1985) and VITÉ *et al.* (1986) concluded that pheromones play only a minor role in processes of colonisation of host trees by these two bark-beetles.

Although research aimed at identifying effective means to control *T. piniperda* has been going on for decades, very little has been done with regard to *T. destruens*.

The aim of the present study, carried out in coastal and hill environments of Tuscany, was to establish monitoring techniques for *T. destruens* using attractant

substances placed in traps or on bait logs to increase their attractiveness. Other trials tested the efficacy of repellent substances used to hinder the bark-beetle's ability to identify and colonise trees in which it usually reproduces.

MATERIALS AND METHODS

Tests of the efficacy of attractant and repellent substances on *T. destruens* adults in the reproductive stage were carried out in 2002-2003 in two pine forests in Tuscany. The first forest, consisting of stone pine trees aged 30-40 years, is located on the Tyrrhenian coast near Marina di Alberese (Grosseto) (E 11°2'40", N 42°39'39"); the second, consisting of maritime pine trees aged about 50 years, is located in the Municipal Park of Poggio Valicaia (Florence), at an altitude of 350 m (E 11°10'27", N 43°43'4").

The use of traps to capture adult insects

The studies were carried out during 2002, at Poggio Valicaia from February to May and at Alberese from February to April. The attractive substance "Tomowit", traded by Witasek as an attractant for *T. piniperda*, was tested in two specially chosen areas, one in the pine forest of Poggio Valicaia (Val.-1) and the other in that of Alberese (Alb.-1). In total, 8 funnel-shaped traps (4 Witasek and 4 Pherotech with 8 elements) were used. In both forests, the traps were set 60 m from a group of pines heavily colonised by the bark-beetle and spaced at least 30 m from each other. The traps were baited with Tomowit, except for two used as controls (one per trap model). The traps were checked every week and were rotated in a clockwise direction at the various installation points, so as to avoid a possible position effect.

In a second area of the Poggio Valicaia pine forest (Val.-2), some attractant substances provided by the University of Lund (Sweden) were also tested. These substances were: racemic α-pinene, a mixture of monoterpenes called "Mt-mix", and trans-verbenol. The combined action of the latter two substances was also tested. Five Pherotech traps were used in the trial; they were checked every week and the same operations described above for the other traps were performed.

In the same study area (Val.-2), tests with Tomowit alone were carried out in October-November 2002 and again in February-April 2003.

In another specially chosen area of the Alberese forest (Alb.-2), 8 Pherotech traps and 8 Theysohn traps were used in October-November 2002 to test the following substances (2 traps per model for each substance): (-)-α-pinene (release rate: 90mg in 24h at +21°C), Tomowit, and a specific experimental attractant for *T. destruens* called "Destruens" (Serbios, Italia). The other 4 traps were used as controls (2 for each model). The traps were set 80 m from a group of pine trees that had been attacked in

the spring of the same year and were spaced 30 m from each other. The traps were checked every two weeks and were rotated at each inspection.

In the same area of the Alberese forest (Alb.-2), the following substances were tested from January to April 2003, in addition to the products tested in the preceding trials (Tomowit, (-)-α-pinene, "Destruens"): (+)-α-pinene (release rate: 80mg in 24h at +21°C) and another experimental attractant for *T. piniperda*, called "Ipm" (Ipm-tech, U.S.A.). In this trial, 18 Pherotech traps were used, with two replications.

During the same period, another trap model known as Intercept-PTBB was used, along with the Pherotech and Witasek traps, in a third area of the Alberese forest (Alb.-3), with a total of 18 traps (6 per model). The attractants Tomowit and Ipm were used in this trial, with two controls for each type of trap; the traps were inspected every two weeks.

During the course of each test, the dispensers containing Tomowit, Destruens and Ipm were replaced every 30 days for the entire flight period of the bark-beetle, while dispensers containing α–pinene were replaced every 15 days.

Tests with repellent substances

Bait logs (diameter 10-15 cm, length 80-100 cm) were used to study the effect of repellent substances on *T. destruens* adults in the reproductive stage; the logs were obtained by felling vigorous maritime pine trees at Poggio Valicaia and stone pines at Alberese. During the tests, groups of 3 logs were prepared for each substance. The tests were carried out in the same areas used for the tests of attractants.

During 2002, the studies were carried out in area Val.-1 from February to May and in Alb.-1 from March to April. The following substances were used in both cases: Green leaf volatiles (Glv's, jans-2-hexen-1-ol), octanol (octanol-1-octanol) and verbenone. In addition, alcohols C_6, C_8 and benzyl alcohol (provided by the University of Lund) were tested in area Val.-2 in early 2002. In both Val.-1 and Alb.-1, with the exception of the group of control logs and another group baited with Tomowit, all the logs were fitted with two dispensers: one for the repellent substance to be tested and another containing Tomowit. In the trials carried out in the other Poggio Valicaia area (Val.-2), the attractant mixture known as Mt-mix was used in addition to the alcohols. We decided to use dispensers with attractants in order to render the influence of the logs more homogeneous with respect to bark-beetle adults. In all three areas, the various groups of logs were placed 30 m from the pine trees attacked by *Tomicus* and were spaced 30 m from each other.

The same test carried out in area Val.-2 in spring 2002 was repeated in area Val.-1 in October-November 2002 and in February-April 2003 using the same substances.

In October-November 2002, several substances other than those previously tested were used in area Alb.-2; these repellents were used alone and without attractive

substances. The aim was to test the effect on *T. destruens* of (-)-β-pinene (release rate: 80mg in 24h at +21°C), racemic limonene (release rate: 60mg in 24h at +21°C) and (-)-α-pinene, in addition to that of verbenone. The logs were placed 50 m from the attacked trees and were spaced at least 30 m from each other.

The same test was repeated in area Alb.-2 in March-April 2003.

During each test, the number of *T. destruens* penetration holes was recorded for each log. The length and diameter of each log was also measured in order to report the values per m^2 of surface area.

Statistical analysis

The data collected during the two-year study were transformed by the formula $x_i = \sqrt{(X+0.5)}$. Analysis of variance (ANOVA) was applied to the transformed data and the levels of significance were then compared with the Tukey test.

RESULTS

To render the results of the trials with attractants and repellents clearer and more efficacious, we decided to report them separately for the two pine forests.

Poggio Valicaia

Capture with attractant substances

In the trials carried out in area Val.-1 of the Poggio Valicaia forest from the end of winter to the beginning of spring 2002, the traps baited with Tomowit did not capture any *T. destruens* adults. Only two specimens of *T. destruens*, one for each trap model (Pherotech and Witasek), were captured in the tests carried out in the same area in autumn 2002, while no beetle was captured in the trials in early 2003.

Only three *Tomicus* adults were caught in the tests of attractants provided by the University of Lund carried out in area Val.-2 between February and May 2002. All three were caught in the Pherotech trap containing racemic α-pinene.

Use of repellent substances

In the trials carried out in area Val.-1 in early 2002, no colonisations were recorded on the logs treated with the different repellents, or on the control logs or those baited with Tomowit; the only exception was a single colonisation on logs protected with octanol and verbenone. In the tests repeated with the same repellents in area Val.-2 in early 2003, there were no colonisations on any of the logs.

In contrast, numerous bark-beetle colonisations were recorded in the trials carried out in area Val.-2 in October-November 2002, but not on the logs treated with verbenone. However, when verbenone was combined with octanol, we recorded the highest level of

attack (9.4 holes/m^2), which was even greater than the values for the control logs (3.3 holes/m^2) and those baited with the attractant Tomowit (6.5 holes/m^2).

In the tests carried out in area Val.-2 during winter-spring 2002, the logs protected with alcohols C_6 and C_8 and benzyl alcohol were not attacked by *T. destruens*, in contrast to the control logs which suffered an average of 1.7 colonisations per m^2.

Nevertheless, the statistical analysis did not reveal significant differences among the repellents used in these tests. However some differences were recorded among the logs treated with the different repellents, thus further investigations about the effect of these substances on the behaviour of *T. destruens* seem worthy to be carried out.

Alberese

Capture with attractant substances

The trials carried out between winter and spring 2002 in area Alb.-1 also resulted in very few capture of *T. destruens* individuals: only two adult bark-beetles were caught in the Pherotech traps baited with Tomowit.

In contrast, many *T. destruens* individuals were caught in area Alb.-2 in autumn 2002. The Pherotech traps containing (-)-α-pinene yielded an average of about 144 bark-beetles, while the Theysohn traps containing (-)-α-pinene caught 63 (fig. 1). The numbers captured with the other substances were considerably lower in both trap models. However, Tomowit was more effective than Destruens, especially when used in the Pherotech traps (on average, 24 individuals with Tomowit vs. 4.5 individuals with Destruens). There were no substantial differences between these attractants when used in the Theysohn traps. The control traps did not catch any bark-beetles.

Overall, the Pherotech traps caught almost double the number of *T. destruens* than the Theysohn traps (on average, 43.2 vs. 23.2). Nevertheless, the difference was not statistically significant. In contrast, the differences between the numbers caught with the various attractants were highly significant (R^2=0.820, F=10.913, df 3,8, $p<0.01$). The Tukey test showed that significantly more bark-beetles were caught with (-)-α-pinene than with Destruens ($p<0.01$), Tomowit ($p<0.05$) or control ($p<0.001$).

In the tests carried out in area Alb.-2 between winter and spring 2003 (using only Pherotech traps), (-)-α-pinene-baited traps yielded an average of 16 individuals, while those with Ipm caught a slightly lower number (15.7) (fig. 2). The number of bark-beetles caught with the other substances was considerably lower (on average, 2 to 5 individuals), while the numbers caught in the control traps were insignificant (an average of 0.3 per trap).

ANOVA revealed highly significant differences (R^2=0.747, F=7.076, df 5,12, $p<0.01$). The Tukey test showed significant differences between (-)-α-pinene and both (+)-α-pinene ($p<0.05$) and control ($p<0.01$). The attractant Ipm was also significantly

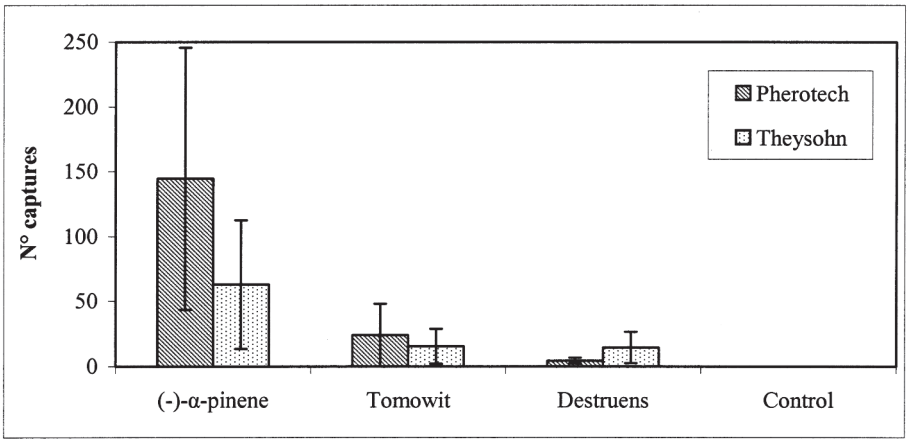

Fig. 1 – Number of *T. destruens* caught with the various attractants in area Alb.-2 of the Alberese pine forest in October-November 2002; bars indicate the Standard Deviation.

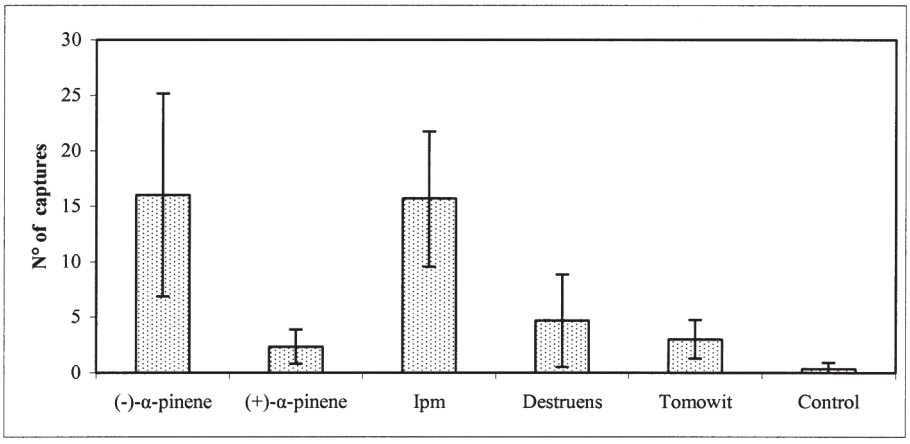

Fig. 2 – Number of *T. destruens* caught in area Alb.-2 from January to April 2003; bars indicate the Standard Deviation.

different from control ($p<0.01$) and (+)-α-pinene ($p<0.05$).

In the parallel tests carried out in area Alb.-3 in winter-spring 2003, Ipm used in Intercept-PTBB traps attracted the highest number of *T. destruens* (on average, 5 individuals per trap), and this attractant was only a little less effective in the Pherotech traps (4.5/trap) (fig. 3).

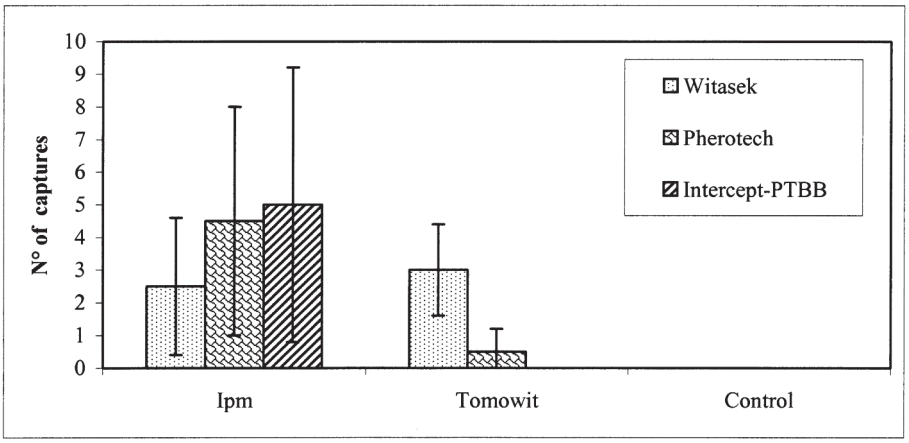

Fig. 3 – Number of *T. destruens* caught in area Alb.-3 from January to April 2003; bars indicate the Standard Deviation.

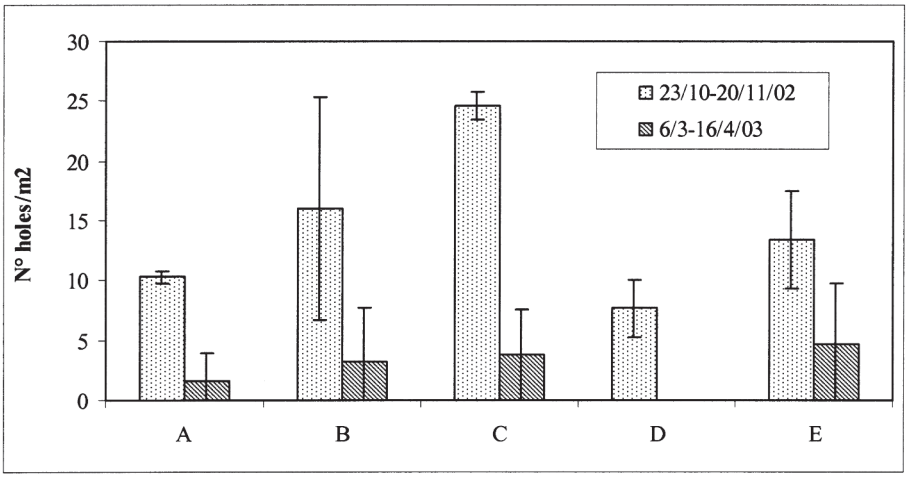

Fig. 4 – Number of *T. destruens* attacks on bait logs in area Alb.-2 in autumn 2002 and early 2003: A = (-)-β-pinene, B = (+)+(-)-limonene, C = (-)-α-pinene, D = Verbenone, E = Control. Bars indicate the Standard Deviation.

ANOVA revealed significant differences between the two attractants, but not between the different trap models: there was a significant difference between Ipm and Tomowit, and between each attractant and the control (R^2=0.763, F=10.789, df 2,9, $p<0.01$). The Tukey test showed that the numbers caught using Ipm were significantly greater than those using Tomowit ($p<0.05$) and the control ($p<0.001$).

Use of repellent substances

In the trials carried out in area Alb.-1 in spring 2002, *Tomicus* attacks on the logs protected with verbenone were the lowest (3.7 holes/m^2), while on the logs used to test the efficacy of the other repellents were much more numerous, as on the logs baited with Tomowit (7.1 holes/m^2) and the control logs (11.3 holes/m^2). However, the highest number of colonisations was recorded on the logs treated with the combination of verbenone and Glv's (18.7 holes/m^2).

In the trials carried out in area Alb.-2 in autumn 2002, logs protected with verbenone had the lowest number of holes/m^2 (7.7) compared with 10.3 holes/m^2 for (-)-β-pinene, 16.0 holes/m^2 for (+)+(-)-limonene, and 24.5 holes/m^2 for (-)-α-pinene; the control logs had 13.4 holes/m^2 (fig. 4).

In the same area in early 2003 (fig. 4) no penetration holes were recorded on logs treated with verbenone.

However, once again, the statistical analysis did not reveal significant differences among the repellents used in these tests. As just said in the case of Poggio Valicaia, the results obtained in the repellency tests carried out in Alberese appeared interesting, therefore further experiments will be planned in the near future.

CONCLUSIONS

The results of our two-year investigation (2002-2003) show some discordance between the two study areas. In the Poggio Valicaia pine forest, very few bark-beetles were captured in the various trap models baited with the different attractants; therefore, the data do not allow an evaluation of the efficacy of the products. In contrast, the results of the trials carried out in the Alberese pine forest demonstrate different capture successes of the various trap models using the same attractants. For instance, the Pherotech traps caught about twice as many *T. destruens* adults as the Theysohn traps, but virtually the same number as the Witasek and Intercept-PTBB traps.

The tests of attractants revealed that (-)-α–pinene, already shown to be attractive to *T. piniperda* (Schroeder, 1988; Zumr, 1989; Lindgren, 1997; Czokajlo and Teale, 1999; Poland *et al.*, 2003), is also effective with *T. destruens*. In fact, this monoterpene was the most effective of all the tested attractants, independently of the type of trap and period of the year. Nevertheless, there was a marked reduction of captures with both (-)-α–pinene and all the other substances in spring with respect to autumn; this could be due, at least partly, to the effect of temperature on the attractive properties of the various products, as observed for α–pinene by Czokajlo and Teale (1999).

The attractant mixture Ipm also provided good results, with efficacy similar to that of (-)-α–pinene.

In contrast, the results obtained with Tomowit were poor, while those with "Destruens", indicated as a specific attractant of *T. destruens*, were even less satisfactory, independently of the trap model and season.

However, even the most active substances produced modest captures of *T. destruens*, as found by other authors for *T. piniperda* (SCHROEDER, 1988; LINDGREN, 1997; CZOKAJLO and TEALE, 1999; POLAND *et al.*, 2003). Therefore, these substances, used in the various types of traps employed in the present research, do not appear to be suitable for mass captures of *T. destruens*, although they might be used successfully to monitor the presence of the bark-beetle in pine forests.

The only repellent that seems to have some activity on *T. destruens* adults in the reproductive stage was verbenone, especially when not combined with another repellent. In fact, the bait logs protected with verbenone alone were not colonised by the bark-beetle (or were only minimally attacked).

The results for both study areas and for the different periods of the year are in line with the results of tests of the repellent action of verbenone against *T. piniperda* (BYERS *et al.*, 1989; KOHNLE *et al.*, 1992).

The absence of *T. destruens* attacks on the bait logs at Poggio Valicaia during the tests of repellents provided by University of Lund (C_6OH, C_8OH and benzyl alcohol) appear to confirm the results of tests of C_6OH and C_8OH against *T. piniperda* (POLAND and HAACK, 2000, SCHLYTER *et al.*, 2000) and of benzyl alcohol against *T. destruens* (GUERRERO *et al.*, 1997). Therefore, further research should be carried out to evaluate the efficacy of these repellents against *T. destruens* in Mediterranean pine forests.

RIASSUNTO

IMPIEGO DI SOSTANZE ATTRATTIVE E REPELLENTI NEI CONFRONTI DI *TOMICUS DESTRUENS* (COLEOPTERA: SCOLYTIDAE) IN PINETE DI *PINUS PINEA* E *P. PINASTER* DELLA TOSCANA

Si riportano i risultati di indagini biennali condotte in pinete di pino marittimo e di pino domestico della Toscana, allo scopo di valutare l'efficacia di semiochimici nell'attrarre o respingere adulti di *Tomicus destruens* nella fase di ricerca delle piante o parti di esse idonee alla riproduzione. Come attrattivi sono stati utilizzati: Tomowit, (-) e (+)-α–pinene, il racemo di α–pinene, Mt-mix, trans-verbenolo, "Destruens" e "Ipm"; come repellenti sono stati sperimentati: Green leaf volatiles (Glv's, jans-2-hexen-1-ol), octanolo (octanol-1-octanol), verbenone, gli alcoli C_6, C_8, l'alcool benzilico, (-)-β–pinene e il racemo di limonene. Tra le diverse sostanze attrattive sperimentate, (-)-α–pinene è risultato il più efficace; catture si sono ottenute anche con l'impiego di Tomowit e con un nuovo formulato denominato Ipm. Tra le sostanze ad effetto repellente provate, il verbenone, quando impiegato da solo, sembra avere una certa attività, mentre perde questa sua proprietà quando viene provato in combinazione con altre sostanze.

Parole chiave: terpeni, monitoraggio, protezione, *Pinus* spp., *Scolytidae*.

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