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Short communication. A simple method for trapping *Hylobius abietis* (L.) alive in northern Spain

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Abstract

Hylobius abietis (L.) is the most economically important pest of coniferous seedlings in clear-cut areas in Europe. Adult weevils cause extensive damage by feeding on the bark of newly planted seedlings on clear felled coniferous stands. During the last 20 years, several studies have tried to find an effective method for trapping *H. abietis* in north Europe, but there is no information for the warmer countries of southern Europe. In this study, α-pinene and turpentine in combination with ethanol as attractants, and pine bark or pine branches as trap substrate were used to trap alive *H. abietis* in NW Spain. The combination of α-pinene and ethanol obtained nearly 7-fold greater captures than the control. However, there were no significant differences between α-pinene and turpentine. The catches were also significantly affected by the substrate used in the traps. Fresh pine bark impregnated with turpentine and ethanol achieved nearly 11-fold greater captures than branches as substrate. The use of pine bark plus turpentine (40 mL) and ethanol (20 mL) appeared as an effective and cheap alternative to capture *H. abietis* in Spain.

Key words: herbivory, α-pinene, turpentine, attractant.

Resumen

FALTA TÍTULO EN CASTELLANO

Hylobius abietis (L.) es la plaga con mayor repercusión económica en plántulas de las repoblaciones forestales de coníferas en áreas recién cortadas en Europa. Los adultos causan daños importantes alimentándose de la corteza de las plantas jóvenes. Durante los últimos 20 años varios estudios han tratado de encontrar un método eficaz de captura de este insecto en el norte de Europa, sin embargo no existe ninguna información de este tipo en el sur de Europa. En este estudio empleamos α-pineno y esencia de trementina en combinación con etanol como atrayentes, y corteza o ramas de pino como sustrato de las trampas. Como era esperado, se encontraron diferencias significativas en las capturas de *H. abietis* entre atrayentes. La combinación de α-pineno y etanol mostró cerca de 7 veces más capturas que el control. Sin embargo, no hubo diferencias significativas entre el uso de α-pineno y de esencia de trementina como atrayentes. También se observaron diferencias significativas en las capturas de *H. abietis* entre los sustratos. Las trampas de corteza de pino impregnada con una gran cantidad de trementina y etanol mostraron cerca de 11 veces más capturas que las trampas con ramas de pino impregnadas de la misma manera. Por lo tanto, el uso de corteza de pino y esencia de trementina (40 mL) y etanol (20 mL) se presenta como una buena y barata alternativa para la captura de ejemplares vivos de *H. abietis* en España.

Palabras clave: herbivoría, α-pineno, esencia de trementina, atrayente.

Introduction

Damage caused by the pine weevil *Hylobius abietis* (L.) (Coleoptera, Curculionidae) is a major problem in clear-cut areas that are replanted with conifer seedlings in large parts of Europe (Orlander and Nilsson, 1999; Wallertz, 2005; Zas *et al.,* 2008). Pine weevils feed on the phloem of young seedlings causing seedling mortality up to 60-80% when seedlings are planted without protection (Orlander and Nilsson, 1999). Successful pest management implies a deep knowledge of the biology and behaviour of the species to be controlled. During late spring, adult pine weevils fly long distances to new breeding habitats, attracted by volatile chemicals emitted from freshly cut stumps (Nordlander *et al.,*

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1986). Female weevils oviposit throughout the summer, laying the eggs on the bark of fresh stumps-roots or in the soil nearby (Nordlander *et al.,* 1997). The time for development from egg to adult depends on the climate and the quality of the host stumps (Leather *et al.,* 1999).

Several investigations have been conducted for estimating the relationship between the relative size of pine weevil populations and seedling damage in different locations (Langstrom, 1982; Nordlander, 1987; Voolma, 2002). Several semiochemicals commonly used to attract bark beetles have been tried as bait to *Hylobius* spp. The combination of ethanol and α-pinene appeared as an efficient bait to trap *Hylobius* spp. in northern coniferous forests (Nordlander, 1987; Erbilgin *et al.,* 2001). In Sweden, Nordlander (1987) found that traps with a combination of α-pinene and ethanol were more effective capturing walking pine weevils than traps with α-pinene or ethanol alone. In the same way, Erbilgin *et al.* (2001) observed that *Hylobius pales* Hbst. were highly attracted to flight traps baited with α-pinene and ethanol at two locations in North America.

Besides, captured *H. abietis* adults have been used in laboratory bioassays for studying feeding rates (Mansson and Schlyter, 2004; Wainhouse *et al.,* 2004), feeding preference (Mansson and Schlyter, 2004) and pine weevil antifeedants (Schlyter *et al.,* 2005). Live *H. abietis* individuals can also be used in bioassays to explore the genetic variation in conifer resistance against these generalist phytophagous insects. Improving tree resistance against insects may be an interesting tool to control pests, particularly within the frame of the Integred Pest Management strategies (Heidger and Lieutier, 2002). In our opinion, *H. abietis* is a suitable insect model for using in resistance studies due to i) easy laboratory handling, ii) to that main damage is caused on young seedlings, and iii) to that feeding rates can be easily evaluated. Moreover, and considering an evolutionary point of view, *H. abietis* frequently causes the death of the tree, which is a variable directly related with plant fitness that thus can be easily assessed in trees.

All the studies for trapping *H. abietis* were carried out in central and northern Europe where climatic conditions, tree species and *H. abietis* populations are highly different to those in southern Europe. It would be necessary to confirm at what extent results from these capture studies may also apply in southern Europe. The aim of the present paper is to find an efficient and economic trap lure to obtain large number of adult pine weevils alive at southwestern Europe for using in laboratory assays.

Material and Methods

The experiment was carried out during June 2007 on five clear-felled forest sites located at Cuspedriños (San Xurxo de Sacos Forest, Galicia, Spain, 42.30° N; 8.30° W; altitude 550 m a.s.l). Before harvesting, stands at all these sites comprised nearly 2000 ha of 35-year-old *Pinus pinaster*. The climate is temperate humid Atlantic, with annual precipitation of about 2,000 mm and mean annual temperatures of 11.3°C. During the sampling period, maximum and minimum temperatures were 26°C and 14°C, respectively.

Tramps consisted of about 1 kg fresh weight of natural harvesting debris as substrate disposed directly on the ground: i) fresh *P. pinaster* bark or ii) fresh pine branches with diameter < 1 cm, both just removed from recently felled logs. Traps were established on 27 June 2007, and sampled everyday at 11 a.m. during the four subsequent days. Beetles attracted within the traps were hand-sorted from the bark or branches, weighted and reared with food in culture bins for laboratory assays. For trapping the pine weevils we used as attractants i) commercial turpentine, obtained from a local drugstore (priced at 3 € 1,000 mL^{-1}) and ii) α-pinene (Merck ref. # 8.18632.0100), both always in combination with ethanol (VWR 70% v:v in distilled water).

We examined the effect of using the available pine debris as trap substrate comparing the pine bark to the pine branches, both using a high liberation rate of turpentine, after spraying 40 mL of α-pinene and 20 mL ethanol directly on the pine debris.

In order to study the effect of the attractant we used 3 types of pine bark traps: control traps without attractant, traps baited with ethanol and turpentine, and traps baited with ethanol and α-pinene. For this objective, the attractants were dispensed in 1 mL polyethylene eppendorf vials, settled in vertical position under the pine bark. For adjusting the liberation rates we followed the recommendations of Nordlander (1987). Based upon several previous trials at field temperature we estimated that one 1 mL eppendorf vial provided a slow release of about 0.25 mL of the monoterpene and ethanol per day. Considering that the adequate slow release is about 1 mL of monotepene and 0.75 mL of ethanol per day (Nordlander, 1987), for the experimental setup we used 4 vials (filled with 1 mL each) of α-pinene or turpentine plus 3 vials of ethanol. Therefore, after the four sampling days, all the initial volume of attractants in the vials was released.

The traps were randomly set on the ground along a 10 m radius circle in each of the 5 sites (5 traps × 5 sites = 25 traps) placed along a 1 km radius circle in the pine forest. The effects of the attractant and the substrate on the cumulative pine weevil catches were independently analyzed by means of ANOVA using the general linear model $Y_{ij} = \mu + S_i + T_j + \varepsilon_{ij}$, where Y_{ij} is the value of the cumulative pine weevil catches, μ is the general mean, S_i and T_j are the main effects of site i ($i = 1$ to 5) and trap j ($j = 1$ to 3 for attractant and $j = 1$ to 2 for substrate) and ε_{ij} is the experimental error. Data were transformed with an arcsine function to achieve normality and homogeneity of variances before analysis, but are presented as untransformed values in the figures. When main effects were significant, differences among means were tested for significance using the Fisher Test ($p < 0.05$). All analyses were performed using Statistica v. 6.0.

Results and Discussion

A total of 153 specimens of *H. abietis* (mean weight = 124.3 ± 2.9 mg) were captured from 27 June to 30 June 2007. The highest catches of pine weevils were obtained during the first and second days (data not shown) and then drastically decreased. The warmer conditions in the first days likely promoted a higher release of the volatile chemicals used to attract the weevils. Release rates strongly influence trapping effectiveness and duration. Nordlander (1987) used a release rate of about 0.5 mL of α-pinene and 1 mL of ethanol during a 5-day period at field, whereas Miller (2006) found effective a release of 2 mL of α-pinene and 1 mL of ethanol per day in multiple-funnel traps at 25-28°C. In our study, we observed that in all cases the attractants used as bait in the traps were exhausted after 72 h, as expected after our preliminary tests.

The catches were significantly affected by the attractants used as bait (Table 1). The combination of ethanol and α-pinene in traps showed nearly 7-fold greater *H. abietis* catches than the control (Fig. 1A). In Europe, Nordlander (1987) found a synergism between α-pinene and ethanol as attractants in pitfall traps, trapping about 6 times more pine weevils than α-pinene alone, 10 times more than ethanol alone and 25 times more than empty traps. In the same way, Zurm and Stary (1992) found that traps baited with α-pinene and ethanol consistently caught more pine weevils than α-pinene alone baited traps. In our study, although the combination of α-pinene, ethanol and pine bark showed the highest catches, differences among α-pinene and commercial turpentine as bait in traps were not significant (Fig. 1A). Commercial turpentine is a cheap product obtained from the distillation of conifer resin. It is composed of terpenes, mainly the monoterpenes α-pinene and β-pinene. Thus, our results suggest that the use of turpentine as bait in *H. abietis* traps might be an interesting economic alternative.

Catches were also significantly affected by the substrate used in the traps (Table 1). Fresh pine bark impregnated with high amount of turpentine and ethanol showed nearly 11-fold greater captures than impregnated branches as substrate (Fig. 1B). Our results agree with previous findings pointing to fresh conifer bark as more attractive to *H. abietis*. Nordlander (1987) found that the use of fresh pine bark in the traps increased by 30% the number of weevils trapped. A similar trend was observed by Zumr and Stary (1992) using spruce bark.

In summary, traps with fresh pine bark impregnated with 40 mL of commercial turpentine and 20 mL of

Table 1. Results of ANOVA, showing the main effects of site and trap on the cumulative pine weevil catches after four days since trap establishment

Effect	DF	MS	F	p
Attractant				
— Site	4	0.87	2.42	0.134
— Attractant	2	1.84	5.12	**0.037**
— Error	8	0.36		
Substrate				
— Site	4	1.27	6.39	**0.049**
— Substrate	1	4.53	22.74	**0.009**
— Error	4	0.20		

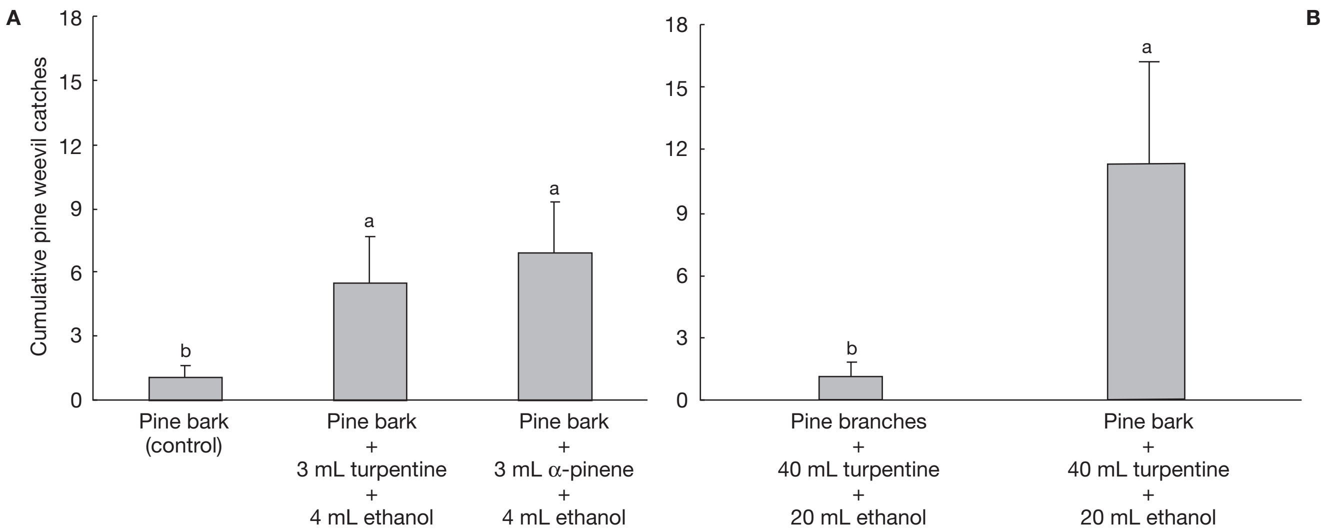

Figure 1. Cumulative catches of *H. abietis* per trap over four days in traps with different (A) attractants and (B) substrates. LS means ± s.e. Different letters within each figure indicate significant differences at $p < 0.05$

ethanol set on the ground could be an effective and cheap alternative to obtain a sizable number of live *H. abietis* adults in Spain. However, the attractants used as bait in the traps must be renewed every 3-4 days to obtain optimal catches, limiting the application of this method for operative use in weevil control.

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