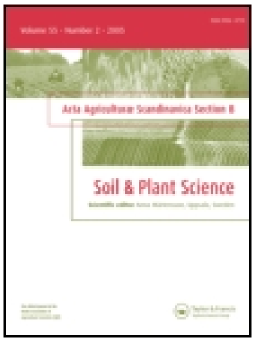

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SHORT COMMUNICATION

Clover root borer *Hylastinus obscurus* (Marsham) (Coleoptera: Scolytidae) has no preference for volatiles from root extracts of disease infected red clover

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Keywords: *Insect pest*, *plant volatiles*, *red clover*, *red clover decline*, *Trifolium pratense*.

Introduction

Red clover, *Trifolium pratense* L., is an important forage legume grown in North America and Europe. Soil-climatic conditions in southern Chile are also favourable for the establishment of red clover stands. Although red clover is considered a perennial plant, red clover stands often decline to unsatisfactory levels within two years after sowing (Cuevas & Balocchi, 1983; Leath, 1985; Steiner & Alderman, 2003). Factors that may contribute to reduced persistence of red clover include root rot severity, and root borer *Hylastinus obscurus* (Marsham) infestations (Graham & Newton, 1959; Carrillo & Mundaca, 1974). At present there is no control for clover root borers other than rotation (Aguilera et al., 1996).

A few studies have been carried out on the chemical interrelationship between the root-rot complex of red clover with borers feeding on clover roots. Leath and Byers (1973) reported that in preference tests performed in Petri dish chambers adult borers were more attracted to water-leached materials from diseased red clover roots than to leached materials from healthy roots. Some volatile compounds identified in a hexane extract of diseased red clover roots were also found attractive to borers in Petri dish preference tests, but in field tests no individual compound or mixture was attractive to adult borers (Kamm & Buttery, 1984).

As the authors in the preceding behavioural studies used still-air bioassays, the present study attempted to determine whether adult borers could discern between odours released from ethanolic extracts of healthy and disease-infected red clover roots by using a four-arm olfactometer, a more powerful statistical design (Hare, 2000) that utilizes moving air.

Material and methods

Plant material and growth conditions

Healthy plant experimental units consisted of single 15 cm diameter plastic pots filled with 400 g of autoclaved soil with 30 red clover seedlings. The red clover cultivar Quiñequeli was used in all experiments. Pots were placed in a high humidity growth chamber at approximately 22°C (range 15–26°) with 16 h daylight. Plants were fertilized every 15 days starting 4 days after planting with Long Ashton solution (Hewitt, 1966) at a rate of approximately 100 ml/pot/day. Three replicate units were maintained. Experiments were conducted at 60 days after planting.

The same procedure was used for three experimental units with autoclaved soil inoculated at 1 ml

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inoculum/100 ml soil before planting. An isolate (of proven pathogenicity) of *Fusarium oxysporum* from diseased red clover roots in southern Chile was maintained on potato-dextrose agar. Specific characterization of this strain as *F. oxysporum* was provided by CABI Bioscience Identification Services, UK Centre (Egham). Inoculum consisted of 3- to 4-week-old cultures of the fungus grown on sterile, autoclaved oat seed.

Root and fungus culture extracts

At harvest (60 d.a.p.), 20 uniform plants per pot were carefully removed from the potting soil, and the root systems were separated and washed with tap water and then dried at 50°C for 24 h. The dried roots of the three replicates in both seedling growth experiments were pooled to obtain approximately 10 g of dried roots from healthy plants and 4 g of dried roots from disease-infected plants. The dried roots were extracted with 250 ml of 96% ethanol for two days. The volume of the extract was reduced on a rotatory evaporator to yield about 0.50 g residue from healthy roots and 0.12 g residue from disease-infected roots. An ethanolic extract from 3- to 4-week-old culture of *F. oxysporum* was similarly obtained.

Insects

Red clover roots infested with adult borers were collected from commercial seed fields near Temuco from early winter to spring 2003. The beetles were removed from the roots with Berlese funnels and stored in freezer containers lined with moist paper towels, provisioned with several clover roots. Beetles were removed from storage and exposed to room temperature 2 h before a bioassay. Each beetle was used only once and then discarded.

Olfactometry

Behavioural studies were performed in an olfactometer as described by Pettersson (1970). One beetle was enclosed in an area permeated by air (200 ml/min) coming in from each of its four arms and drawn out through a hole above the centre of the area. Extract samples (2 mg) were applied to filter papers, which were placed at the ends of two opposing arms, and untreated filter papers were placed in the two remaining control arms. The beetle was constantly observed for a period of 30 min. Each experiment was replicated 12 times with different beetles and the times spent in the stimulus and control arms were compared using the Wilcoxon matched-pairs test.

Results and discussion

In the growth chamber step, root dry weights were reduced by inoculum of *F. oxysporum*. In the olfactometer, adult borers showed a significant preference for the odour extracts from both healthy and disease-infected red clover roots when tested against clean air (Figure 1). In a choice test, *H. obscurus* did not discriminate between volatiles from infected and uninfected red clover root extracts (Table I).

These findings are in contrast to reports of others researchers that in Petri dish bioassays borers preferred plant materials or extracts from diseased red clover roots over materials from healthy roots (Leath & Byers, 1973; Kamm & Buttery, 1984). It is important to note that the Petri dish experiments used still air and involved short-range contact with the odour source. In contrast, our experiment was conducted in a Pettersson's olfactometer, with utilization of moving air, which evaluates borer responses to short-range semiochemicals without allowing direct contact with the odour source. These differences in experimental conditions may explain why borers in the two-bioassay methods responded differently to like odours from root materials.

Here we suggest that the ethanolic extracts from red clover roots, whether infected or uninfected, emit qualitatively similar chemical blends such that borers are unable to distinguish between volatiles from each extract. This implies that *F. oxysporum* infection does not produce significant chemical differences in the odour blends released from root

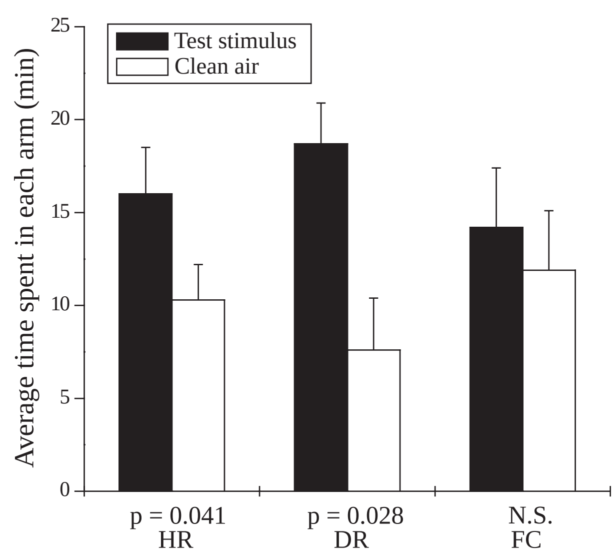

Figure 1. Response of *H. obscurus* towards clean air versus volatiles released from ethanol extracts of healthy (HR) and diseased (DR) red clover roots, and from an extract of *F. oxysporum* culture (FC) in olfactometer tests, expressed as average numbers with S.D. Comparisons made with Wilcoxon matched-pairs tests. N.S. $P \geq 0.05$; $n = 12$ in all comparisons.

Table I. Response of *H. obscurus* in olfactometer choice tests to volatiles released from ethanol extracts of healthy and diseased red clover roots. Comparisons made with Wilcoxon matched-pairs tests, $n = 12$.

Stimulus applied[a]	Average time spent in each arm (min) ($\bar{X} \pm$S.D.)
Healthy roots extract	12.6 ± 1.9
Diseased roots extract	12.6 ± 2.3

[a] 2 mg extract samples.

extracts. It is interesting to note that borers showed no preference when exposed to an extract of *F. oxysporum* culture vs. clean air (Figure 1). Recently, Steiner and Alderman (2003) reported that in clover seed fields with lime application in Oregon both root diameter and root rot index increased with increasing soil pH, but the percentage of roots with root borer infestations remained unaffected, and the authors concluded that root borer infestation, not the severity of *Fusarium solani* infection, was a primary cause of root health decline in Oregon red clover seed fields. Our results suggest that *F. oxysporum* infection is not a critical factor responsible for the infestation of clover roots by *H. obscurus*.

This study used ethanolic extracts from red clover roots in determining behavioural responses of *H. obscurus* because ethanolic extracts provided a broad range of compounds, allowing a gradual release of volatiles in the olfactometer that may be important in determining their possible attractiveness to *H. obscurus*. Further studies are needed to elucidate the importance of the different compounds in the odours released from red clover root extracts on the behaviour of *H. obscurus*. This should lead to a better understanding of the factors affecting red clover persistence.

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