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SHORT COMMUNICATION

Disruption of pheromone communication in the European pine sawfly, *Neodiprion sertifer*, at various heights

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Introduction

Mating disruption is one of the most promising applications of insect sex pheromones in plant protection. Mainly moths (Lepidoptera) are concerned, but some experiments have been done with bugs (Hemiptera, Heteroptera), beetles (Coleoptera), and sawflies (Hymenoptera, Symphyta) (Hardie & Minks, 1999). Much research has been devoted to understanding the mechanisms behind successful mating diruption, but no general or conclusive results have been presented yet (Wyatt, 1996; Howse et al., 1998). It should also be noted that the vast majority of studies have been done in agricultural, i.e., two-dimensional, or in low three-dimensional habitats, such as vineyards or orchards. In forest habitats comparatively few attempts have been made, notably with the gypsy moth, *Lymantria dispar* (L.) (Schwalbe et al., 1983), Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough) (Hulme & Gray, 1994), and with the target of the present study, the European pine sawfly, *Neodiprion sertifer* (Geoffroy) (Hymenoptera, Diprionidae). This and many other pine or conifer sawflies (Hymenoptera, Diprionidae) cause severe defoliation of pine (*Pinus* spp.) forests over large areas in Europe, Asia, and North America (Smith, 1993; Day & Leather, 1997). The larvae consume the needles, which causes decreased growth and sometimes death of the trees. Outbreaks are regularly controlled by aerial application of chemical insecticides.

The female attracts males with a pheromone and thus there is a possibility to disturb mating by application of the mating disruption technique. Even unmated females of this arrhenotokous species, however, may lay eggs that develop to males only. The main result after a mating disruption treatment of one generation therefore would be a male-biased sex ratio, and a drop in the population density should be expected after treatment of two generations (Mertins et al., 1975). In the first mating disruption experiments with *N. sertifer* almost complete trap catch reduction was obtained in small plots (0.5 ha). Due to a general collapse of the population in this area, however, no evaluation of sex ratio or population density could be done during the following generation (Anderbrant et al., 1995a). In the following experiments, the size of the test plots was increased to around 4.5 ha. Also here a dramatic decline in trap catch was recorded, but no apparent effects on sex ratio, larval density, or defoliation could be detected (Anderbrant et al., 1995b). These two studies used the attractive pheromone isomer (1S,2S,6S)-trimethyltetradecyl acetate, either alone or in its erythro blend, as disruption agent. In a third study, the antagonistic isomer (1S,2R,6R) was used, either alone or in combination with the attractive isomer, but failed to improve the effect of the mating disruption treatment (Anderbrant et al., 1998). The following hypotheses were put forward to explain the negative results. (1) Trap shut-down did not mirror a reduction in mate finding. This was tested and rejected as only few of the females observed inside the treated area mated as compared to females outside this area (Östrand et al., 1999). (2) The treated area was too restricted in size, so that mated females dispersed into all parts of the treated area, and thus obscured the effects of the treatment. The results, however, remained unchanged even when using a 25-ha large area (Anderbrant et al., 2002). (3) The vertical coverage was not sufficient because disruption dispensers were applied only at one height in a three-dimensional habitat. A test of this hypothesis is reported here.

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Methods

Previous mating disruption experiments with *N. sertifer* used dental cotton rolls covered with a cardboard rain and sun shelter for releasing the disruption pheromone. In order to simplify the dispenser application, an experiment to test the effectiveness of the dispenser without a shelter was designed, using modified Lund-I sticky traps (Anderbrant et al., 1989). In two of the traps the cardboard roof was entirely removed, and in two traps it was replaced by a small cardboard shelter above the dispenser. The same dental cotton rolls as before (Celluron® no. 2, Paul Hartmann, S.A., France) were used. Each trap was loaded with 100 µg of the attractive (1S,2S,6S)-trimethyltetradecyl acetate, > 99% stereochemically pure and synthesised according to Högberg et al. (1990). Thus, in both trap types the sticky bottom was exposed to rain and sunlight. Traps were placed in pines about 2 m above ground in a young Scots pine, *Pinus sylvestris* L., plantation near Valdmarsvik in the province of Östergötland, south-eastern Sweden, from 23 August to 7 October 1993. Intertrap distance was about 50 m. Traps were re-randomised each time they were inspected. The shelter above the dispenser did not affect the trap catch ($P > 0.1$, t-test). On average, 147 ± 121 (SD, $n = 28$) males were caught in the traps without a shelter compared to 95 ± 76 ($n = 28$) in traps with shelter. We therefore used unprotected cotton roll dispensers in the following mating disruption experiment.

The test, designed to document the vertical effectiveness of the mating disruption treatment, was done in a stand of Scots pines, 6–8 m in height, in the area mentioned above. Two ha (140 × 140 m) were treated with pheromone. The mating disruption dispenser consisted of a dental cotton roll impregnated with 8 mg of erythro-1,2,6-trimethyltetradecyl acetate containing less than 0.03% of the antagonistic (1S,2R,6R)-isomer (Hedenström & Högberg, 1994). The dispensers were hung 10 m apart in a square grid at about 2 m height in pine trees on 10 August 1994. Based on measurements in the laboratory and field (Anderbrant et al., 1992), the release of the active (1S,2S,6S)-isomer was estimated at about 180 mg ha^{-1} for the whole season (c. 60 days).

The ability of the males to find an odour source was monitored by Lund-I sticky traps (Anderbrant et al., 1989), baited with 100 µg of the acetate of the attractive (1S,2S,6S)-isomer (see above). One trap was placed at each of three heights, 1.5, 3.5 and 5.5 m, on 16 metal poles fixed in the ground on 11 August. The poles were arranged along two perpendicular lines through the centre of the treated area. The distance between poles was 40 m resulting in eight poles inside the treated area and eight poles outside this area (untreated control). Trap catches were checked and sticky bottoms replaced on 20 August, 4 September, and 6 October, when the experiment ended. Pheromone dispensers in the traps were renewed on 4 September. Trapping periods had different lengths, therefore the data were recalculated to obtain daily catches before analysis. Mean daily trap catches outside the treated area were $\log(x + 1)$ transformed to make the variances more homogeneous (Levene's test, SPSS 10.0).

The trap catch reduction caused by the treatment was calculated as follows. For each period and height the average catch outside the treatment was calculated (eight traps per height). Then trap capture inside the treatment was expressed as a percentage of the capture outside the treatment. One hundred percent minus this percentage gives the disruption effect.

Results and discussion

The traps inside the treated area caught only few male sawflies compared to the traps outside the treatment (Figure 1). There was an obvious effect of the pheromone treatment at all heights. ANOVA using transformed daily trap catches outside the treated area, with trap height and trapping period as independent factors, showed a significant effect of trapping period ($P < 0.05$), but not of height or of the period × height interaction. The large differences between variances among catches at different heights and periods within the treatment made a similar analysis impossible. As seen in Figure 1, however, the catches increased about an order of magnitude during the last trapping period.

The trap catch reduction was above 97% for all heights during the first two periods (Figure 2). During the third period the effect declined, especially at the 3.5 and 5.5 m levels, where the disruption was about 90%. This clearly shows that pheromone released at one height readily reaches other vertical strata. The effect on male trap catch was almost identical from the level of the dispensers up to the upper level of the trees. This is an important factor to consider when using pheromone-based control strategies in forestry or in orchards, but up to now there have been few direct investigations of this phenomenon. Most activity of tree defoliating insects occurs, of course, at the canopy level and trap catches of *N. sertifer* were sometimes more than 10 times as large in the canopy than at 2.5 m height (Simandl & Anderbrant, 1995).

This study was made in a plantation very similar to those used in the mating disruption experiment reported by Anderbrant et al. (1995b). It was hypothesized that the lack of an effect on population density or future sex ratio could be due to bad vertical coverage of the disruptive

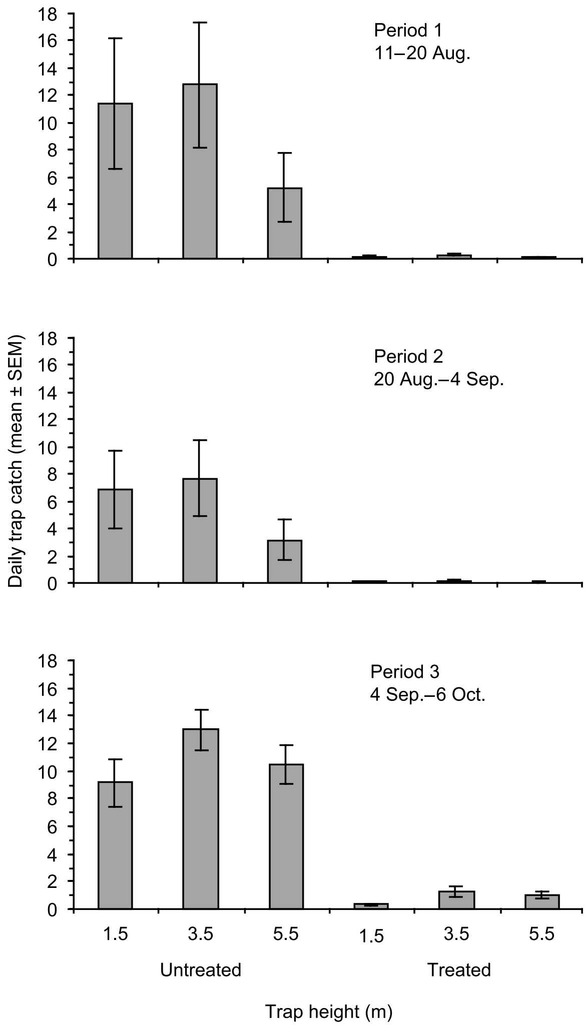

Figure 1 Average daily catches of traps inside and outside the pheromone treated area at various heights and during three different periods.

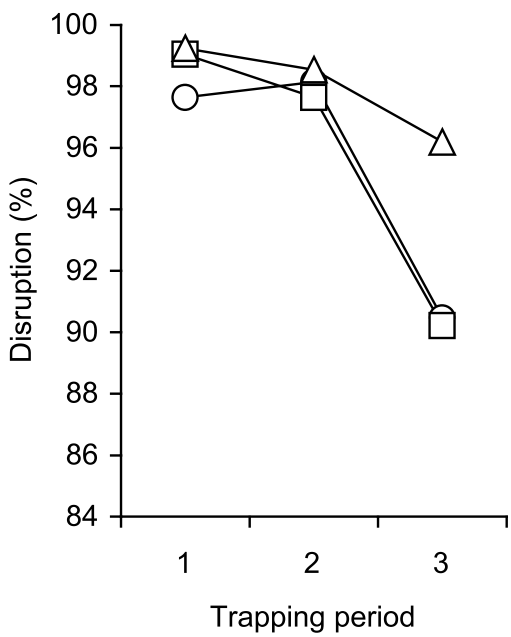

Figure 2 Effect of mating disruption, as estimated from trap catch reduction, at 1.5 m (△), 3.5 m (□), and 5.5 m (○), during the three trapping periods 11–20 August, 20 August–4 September, and 4 September–6 October.

pheromone. The present study gives clear evidence that this hypothesis was not correct. In a recent publication Martini et al. (2002) showed that mating disruption of *N. sertifer* can both alter the sex ratio and reduce the populations in small isolated pine stands. Thus, the assumption that it is the movement of mated females from surrounding areas that obstructs the effect of mating disruption in non-isolated areas still remains.

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