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*Published in:*  
IOBC/WPRS Bulletin

2002

[Link to publication](#)

*Citation for published version (APA):*

Anderbrant, O., Hedenström, E., & Högberg, H.-E. (2002). Pheromone mating disruption of the pine sawfly *Neodiprion sertifer* : is the size of the treated area important? *IOBC/WPRS Bulletin*, 25(9), 111-116.  
<http://phero.net/iobc/samos/bulletin/anderbrant.pdf>

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## **Pheromone mating disruption of the pine sawfly *Neodiprion sertifer*: is the size of the treated area important?**

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**Abstract:** Few attempts to control forest insects by means of pheromone mating disruption have been reported. The first such experiments with the European pine sawfly, *Neodiprion sertifer* (Hymenoptera: Diprionidae), resulted in nearly complete trap catch reduction, but no effects on population density or sex ratio were noted. Unmated females lay eggs, which develop into males only. Therefore, if mating disruption would be successful, a more male-biased sex ratio is expected the next generation. One possible explanation for the early results is that mated females disperse into the treated area, and thus obscure the effects of the treatment. In order to reduce the effect of such immigrating females, the treated area was increased in the experiment described here from the earlier used 0.5 – 4 ha to 25 ha. The acetate of erythro-3,7-dimethyl-2-pentadecanol was used for disruption and released from dispensers every 10 m. The influence on male orientation was monitored by pheromone traps, baited with the acetate of (2S,3S,7S)-3,7-dimethyl-2-pentadecanol and placed at 100 m interval along two perpendicular, 1500 m lines intersecting the treated area. The trap catch reduction was near 100% during the first month, but then declined to around 90% during the second month. Mating frequencies were checked by comparing the sex ratio of the next generation from within and outside the treatment area. No effect of the treatment on the sex ratio was detected, and the frequency of mated females could be assumed to be independent of treatment. Alternative hypotheses to explain the failure of pheromone mating disruption in *N. sertifer* are discussed.

**Key words:** Hymenoptera, Diprionidae, pest management, sex attractant, 3,7-dimethyl-2-pentadecanol

### **Introduction**

Many species within the sawfly family Diprionidae, pine or conifer sawflies, cause severe defoliation of pine (*Pinus* spp.) forests over large areas in Europe, Asia and North America (Smith 1993, Day & Leather 1997). Outbreaks are regularly controlled by aerial application of chemical insecticides such as Dimilin and various pyrethroids. Recently, attempts have been made to use the female produced sex pheromone for controlling populations of *Neodiprion sertifer* Geoffroy, one of the most widespread

and economically important diprionids. The method used has been mating disruption. In such experiments a relatively large amount of the pheromone is released in order to obstruct the mate finding behaviour of the male. Unmated females lay eggs, which develop into males only. Therefore, if mating disruption was successful, no drastic population decline would be expected but instead a more male-biased sex ratio would occur in the next generation.

In the first mating disruption experiments with *N. sertifer* a near complete trap catch reduction was obtained in small (0.5 ha) plots. However, due to a general collapse of the population in the area, no evaluation of sex ratio or density could be done of the next generation (Anderbrant *et al.* 1995a). In the following experiments the treated areas were increased to around 4.5 ha. Also in this case 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, the acetate of (2*S*,3*S*,7*S*)-3,7-dimethyl-2-pentadecanol, either alone or in its *erythro*-blend, as disruption agent. In a third study, the antagonistic (2*S*,3*R*,7*R*)-isomer was used, either alone or in combination with the attractive isomer, but this failed to improve the mating disruption (Anderbrant *et al.* 1998).

One hypothesis that could explain these negative results is that, due to the high population density during such epidemic conditions that prevailed during these experiments, males are still able to locate females. However, observations of females either inside or outside a treated area clearly showed that only a small fraction of those inside mated compared with those outside (Östrand *et al.* 1999). An alternative hypothesis is that mated females disperse into the treated area, and thus obscure the effects of the treatment. In order to reduce the effect of such immigrating females, the treated area was increased in the experiment described here from earlier 0.5 – 4 ha to 25 ha. The success of the pheromone treatment was monitored by traps within and outside the treated area and by collection of next generation larval colonies for a check of the sex ratio.

## Materials and methods

The study was performed in a Scots pine, *Pinus sylvestris* L., plantation near Valdmarsvik in the province of Östergötland, southeast Sweden, during 1993. The treated area consisted of several stands mainly with young pines, 2 – 5 m in height. Some stands with older trees or mixed with Norway spruce, *Picea abies* Karst., were also included.

The mating disruption dispenser consisted of a dental cotton roll (Celluron No. 2, Paul Hartmann, S.A., France) impregnated with 8 mg of the acetate of erythro-3,7-dimethyl-2-pentadecanol (Hedenström & Högberg 1994). The dispensers were hung in trees at about 2 m height under a sun- and rain-protection made from wax-

impregnated cardboard. Dispensers were placed 10 m apart in a square grid 500 by 500 m on the 27 July to 1 August. Based on measurements in the laboratory and field (Anderbrant et al. 1992), the release of the active (2S,3S,7S)-isomer was estimated at about 180 mg ha<sup>-1</sup> for the whole season (60 d).

The ability of the males to find an odour source was monitored by Lund-I sticky traps (Anderbrant et al. 1989) placed 100 m apart along two perpendicular lines intersecting each other at the centre of the treated area. Eight traps were inside the treated area, four were at the border and 20 traps were outside. The traps were placed in pines about 2 m above the ground and were loaded with 100 ng of the acetate of the attractive (2S,3S,7S)-isomer, synthesised according to Högberg et al. (1990). Sticky bottoms were replaced on 23 August and 7 September, and baits were renewed on 7 September. The trapping was finished on 6 October.

Larval density within the plantation was recorded in spring before the experiment. Two trees every 50 m along two lines were inspected and the number of colonies counted. However, because nearly all of these trees were located within an area, which later was chosen for treatment, a complementary census of the defoliation was performed. This was made according to the method described in Anderbrant et al. (1995b), using four trees every 100 m along half the length of each transect, from the centre to the outer end. In total 40 trees outside and 20 inside the treatment were inspected.

The sex ratio of the generation following the mating disruption treatment was estimated by collection of larval colonies, which were reared until cocoon formation. Rearing took place in ventilated cardboard boxes and larvae were moistened daily and fed fresh pine twigs when needed. Twentyfour colonies were collected from each of the areas inside and outside the treatment. This was done in June 1994 and the colonies were reared individually. In addition, approximately 15 colonies were collected from each of five sites inside the treated area and four sites outside it, along one of the transects. All colonies collected from one individual site were reared together until cocoon formation. The cocoons were sex determined based on size, females being about twice as large as males.

## Results and discussion

Similar to the results from previous studies, the pheromone treatment caused a nearly complete trap shut down (Figure 1): Average trap catches of male *N. sertifer* for the whole season were  $612 \pm 298$  (SD, n=20),  $180 \pm 161$  (n= 4) and  $38 \pm 47$  (n=7, one trap had fallen down and was excluded from the analysis) for traps outside, at the border and inside the treated area, respectively. The catches, transformed to  $\log(\text{catch}+1)$ , inside and outside were significantly different ( $P=0.002$ , t-test, unequal variances). The trap catch reduction was complete during the first three weeks, but during the last month of trapping it was only just over 90 % (Figure 2).

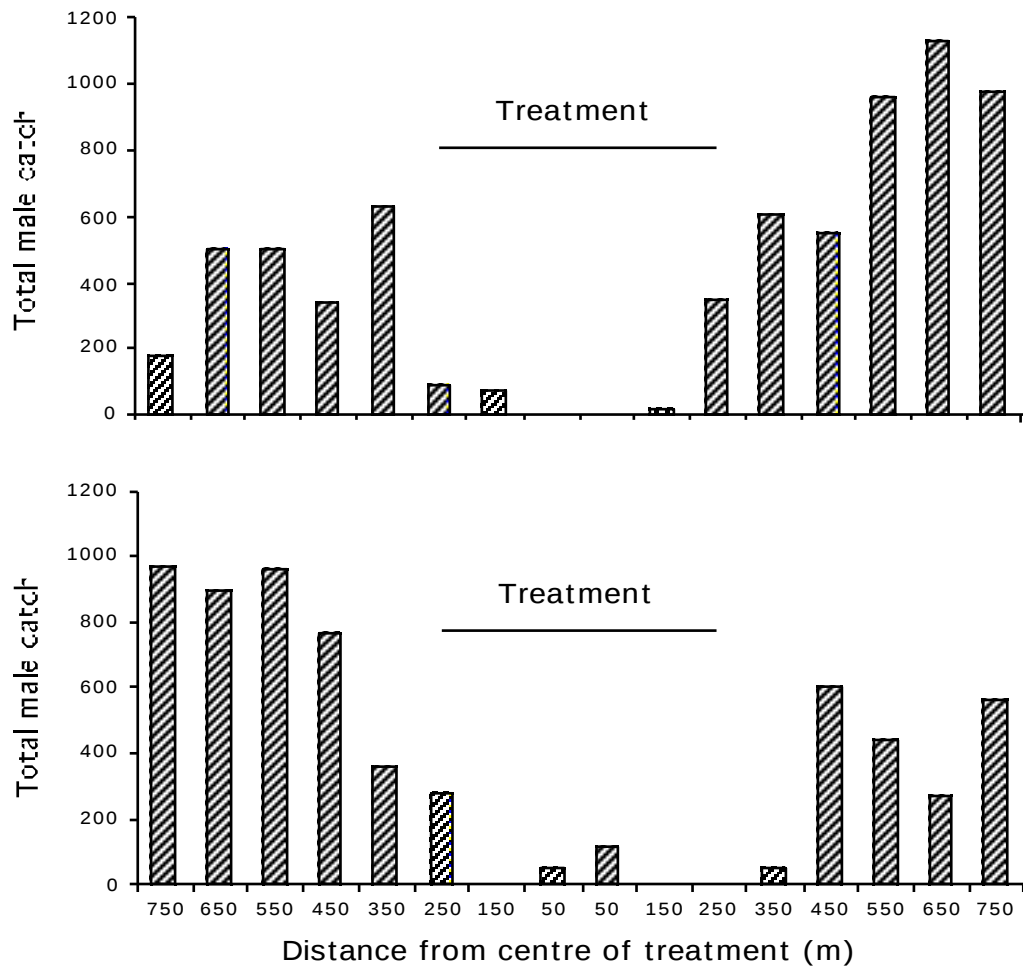


Figure 1. Total catch in pheromone traps along the two perpendicular transects through the mating disruption treatment.

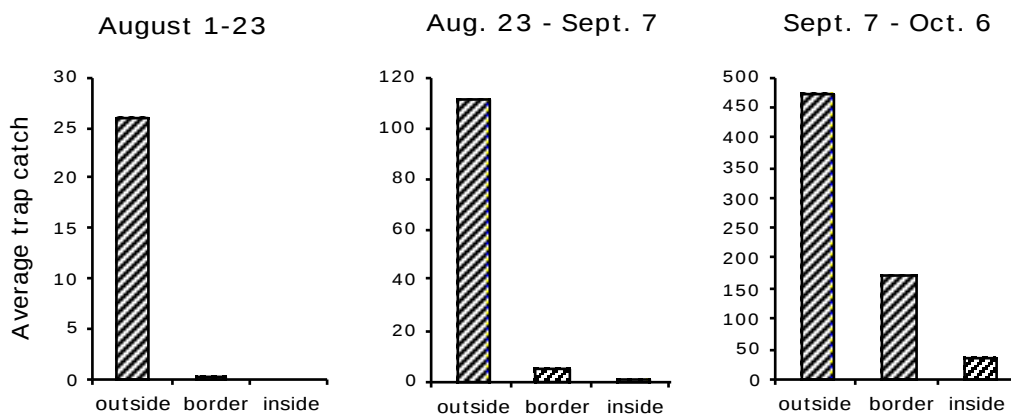


Figure 2. Catch in pheromone traps positioned along the transects outside, inside or at the border of the mating disruption treated area during different periods since start of the experiment.

On the average 7.4 larval colonies per tree ( $n=48$ ) were recorded in the plantation before the treatment. Considering that most trees were small (mean 2.4 m in height) this represents a high population level and some of the trees were completely defoliated. This was confirmed by the defoliation census performed in the autumn: The proportion of inspected shoots that was consumed by the larvae inside the treated area was  $0.37 \pm 0.36$  ( $n=20$ ) compared with  $0.73 \pm 0.32$  ( $n=34$ , six trees were omitted from the analysis as their height made the inspection unreliable) outside it. Although this difference was statistically significant,  $P < 0.05$  (t-test of arcsine square-root transformed proportions), the population densities before the application of mating disruption were at the same high level inside and outside the treated area.

The survival rate was rather low of the larvae collected for determination of sex ratios from the generation following the disruption experiment. In many of the individually reared colonies less than 10 individuals survived to cocoon formation (Figure 3). However, it was quite obvious that in all of the colonies, with a reasonable number of survivors, both sexes were represented. The average sex ratio (proportion of males) was not significantly different outside,  $0.49 \pm 0.28$  ( $n=24$ ) compared to inside  $0.46 \pm 0.30$  ( $n=24$ ) of the treatment. The results from the rearing of groups of colonies were similar (Figure 3), with a mean sex ratio of  $0.52 \pm 0.16$  ( $n=4$ ) outside and  $0.56 \pm 0.05$  ( $n=5$ ) inside the treated area.

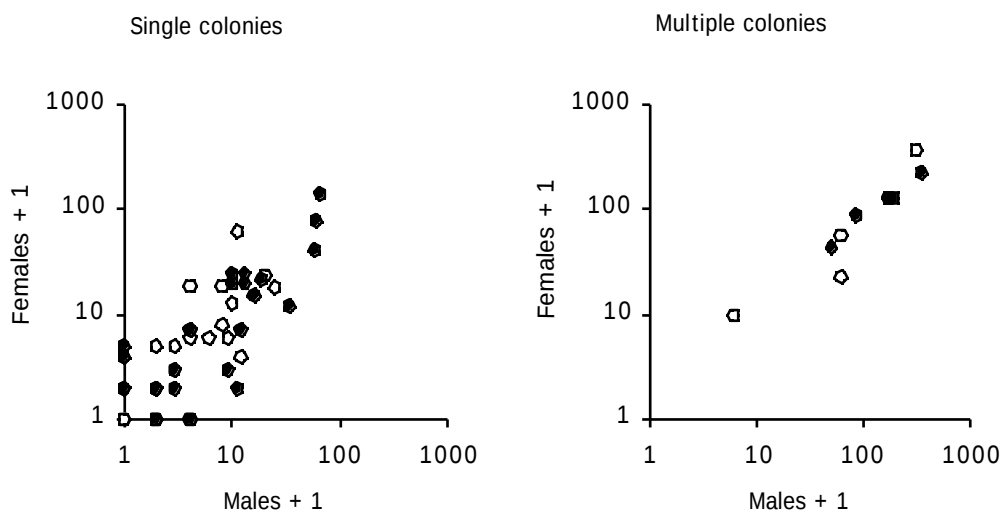


Figure 3. Number of male and female cocoons in colonies collected inside (filled) or outside the mating disruption treated area. Colonies were reared either singly or several together.

The results obtained in this experiment confirm those from earlier attempts to disturb the mating in *N. sertifer* (Anderbrant et al. 1995a, b, 1998); it is possible to reduce the

catches to very low levels without affecting the proportion of mated females inside the treatment, as measured by the sex ratio of colonies. However, it has also been clearly shown that females inside pheromone treated areas are only rarely able to attract males (Östrand *et al.* 1999). Instead the females seem to disperse if they have not been able to attract males during the first days in the area. These contradicting results may be explained by a massive immigration of mated females into the treated area. Because no female attractants are known, this hypothesis is difficult to test. An alternative hypothesis is that even if the movement of females was higher inside than outside the pheromone treatment (Östrand *et al.* 1999), the females sooner or later mated while still inside the treatment. A test of this hypothesis requires tracking the females inside a pine plantation, and a suitable technique for doing this will certainly be difficult to develop.

The pheromone dose used in this experiment is about 100 times lower than what is normally used in many lepidopteran mating disruption experiments (e.g. Leonhardt *et al.* 1996). Although the trap shut down and the reduction in mating frequency seemed to be nearly complete in the *N. sertifer* experiments, there is still a possibility that the final result would be different if higher pheromone doses were used. One could also hypothesise that the "restlessness" in females is not only a response to the lack of courting males, but also to the pheromone *per se*. A prerequisite for this is that females can perceive their own pheromone. Although this is the case for a few lepidopteran species (see e.g. Ljungberg *et al.* 1993), it is as yet unknown if it occurs among pine sawflies. It should also be observed that the experiments were done under epidemic conditions, while most successful disruption attempts involving moths have occurred when the population densities have been relatively low.

In conclusion, our results indicate that the size of the treated area, up to 25 ha, is of little importance for the success of mating disruption in *N. sertifer*. Possible improvements include use of higher dose and, maybe more important, treatment of less dense populations. Also treatment of isolated stands, where immigration is negligible, would possibly generate results that are easier to interpret.

## Acknowledgements

We thank Fredrik Östrand, Rolf Wedding and Erling Jirle for their excellent help with the fieldwork. Funding was provided by Carl Trygger Foundation, C. F. Lundströms Stiftelse, Stiftelsen Futura and the Swedish Council for Forestry and Agricultural Research (SJFR).

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