Performance of Pine Sawflies under Elevated Tropospheric Ozone

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Concentration of the phytotoxic air pollutant, ozone (O_3) is continually increasing in the lower layer of the troposphere. The purpose of this study was to compare performance of pine sawflies on Scots pine seedlings in ambient and future levels of ozone. Scots pine seedlings were grown in field fumigation system where the ozone doses in fumigated plots were 1.5–1.6 times the ambient level. Larvae of the European pine sawfly (*Neodiprion sertifer*) and *Gilpinia pallida* were reared on the foliage of Scots pine. The levels of resin acids and monoterpenes in foliage were analysed. There were no significant effects of ozone fumigation on sawfly performance or levels of defence compounds in pine foliage. The results suggest that the elevated ozone concentrations do not strongly affect the needle quality of young Scots pine and the importance of these two diprionid sawfly species as forest pests.

Keywords air pollution, *Neodiprion sertifer*, *Gilpina pallida*, Scots pine, resin acids
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1 Introduction

Pine sawflies are forest pests of greatest economic significance in Fennosscandia. Occasionally outbreaks of the European pine sawfly (*Neodiprion sertifer* Geoffr.) can spread over tens of thousands of hectares in Scots pine (*Pinus sylvestris* L.) forests. Outbreaks may last several years. Large-scale outbreaks appear at 20 to 30 years' intervals, while local outbreaks occur more frequently (Juutinen and Varama 1986). *Gilpinia pallida* (Klug) causes defoliation to young pine stands. Environmental stresses may reduce the capacity of host trees to produce defensive substances leading to greater susceptibility of trees to sawfly damage (Larsson and Tenow 1984).

Ozone is a phytotoxic air pollutant that show an elevating trend in the lower layers of the troposphere (Runeckles and Krupa 1994). It is known to decrease plant carbon gain by reducing photosynthetic rate and leaf area and accelerating senescence of plants (Chappelka and Chevone 1992, Greitner at al 1994). Ozone-induced disturbances in metabolism of conifers are indicated e.g. by reduced levels of carbohydrates (Paynter et al 1990, Friend et al. 1992, Kainulainen et al 1994), increased levels of free amino acids (Dohmen et al 1990), and induced formation of pathogenesis-related proteins (Kärenlampi et al 1994).

Carbohydrates (e.g. soluble sugars) promote growth of insect larvae (Albert and Parisella 1985). Starch content has been generally considered a good indication of conifer vigor after stress (Webb 1981). Herbivorous insects exposed to air pollution stress have been shown to change in feeding or oviposition behaviour. The Mexican bean beetle preferred bean plants exposed to sulfur dioxide or ozone (Endress and Post 1985), the gypsy moth had a variable preference response to oak seedlings under elevated ozone concentrations (Jeffords and Endress 1984) and willow leaf beetle showed an increased feeding preference for ozone-fumigated eastern cottonwood (Jones and Coleman 1988). Ozone may cause a significant reduction in the rate of photosynthesis, which decreases leaf carbon concentrations. According to the theory of carbon/nutrient balance (Bryant et al. 1983, Herms and Mattson 1992) leaf carbon concentrations can directly affect the level of carbon-based defensive compounds, e.g. monoterpenes and resin acids. The mechanism of ozone as an environment stress factor is not clearly known, but raising ozone levels can be predicted to decrease foliage carbon/nitrogen ratio and promote the consumption of herbivores.

The aim of the present study is to unravel the possible effect of elevated tropospheric ozone concentration on the larval performance of two pine sawfly species on Scots pine in open field, and by means of these experiments to estimate the pest status of the sawfly species under future atmospheric conditions.

2 Materials and Methods

The effect of tropospheric ozone on needle quality of Scots pine was tested by rearing two species of diprionid sawflies (*N. sertifer* and *G. pallida*) (Hymenoptera: Diprionidae) on 3-year-old seedlings in an open-field fumigation system at the University of Kuopio. Bare-rooted pine seedlings (seed origin: Leppävirta) were planted in 7.5 l plastic pots in a mixture of sand and fertilized and limed peat (2:1, v/v) in 1992, and in quartz sand in 1994. Seedlings were sufficiently watered during growing season and fertilized with full-nutrient fertilizer (N 32 kg ha^{-1} in 1992 and N 45 kg ha^{-1} in 1994).

There were two replicate plots with a diameter of approx. 10 m in with ambient ozone levels and two similar plots with elevated ozone levels. Ozone was produced from pure oxygen with ozone generators (Model 500 and OZ 500, ozone generator, Fisher, Bonn Germany) and released to exposed area through perforated plastic tubes in upwind position. The target ozone concentration at the center of exposed plots was 2 times the ambient level, monitored near control plots. The ozone fumigation started in May in both years. The total ozone dose in fumigated plots was 1.5 and 1.6 times the ambient dose, in 1992 and 1994, respectively (Table 1). The ozone concentrations in the center of the plots were measured with an ozone analyzer (Model 1008-RS, Dasibi Environmental Corp., Glendale, California). More details of the fumigation system are given by Wulff et al. (1992).

Table 1. Mean monthly ozone concentrations (ppb, 7 h mean) at the open-field experiments. Maximum daily 7h-means of ozone concentrations are presented in parentheses.

Month	Ozone concentration		
	Ambient	O_3 block 1	O_3 block 2
1992			
May	41 (58)	51 (84)	48 (74)
June	35 (53)	47 (68)	56 (103)
July	27 (47)	41 (75)	48 (77)
August	24 (43)	33 (65)	38 (58)
September	22 (39)	35 (58)	36 (64)
1994			
May	31 (40)	45 (64)	45 (61)
June	30 (41)	48 (64)	48 (66)
July	33 (52)	53 (73)	51 (79)
August	30 (44)	49 (71)	50 (73)
September	23 (35)	39 (62)	40 (65)

Ten Scots pine seedlings per plot were used in bioassays with *N. sertifer* and 16 seedlings with *G. pallida*, in 1992 and 1994, respectively. In 1994, half of the seedlings per plot were treated with the fungicide propiconazole to control ectomycorrhizal infection. The interactions between larval performance and mycorrhizal status of pine roots will be reported later.

The sawfly species used in the bioassays are gregarious and univoltine (Schwenke 1982). The sawfly cultures were collected from field. *N. sertifer* originated from northern Finland and *G. pallida* from southern Finland, since either of these species has not appeared on outbreak level in the Kuopio area recently. In 1992, relative growth rate (RGR = $(\ln(w_2) - \ln(w_1)) / t$, w_2 = final mass, w_1 = initial mass, t = duration of the growth trial (days)) and survival of *N. sertifer* larvae was determined. The bioassay lasted for 17 days, starting in June 25. The larvae were reared in mesh bags in groups of 10 larvae on 10 pine seedlings per plot. Bioassay with third instar larvae of *G. pallida* larvae was accomplished with detached branches in plastic containers (Lyytikäinen 1993) during a seven-day period, starting in August 8, 1994. The effect of dead larvae was estimated by making an assumption, that they lived until the middle of a bioassay period. Relative growth rate (RGR), survival, total consumption (TC) were determined. Food utilization indices, relative consumption rate (RCR), efficiency of conversion of ingested material (ECI) and approximate digestibility (AD) were calculated according to Waldbauer (1968):

TC = dry weight of plant material eaten

RCR = dry weight of plant material eaten / (mean larval dry weight × time)

ECI = 100 × larval dry weight gain / dry weight of plant material eaten

AD = 100 × (dry weight of plant material eaten – dry weight of frass) / dry weight of plant material eaten

For TC measurement average needle weight of each seedlings was determinated by weighing. The weight of totally consumed needles was estimated by multiplying the average weight by the numbers of eaten needles. The weight of consumed biomass of partly eaten needles was estimated by measuring the over-left needle parts, and using the difference between an average needle and an uneaten needle part as an estimate.

To determine the chemical quality of needle material with gas chromatography-mass spectrometry (Kainulainen et al. 1994), current needles for resin acid analysis were sampled in mid-September 1992, and at the time of *G. pallida* larval feeding in 1994 in liquid nitrogen.

3 Results

Ozone did not have significant ($p > 0.05$) effect on the survival and the relative growth rate of *N. sertifer* -larvae (Fig. 1). The larval weights of *G. pallida* tended to be lower in elevated ozone concentration, but the relative growth rate (RGR) attained higher. However, there were no significantly ($p > 0.05$) different values under the same treatment (Fig. 2). The treatments had no effects

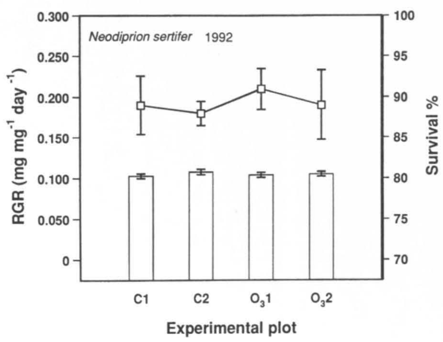

Fig. 1. Relative growth rate (± S.E.) (bar) and survival (± S.E.) (line) of *Neodiprion sertifer* larvae in 1992. Experimental plots: C1 = ambient air, plot 1, C2 = ambient air, plot 2, O_31 = elevated ozone, plot 1, O_32 = elevated ozone, plot 2. n = 10.

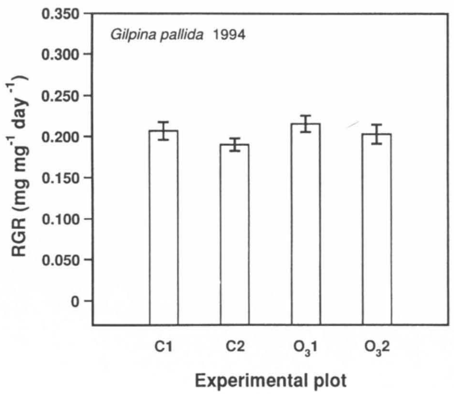

Fig. 2. Relative growth rate (± S.E.) *Gilpina pallida* larvae in 1994. Experimental plots are explained in Fig 1. n = 16.

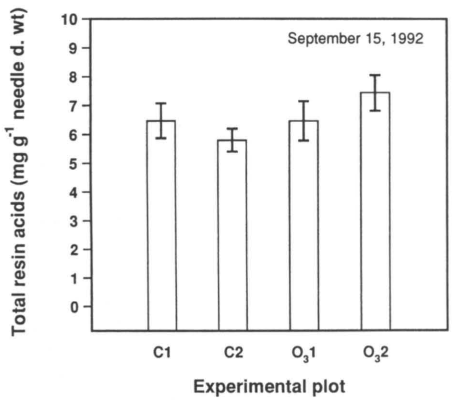

Fig. 3. Levels of total resin acids (± S.E.) in the current-year needles of Scots pine seedlings at the end of the growing season 1992. Experimental plots are explained in Fig 1. n = 6

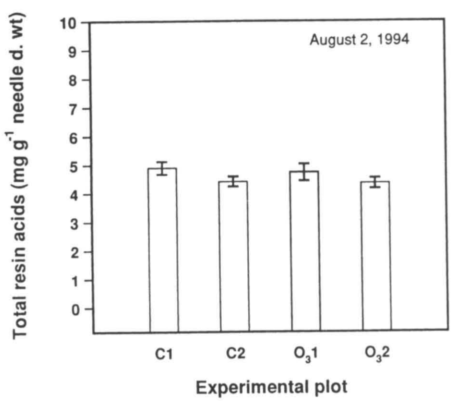

Fig. 4. Levels of total resin acids (± S.E.) in the current-year needles of Scots pine seedlings during feeding experiment in 1994. Experimental plots are explained in Fig 1. n = 16.

on larval survival, since only very few larvae were dead during a bioassay period. The values of relative consumption rate (RCR), total consumption (TC), approximate digestibility (AD) and growth efficiency (ECI) declined slightly in elevated ozone concentration, but did not reach a statistical significance.

There were no differences in total (Figs. 3 and 4) or individual resin acid concentrations between the treatments.

4 Discussion

The current results from the field experiments suggest that the elevated ozone concentrations in the field do not strongly affect the needle quality of young Scots pine and the performance of these two diprionid sawfly species. Spatial variation of microclimate between the plots in the experimental field may affect suitability of pine foliage to sawflies more than ozone dose.

One further explanation for the lack of responses in sawflies may be their chewing feeding behaviour. Insects belonging to different feeding guilds may have differences in stress response (Larsson 1989). Cambium feeders, represented by bark beetles and sucking insects like aphids have been more sensitive to stress-induced changes in host plants than chewing insects that do not separate chemical fractions in their food as efficiently. Studies of herbivorous insects on plants exposed to air pollutants, especially to SO_2 and NO_2 have supported this hypothesis (Whittaker 1994).

Scots pine seems to be relatively resistant against ozone, because ozone exposures in chamber experiments does not affect aphid growth (Brown et al. 1993) or reproduction (Kainulainen et al 1994). However, the effect of ozone fumigations on insect performance has been complex and it seems to depend on the exact pattern of fumigation (Whittaker 1994). Therefore, comparison of insects performance in current ozone levels to elevated levels may give more reliable estimates of the ecosystem effects of ozone than comparison of filtered air to constant ozone concentration in chamber experiments using short term fumigations. The seedlings in this study were exposed to elevated ozone concentrations several weeks and the levels followed the natural diurnal variation of ozone levels.

Outbreak species may be less affected by foliage quality than uncommon species (Hanski and Otronen 1985, Lyytikäinen 1994). Different types of stress with variability in time and dosage scale may result in different plant responses, e.g. variations in concentrations of phenolic compounds (Larsson et al. 1986). Such environmental stresses that alter plant carbon/nutrient balance would affect most strongly plant-herbivore interactions. It is obvious, that applied ozone fumigation or propiconazole treatment are not this kind of stresses. This conclusion is supported by the fact that we did not find any ozone-induced response in the levels of oleoresins in pine foliage.

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