A Plant Notices Insect Egg Deposition and Changes Its Rate of Photosynthesis

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Scots pine (Pinus sylvestris) is known to change its terpenoid metabolism in response to egg deposition by the sawfly Diprion pini (Hymenoptera, Diprionidae). Three days after egg deposition, parts of the pine twig adjacent to the egg-laden one are induced to emit volatiles, which attract egg parasitoids. In this study, we investigated whether egg deposition by this sawfly affects pine photosynthesis. Measurements of photosynthesis were taken from untreated control twigs and from pine twigs adjacent to egg-laden ones (i.e. systemically oviposition-induced twigs) for a period of 3 d starting after egg deposition. The net photosynthetic rate of oviposition-induced pine twigs was lower than that of untreated control twigs, whereas the respiration rate of pine twigs was not affected by egg deposition. CO₂ response curves of oviposition-induced twigs tended to be lower than those of controls. The potential rate of electron transport (Iₘₚₙₐₓ) and the maximum rate of Rubisco activity (Vₘₚₙₐₓ) were calculated from the data of the CO₂ response curves. Iₘₚₙₐₓ of oviposition-induced twigs was significantly lower than that of controls at day 1 after egg deposition, while the difference diminished from day 2 to day 3. A similar pattern was observed for Vₘₚₙₐₓ. Light response curves of oviposition-induced twigs were significantly lower than those of untreated ones during 3 d of measurements. Stomatal conductance was slightly lowered by egg deposition. When considering photosynthetic activity as a physiological currency to measure costs of induction of plant defense, the effects of insect egg deposition on gas exchange of pine are discussed with respect to known effects of insect feeding on the photosynthesis activity of plants.

Induced responses of plants to herbivore feeding damage have been studied extensively. These responses include changes in plant chemical composition, phenomenology, morphology, growth, and photosynthesis (for review, see Karban and Baldwin, 1997). Effects of herbivory on photosynthesis have been studied both on a local scale at the damaged leaf tissue and on a systemic scale by investigating photosynthetic activity of undamaged leaves adjacent to the damaged ones. Local effects measured at leaf tissue structurally damaged by mesophyll feeders, such as Diptera, Hemiptera, or Acari, or at leaf tissue right next to feeding holes produced by chewing insects often show that photosynthetic activity is reduced (Welter, 1989; Zangerl et al., 2002; Haile and Higley, 2003). Many studies on systemic effects of herbivory measured at undamaged leaves adjacent to the sites where chewing herbivores removed leaf material show an enhancement of the photosynthetic rate (Welter, 1989; Zangerl, 1999).

In comparison to the effects of insect feeding on plant metabolism, little is known about how a plant responds to insect egg deposition. Gall insects are known to disturb the inner architecture of a leaf by inserting their eggs (Hilker et al., 2002b). In a few cases, insect egg deposition was shown to induce a hypersensitive response of plant tissue, a well-known response of plants to phytopathogens (Shapiro and DeVay, 1987; Balbyshev and Lorenzen, 1997). Specific pea (Pisum sativum) lines were shown to form neoplasms in response to bruchid egg deposition (Doss et al., 1995, 2000). During the last years, several studies have shown that insect egg deposition induces a change of plant volatiles, thereby attracting egg parasitoids. This induction of volatiles by insect egg deposition is known to occur locally at the site of egg laying and systemically at plant tissue adjacent to the oviposition site (Meiners and Hilker, 2000; Hilker et al., 2002a; Colazza et al., 2004). In contrast to insect feeding, egg deposition of free-living herbivorous insects is investigated with respect to its effects on the plant’s primary metabolism.

In this study, we investigated whether gas exchange of Scots pine (Pinus sylvestris) is affected by egg deposition of the sawfly Diprion pini, which often occurs in high population densities on pine (Pschorr-Walcher, 1982, 1988). Larvae of D. pini may heavily damage pine forests by their feeding activity, while adults do not feed upon the plant. The secondary metabolism of Scots pine is known to be changed by egg deposition of D. pini. Pine twigs carrying eggs are induced to emit volatiles that attract the eulophid egg parasitoid Chrysonotomia ruforum, which kills the eggs of the herbivore. Hilker et al. (2002a, 2002b) interpreted this oviposition-induced release of volatiles attracting egg parasitoids as a preventive induced defense strategy acting prior to feeding damage. The attractive volatiles are emitted both from parts of the...
pine twigs carrying eggs (local induction) and from parts free of eggs but adjacent to the egg-laden parts (systemic induction; Hilker et al., 2002a). The terpenoid volatile pattern of systemically oviposition-induced pine twigs changes quantitatively after an induction time of 3 d compared to controls (Mumm et al., 2003).

To examine the effect of egg deposition by *D. pini* on the primary metabolism of Scots pine, net photosynthesis activity of systemically oviposition-induced pine twigs and of untreated, egg-free ones were compared. To obtain information about the effect of egg deposition on the potential electron transport (*J* max), Rubisco activity (*V* max), and stomatal conductance, CO₂ response curves were measured. In order to gain further information about the light response, pine twigs were also subjected to decreasing light intensities and light response curves were generated.

**RESULTS**

**Continuous Measurement of Gas Exchange**

The net photosynthetic rate of Scots pine twigs was significantly affected by egg deposition of the sawfly *D. pini* (Fig. 1). Systemically oviposition-induced twigs showed a significantly lower rate than controls during all days of the measurements. Both in treated and control twigs, the net photosynthetic rate and respiration rate decreased during the 3-d measurement period. There was no interaction observed between treatment and time (days; Table I). Thus, the oviposition-induced twigs showed no stronger decrease of the net photosynthetic rate than the controls with increasing time. The respiration rate, which also decreased during the period of measurement, was not affected by the oviposition treatment.

**CO₂ Response Curves**

Both oviposition-induced and control pine twigs significantly responded to increasing CO₂ concentrations by enhancing their net photosynthetic rates (Fig. 2). However, the CO₂ response of oviposition-induced twigs was slightly lower than that of controls (*P* = 0.055; Table II). The CO₂ response of the pine twigs did not change from day 1 to day 3. A combined effect of treatment and time was detected, i.e. the slight difference of oviposition-induced and control twigs decreased from day 1 to day 3.

**A* max, A* 350, J* max, and V* cmax**

The parameters *A* max, *A* 350, and *J* max calculated from the CO₂ response curves differed significantly at day 1 between oviposition-induced twigs and controls, while *V* cmax only tended to be lower in oviposition-induced twigs than in untreated controls. At day 2, differences between oviposition-induced and control twigs decreased with respect to these four parameters and vanished at day 3. The *V* cmax/*J* max ratio remained statistically unchanged on all days (Table III).

**Light Response Curves**

Both treated and control twigs significantly raised their net photosynthetic rates with increasing light intensity (Fig. 3). However, the light response of oviposition-induced twigs was significantly lower than that of untreated twigs. Light response did not significantly change from day 1 to day 3, indicating that the responsiveness of the twigs to light was stable during the measurement. A slight combined effect of treatment and time was detected, i.e. the difference of oviposition-induced twigs and controls (*P* = 0.07; Table II) tended to decrease from day 1 to day 3.

![Figure 1. Net photosynthetic rate (mean ± se) of systemically oviposition-induced twigs of Scots pine and untreated controls. Continuous measurements during 3 d. Light saturation of approximately 1,100 μmol m⁻² s⁻¹ during the light period (18 h), no light during the dark period (6 h), and a CO₂ concentration of 350 μmol mol⁻¹ were given.](image-url)
light response of oviposition-induced and untreated twigs was significantly different on day 1 when comparing data measured at light saturation and an ambient CO₂ concentration of 350 μmol mol⁻¹ (A₃50; Table III).

Stomatal Conductance

Stomatal conductance was slightly, but not significantly, lower in oviposition-induced twigs than in controls (Table IV; Fig. 2). From day 1 to day 3, stomatal conductance decreased significantly in both treated and control twigs. A combined effect of treatment and time was detected, i.e. the slight difference of oviposition-induced twigs and untreated controls decreased from day 1 to day 3.

DISCUSSION

This study investigates the effect of egg deposition by a free-living herbivorous insect on plant photosynthesis activity. Our data clearly show that insect egg deposition on Scots pine induces a decrease of...
photosynthetic activity in parts of the pine twig immediately adjacent to the site of egg deposition. These data raise numerous physiological and ecological questions. How can this reduction of photosynthetic activity be explained from a physiological perspective? Which factors cause this reduction? From an ecological point of view, is there a causal link between the reduction of photosynthetic activity and the induction of terpenoid volatiles by egg deposition? Can insects detect the differences of gas exchange of oviposition-induced and noninduced pine twigs and exploit the detected differences?

Physiological processes caused by water deficiency might have affected the photosynthetic activity of oviposition-induced pine twigs. The female pine sawfly slits the pine needle tangentially prior to egg laying, thereby causing desiccation of egg-laden pine foliage (Codella and Raffa, 2002). This water loss might explain the slightly lower stomatal conductance of oviposition-induced pine twigs compared to egg-free ones and thus contribute to the down-regulation of photosynthesis activity in egg-laden pine twigs. The difference between stomatal conductance of egg-laden pine twigs and egg-free ones diminished from day 1 to day 3. This may be due to the fact that we studied cut pine twigs. It is well known that cutting twigs leads to accelerated aging, water deficiency, and subsequent decrease of photosynthesis (e.g. Moldau et al., 1993; Richardson and Berlyn, 2002). Aging and water deficiency of both test and control twigs are indicated by the decrease in the net photosynthetic rate and stomatal conductance during the 3-d measurement period. Thus, effects on stomatal conductance and photosynthetic activity induced by egg deposition might interfere with effects caused by cutting the twigs.

Even though egg deposition by sawflies may cause desiccation of pine needles and thus affect stomatal conductance and photosynthetic activity, water deficiency does not seem to be the only factor causing a decrease in photosynthetic activity of egg-laden pine

<table>
<thead>
<tr>
<th>Table III. Different parameters calculated from CO2 and light response curves</th>
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</thead>
<tbody>
<tr>
<td>Calculation of parameters after the C3 photosynthesis model (Farquhar et al., 1980; Medlyn et al., 2002). See Figure 2, A to C, and Figure 3. Mean values ± sd of systemically oviposition-induced twigs of Scots pine and untreated controls are given. Levels evaluated by the Wilcoxon matched pairs test are presented. Amax, Light-saturated (PPFD 1,100 μmol m−2 s−1) rate of net photosynthesis, measured at CO2-saturated (2,000 μmol mol−1) concentration; A350, light-saturated rate of net photosynthesis, measured at CO2 concentration of 350 μmol mol−1; Imax, potential electron transport rate at Amax conditions; Vmax, maximum rate of Rubisco activity at Amax conditions.</td>
</tr>
<tr>
<td>Measurement</td>
</tr>
<tr>
<td>--------------</td>
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<tr>
<td>CO2 response curve</td>
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<td></td>
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<td></td>
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<tr>
<td></td>
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<tr>
<td>Light response curve</td>
</tr>
</tbody>
</table>

Table II. Repeated-measures ANOVA of CO2 response and light response

Net photosynthetic rate (CO2 and light response curves) during a 3-d measurement period of systemically oviposition-induced twigs of Scots pine and untreated controls were compared (Figs. 2 and 3). df, Degrees of freedom; MS, mean squares; F, F value, F test.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Source</th>
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<th>MS</th>
<th>F</th>
<th>P</th>
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<td>103.613</td>
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<td>7</td>
<td>1,105.203</td>
<td>47.706</td>
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<tr>
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<td>Treatment × day</td>
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<td>45.027</td>
<td>4.128</td>
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<td>Treatment × CO2</td>
<td>7</td>
<td>36.322</td>
<td>4.943</td>
<td>0.001</td>
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<tr>
<td>Light response curve</td>
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<td>6.582</td>
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<td></td>
<td>Day</td>
<td>2</td>
<td>0.791</td>
<td>0.602</td>
<td>0.563</td>
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<td>Light</td>
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<td>225.907</td>
<td>44.136</td>
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<td>Treatment × day</td>
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<td>3.343</td>
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<td>11.138</td>
<td>6.207</td>
<td>0.001</td>
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</table>

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twigs. Rubisco amount, activity, or kinetic properties (as described by $V_{\text{cmax}}$; Sage, 1994) and the regeneration capacity of this enzyme (as described by $J_{\text{max}}$; Long, 1991) affect photosynthetic activity. Insect egg deposition on Scots pine led to a significant decrease in $J_{\text{max}}$ at day 1 of the measurements. Also, $V_{\text{cmax}}$ tended to be lower in oviposition-induced twigs compared to controls 1 d after egg deposition (Table III). However, the effects of egg deposition on Rubisco diminished on days 2 and 3 of the measurements. While feeding damage by herbivores is known to affect expression of genes related to photosynthesis (Arimura et al., 2000), it is unknown so far whether insect egg deposition and the specific plant wounding associated with oviposition are able to influence expression of genes involved in the regulation of photosynthesis.

Methyl jasmonate can down-regulate genes involved in photosynthesis such as Rubisco, whereas genes encoding enzymes of secondary metabolism are up-regulated (Reinbothe et al., 1994; Hermann et al., 2001; Cheong and Yang, 2003). Secondary metabolism of terpenoids in pine and numerous other plants is well known to be up-regulated by methyl jasmonate (Mumm et al., 2003, and refs. therein). Application of methyl jasmonate to Scots pine has the same effect on egg parasitoids as oviposition. Also, treatment of pine twigs with methyl jasmonate induces the emission of specific terpenoid volatiles that render pine odor attractive to the egg parasitoid C. ruforum (Hilker et al., 2002a), even though the volatile pattern induced by methyl jasmonate is not fully identical with the volatile pattern induced by insect egg deposition (Mumm et al., 2003). We still do not know whether the reduction of photosynthesis activity in systemically oviposition-induced pine twigs is also mediated by methyl jasmonate.

From an ecological perspective, photosynthesis activity is one currency among several others to measure costs of plant defense (Cipollini et al., 2003, and refs. therein). Several studies address costs and benefits of plant responses induced by herbivore feeding damage (Dicke and Sabelis, 1992; Karban et al., 1997; Agrawal et al., 1999; Heil and Baldwin, 2002; Dicke and Hilker, 2003; Zangerl, 2003): this study investigates what oviposition-induced plant responses cost. Gershenzon (1994) considered the metabolic costs of terpenoid accumulation in higher plants. Scots pine is known to emit increased amounts of ($E$)-$\beta$-farnesene after egg deposition of D. pini (Mumm et al., 2003). This change of the terpenoid volatile pattern attracts egg parasitoids, killing the eggs (Hilker et al., 2002a; Mumm and Hilker, 2005). Is this defense costly?

A greater allocation of resources to defense may lead to a reduced allocation of resources to tolerance, and vice versa, because defense is expected to involve metabolic costs at the expense of growth (Herms and Mattson, 1992). Induced plant resistance to herbivores has been described by two basic forms, defense and

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Light response (net photosynthesis rate) of systemically oviposition-induced (black squares) twigs of Scots pine and untreated controls (white squares) at day 1 to day 3 (A–C). Mean values ± s.e. are given. Ambient CO$_2$ concentration, 350 µmol mol$^{-1}$ with decreasing PPFD in eight steps (each lasting 10 min) from 1,100 to 0 µmol m$^{-2}$ s$^{-1}$. Days 1 and 2 with $n = 8$; day 3 with $n = 7$.

<table>
<thead>
<tr>
<th>Source</th>
<th>$df$</th>
<th>MS</th>
<th>$F$</th>
<th>$P$</th>
</tr>
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<tbody>
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<td>9,107.855</td>
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<td>Day</td>
<td>2</td>
<td>5,011.298</td>
<td>822.155</td>
<td>0.015</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>7</td>
<td>118.707</td>
<td>31.989</td>
<td>0.003</td>
</tr>
<tr>
<td>Treatment $\times$ CO$_2$</td>
<td>2</td>
<td>1,994.224</td>
<td>211.947</td>
<td>0.003</td>
</tr>
<tr>
<td>Treatment $\times$ day</td>
<td>7</td>
<td>5.138</td>
<td>2.717</td>
<td>0.095</td>
</tr>
</tbody>
</table>

**Table IV.** Repeated-measures ANOVA of stomatal conductance

Stomatal conductance during a 3-d measurement period of systemically oviposition-induced twigs of Scots pine and untreated controls were compared (Fig. 2, D–F). $df$, Degrees of freedom; MS, mean squares; $F$, $F$ value; $P$, $P$ test.
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However, studies on the oviposition behavior of the concentrations characteristic of a flowering plant. Agrawal et al., 2004). In these terms, induction of synthesis to compensate for the herbivore damage (Karban and Baldwin, 1997; Nabeshima et al., 2001; AGR and Higley, 2003). However, while our results show that insect egg deposition on pine leads systemically to a reduction of photosynthetic activity, herbivore feeding often induces systemically or at the canopy level an increase of photosynthesis (Welter, 1989; Zangerl, 1999; Nykänen and Koricheva, 2004). A systemic enhancement of photosynthesis activity close to sites damaged by chewing herbivores may be due to extrinsic and intrinsic factors. An important extrinsic factor is the greater exposure of the remaining leaves to light because of the removal of other leaf tissue. Intrinsically, the change of source/sink relationships by leaf damage may result in increased photosynthesis rates (Zangerl, 1999). Herbivores that remove leaf tissue alter the amount of source tissue without affecting the amount of sink tissue, e.g. roots and stems. Photosynthesis of the remaining tissue of undamaged leaves adjacent to damaged leaves may increase to compensate for the demands of the sink tissue (Welter, 1989).

A possible adaptation to reduction of photosynthesis in oviposition-induced pine twigs does not only need consideration from the plant’s perspective, but also from that of the insect. Could it be possible that the egg parasitoid or the herbivorous sawfly uses the photosynthesis changes for orientation or host location? Some herbivorous insects have been shown to be very sensitive toward CO₂ gradients. For example, the moth Cactoblastis cactorum is able to detect CO₂ concentration 5 mm above the host plant surface. Female moths probe the plant surface with their CO₂ sensor, thus probably examining the suitability of the host plant. Most eggs are laid on the most vigorous plants (Stange et al., 1995). Furthermore, Thom et al. (2004) showed that CO₂ plays an important role in the foraging behavior of the nectar-feeding moth Manduca sexta, which prefers surrogate flowers that emit CO₂ concentrations characteristic of a flowering plant. However, studies on the oviposition behavior of the butterfly Pieris rapae could not detect any preferences for plants with higher gas exchange activities (Langan et al., 2001, 2004). For parasitoids of eggs of herbivorous insects, no sensitivity for CO₂ has been reported. The elucidation of the use of reduced CO₂ assimilation in systemically oviposition-induced pine twigs will need further study, as well as the sensitivity of egg parasitoids for CO₂.

MATERIALS AND METHODS

Plants and Insects

Branches of Scots pine (Pinus sylvestris) were detached from 15- to 30-year-old trees in a forest near Berlin, placed in water, and brought into the laboratory where the stems were cleaned and sterilized according to the method of Moore and Clark (1968) prior to measurement. The sawfly Diprion pini was reared continuously in the laboratory on cut pine twigs as described by Bomhoff and Ramakers (1976) and Eichhorn (1976) at 25°C ± 1°C, 18-h-light/6-h-dark cycles.

Plant Treatment

Two small pine twigs (about 20 cm in length) were cut from a branch. The cut end was placed in water. One twig was used for induction by oviposition (treatment); the other was kept untreated as a control. Since a test and a control twig were always cut from the same branch, they were considered a paired sample.

For treatment, females of D. pini laid eggs on the lower half of a twig for a period of 1 d at the abiotic rearing conditions given above. When at least four egg masses had been laid onto the lower half of the twig, the upper, egg-free half of the twig was used to start measurements of photosynthesis (i.e. day 0; see description of measurements below). Thus, the upper, systemically oviposition-induced part of the twig was used for measurements (for further treatment details, see Hilker et al., 2002a; Mumm et al., 2003). Control twigs were kept at the same conditions as treated twigs, but without any contact with sawflies. For measurements, the upper halves of control twigs were also used.

Gas Exchange Analyzer

Gas exchange was measured using compact mini cuvette systems (CMS 400; Walz, Effeltrich, Germany) equipped with input humidity control (KF-18/2 and RSV-42; Walz) measuring gas cooler and lighting units (FL 440; Walz) in constant environmental conditions (25°C, vapor pressure deficit 1.4 kPa, photosynthetic photon flux density [PPFD] approximately 1,100 mol m⁻² s⁻¹, wind speed 1.9 m s⁻¹). Two mini cuvette systems were available. With one of the systems, gas exchange of the treated twig was measured, and with the other, measurements of the control were conducted simultaneously. The systems were connected to differential nondispersive infrared gas analyzers (IRGA) for water vapor and CO₂ (BINOS 100; Fisher-Rosemount, Hasselroth, Germany), respectively.

Pellet-controlled climate units (KG 022; Walz) with flanged Plexiglas cuvettes (MK-022/A; Walz) were provided with air taken from outside the laboratory. Relative humidity (55%) inside the Plexiglas cuvette (500 cm³) was controlled by passing saturated air with water vapor through an input humidity control (dew-point temperature 15.4°C). The CO₂ concentration was controlled by passing air over soda lime columns, retaining the naturally occurring CO₂ and adding the concentration needed from a CO₂ gas container. CO₂ partial pressure was varied to eight CO₂ concentrations (50, 150, 250, 350, 550, 700, 1,000, and 2,000 μmol mol⁻¹ CO₂) by using a CO₂/N₂ gas-mixing system (GMA-2; Walz). Nonlinearity of the differential IRGA systems to background CO₂ concentration was accurately described with nonlinear equations. Calibration was accomplished with precision mixing pumps (Type 1 SA 27/2a; Wooshoff, Bochum, Germany).

The flow rate through the cuvettes was regulated by thermal mass flow meters (1,000 cm³ min⁻¹). The setup was illuminated by a halogen lamp (FL 440; Walz) providing about 1,100 μmol m⁻² s⁻¹ during the light phase (18 h/day). Environmental conditions inside the cuvette and leaf temperature were...
monitored continuously with a microprocessor-controlled data acquisition system.

A mini cuvette was placed over the upper, egg-free part of the treated twig. The lower, egg-laden part of the twig was left outside the cuvette. The opening where the upper half of the twig entered the mini cuvette was closed by a sealant (Terosit; Teroson GmbH, Heidelberg). Accordingly, the upper half of the untreated control twig was similarly placed into the cuvette of the second measurement system with the lower half left outside. The cut ends of the twigs were supplied with tap water during measurement.

Continuous Measurements of Gas Exchange

Measurements of a systemically oviposition-induced twig (n = 8) and the respective control (n = 8) taken from the same branch were conducted simultaneously. The day when oviposition-induced and untreated control pine twigs were placed in the mini cuvette systems is referred to as day 0. From this time on, the gas exchange was continuously measured for a period of further 3 d. Changes in the difference between the controlled input of CO2 and water partial pressures into the cuvette and outputs from the cuvettes were monitored continuously with the IRGA. At day 3, one of the control twigs no longer showed photosynthesis. Since test and control twigs were considered paired samples, this pair was removed from further statistical analyses (thus, n = 7 at day 3 of the measurements).

CO2 and Light Response Curves

On days 1 to 3, each morning 3 to 4 h after the onset of the light cycle, a light response curve was determined. For this purpose, the PPFD was lowered in eight steps (each lasting 10 min) from 1,100 to 0 μmol m⁻² s⁻¹ with a constant CO2 concentration of 350 μmol mol⁻¹. The measurement was conducted at the end of each 10-min period. After measuring these light responses, plants were provided with light of approximately 1,100 μmol m⁻² s⁻¹ and a CO2 concentration of 350 μmol mol⁻¹ for 1 h. After this acclimatization period, measurements for the CO2 response curve were conducted. For this purpose, the CO2 concentration was changed in eight steps (each lasting 10 min) from 50 to 2,000 μmol mol⁻¹ with light saturation of approximately 1,100 μmol m⁻² s⁻¹. Again, the measurement was conducted at the end of each 10-min period. After measurements for light and CO2 response curves, the continuous measurements of water vapor and CO2 were restarted.

Data Calculation and Statistics

All data were calculated on the basis of projected leaf area measured with a leaf area meter (model Li-3100; LI-COR, Lincoln, NE). The net photosynthetic rates were calculated after von Caemmerer and Farquhar (1981) and Field et al. (1989). The potential electron transport rate (Iₘₐₓ) and maximum rate of Rubisco activity (Vₘₐₓ) were calculated from data of the CO2 response curves without stomatal influences (Farquhar et al., 1980; Farquhar and von Caemmerer, 1982).

Data obtained from simultaneously conducted measurements of a treated twig and its respective control from the same branch were considered paired samples. Data on photosynthesis and respiration rates of continuous measurements for the CO2 response curve were conducted. For this purpose, the CO2 concentration was lowered in eight steps (each lasting 10 min) from 50 to 2,000 μmol mol⁻¹ with light saturation of approximately 1,100 μmol m⁻² s⁻¹. Again, the measurement was conducted at the end of each 10-min period. After measurements for light and CO2 response curves, the continuous measurements of water vapor and CO2 were restarted.

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LITERATURE CITED


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