Herbicide Chlorsulfuron Decreases Assimilate Transport Out of Treated Leaves of Field Pennycress (Thlaspi arvense L.) Seedlings

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ABSTRACT

Treatment of field pennycress (Thlaspi arvense L.) leaves with the herbicide chlorsulfuron resulted in a decrease in the export of assimilate. Twelve hours after a spot application of 1 microgram, assimilate translocation was 70% of that in control leaves. In excised leaves treated with chlorsulfuron the total amounts of sugars and free amino acids were 150 and 170%, respectively, of the amounts in control leaves, 30 hours after herbicide treatment. The amount of sucrose was 247% of that in control leaves. The increase in the concentration of sucrose in the chlorsulfuron-treated leaves, combined with the absence of an effect of chlorsulfuron on carbon dioxide fixation, suggests that the decrease in assimilate transport is not due to an effect on the synthesis of assimilates, but rather to an effect on their movement out of the leaves. Supplying branched-chain amino acids to the field pennycress seedlings prior to the application of chlorsulfuron prevented the occurrence of the effects described.

dose was translocated out of treated leaves of several species in a 24-h period. Export of less than 5% of the absorbed chlorsulfuron in 24 h has been reported for Canada thistle and perennial sow thistle (Sonchus arvensis) (8) and for Tartary buckwheat (Fagopyrum tataricum [L.] Gaertn.) (2).

The limited phloem mobility of chlorsulfuron cannot be explained in terms of the ability of plant tissue to accumulate the herbicide (6, 7) but, instead, is attributed to an effect on assimilate translocation. The objective of the research described in this paper was to understand the effect of chlorsulfuron on the translocation of assimilates out of treated leaf tissue of field pennycress (Thlaspi arvense L.) seedlings. In addition, the rates of uptake and of translocation of the herbicide and the extent of its metabolism were determined.

MATERIALS AND METHODS

Plant Material

Field pennycress (Thlaspi arvense L.) seedlings were grown from seed in 175-mL styrofoam cups filled with horticultural-grade vermiculite. The cups were subirrigated with half-strength Hoagland solution (15) modified to contain 1.5 μg/mL iron. The plants were grown in a growth cabinet at 23°C/19°C day/night temperatures and with an 18-h photoperiod. Fluorescent lights supplied a photosynthetic photon flux density of 800 μE m⁻²s⁻¹. The RH was 50%.

In experiments that included the branched-chain amino acids L-valine, L-leucine, and L-isoleucine, these amino acids were supplied to the seedlings through the roots. Six to 8 h before herbicide treatment the vermiculite was washed off the roots and the seedlings were mounted with styrofoam plugs in holes in a sheet of PVC (6 mm thick) in such a manner that the roots were hanging in half-strength Hoagland solution, with or without 1 mM concentrations of each of the three amino acids.

Herbicide Application

Chlorsulfuron, ¹⁴C-labeled (phenyl-¹⁴C [U]; specific activity 152.1 Bq nmol⁻¹; radiochemical purity 98.9%) or technical product (95% pure), was applied in 8 to 10 droplets (total volume 10 μL) of application solution consisting of 10 mM Na₂HPO₄-citric acid buffer (pH 8.0), with 10% (v/v) tetrahydrofuran and 0.1% (v/v) Citowett Plus surfactant. The droplets were placed on the third true leaf of seedlings that had five to seven true leaves. Control treatments consisted of 8 to
Absorption and translocation of [14C]chlorsulfuron in field pennycress seedlings. The data, means and standard errors, are the results of two runs with three seedlings per treatment. 'Recovered' radioactivity includes surface wash and all radioactivity in the tissue.

10 droplets (10 μL total volume) of application solution without chlorsulfuron.

Assimilation Chamber

The 14CO2-labeling experiments were conducted in a custom-built circular assimilation chamber that could accommodate up to six intact field pennycress seedlings in such a manner that the roots, a single leaf of each seedling, and the remaining parts of the shoots were in three completely separate compartments (24). Sealing between the inner compartment and the outer compartment, and between the outer compartment and the root compartment, was done with a cellulose filler around the stems and the petioles. Hydrocarbon-free air of known CO2 concentration was supplied independently to the single-leaf compartment and the shoot compartment at a rate that maintained a CO2 concentration of 400 ± 25 μL/L within each compartment. The roots of the seedlings were immersed in 15 mL nutrient solution. The entire chamber was placed in a temperature-controlled water bath at 25 ± 0.5°C. Incandescent and fluorescent lights supplied a photosynthetic photon flux density of 300 μE·m⁻²·s⁻¹ at the level of the seedlings in the chamber. The RH in the chamber was 40%.

14CO2-Labeling

14CO2 was generated from [14C]NaHCO3 and lactic acid outside the chamber, and was circulated via a peristaltic pump through a closed loop connected with the appropriate compartment. During the 30-min labeling period the CO2 concentration within the compartment was kept constant by pump-
CHLORSULFURON DECREASES ASSIMILATE TRANSPORT

Figure 3. Exudation profiles of excised field pennycress leaves treated with 0 or 1 μg of chlorsulfuron, excised, and exposed to 14CO2 (30 min pulse) 6, 12, or 24 h later. The data, means, and standard errors, are expressed as percentages of the cumulative total amount of 14C activity or sugars exuded by control leaves during the exudation period, and are the results of two runs with three leaves per herbicide dose.

Table I. Effect of Chlorsulfuron on Assimilation and Exudation by Excised Leaves of Field Pennycress Seedlings, and Allocation of 14C Activity in Sugar and Amino Acid Fractions Extracted from These Leaves following Exposure to 14CO2

The leaves were excised and exposed to 14CO2 (30 min pulse; 330 min chase period) 24 h after the application of 0 (blank) or 1 μg of chlorsulfuron. The data are mean results of two runs with three leaves per treatment.

<table>
<thead>
<tr>
<th>Parameter and Fraction</th>
<th>Units*</th>
<th>Chlorsulfuron</th>
<th>Herbicide Effect as Percentage of Controlb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Blank</td>
<td>1 μg</td>
</tr>
<tr>
<td>Assimilation</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>14C activity assimilated</td>
<td>dpm mg⁻¹</td>
<td>31,759</td>
<td>33,943</td>
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<td>Exudation</td>
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<tr>
<td>Total 14C activity</td>
<td>dpm mg⁻¹</td>
<td>1,477</td>
<td>763</td>
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<tr>
<td>Total sugars</td>
<td>nmol mg⁻¹</td>
<td>2.02</td>
<td>1.06</td>
</tr>
<tr>
<td>Leaf extracts</td>
<td></td>
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</tr>
<tr>
<td>Sugars</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total sugar</td>
<td>nmol mg⁻¹</td>
<td>34.0</td>
<td>49.4</td>
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<tr>
<td>Total 14C activity</td>
<td>dpm mg⁻¹</td>
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<td>Specific activity</td>
<td>dpm nmol⁻¹</td>
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<td>Amino acids</td>
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<td></td>
</tr>
<tr>
<td>Total amino acids</td>
<td>nmol mg⁻¹</td>
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<td>28.6</td>
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<tr>
<td>Total 14C activity</td>
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<td>2,315</td>
</tr>
<tr>
<td>Specific activity</td>
<td>dpm nmol⁻¹</td>
<td>138</td>
<td>88</td>
</tr>
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</table>

*Weights (mg) refer to tissue fresh weights.  
NS, not significant; * significant at P ≤ 0.05; ** significant at P ≤ 0.01.
by rinsing the treated leaf three times with 5 mL 10% (v/v) ethanol (5). Total radioactivity in the rinse solutions was quantified by LSS. The vermiculite was washed off the roots and the plants were divided into four parts, i.e., roots, treated leaf, shoot apex, and the remaining part of the shoot. The plant parts were stored at −20°C until they were combusted.

**Metabolism of Chlorsulfuron**

The extent of chlorsulfuron metabolism in seedlings was determined (3) 12 or 24 h after application of 500 Bq [14C]-chlorsulfuron. Plants were ground twice in deionized water, the homogenate then was centrifuged (10,000 g, 10 min) and filtered. Protein was removed by precipitation with cold acetone and further centrifugation (1,000 g, 10 min). The acetone was evaporated, the remaining extract was lyophilized, and the dried residue was dissolved in 1 mL deionized water. Chlorsulfuron and its metabolites were separated by loading an aliquot of the extract on a C18 reverse-phase preparative chromatography column and were eluted with a water/methanol (with 0.1% [v/v] formic acid) step gradient. Only the fraction that was eluted at 45% (v/v) methanol contained unmetabolized chlorsulfuron.

**Exudation of Assimilate by Excised Leaves**

Six 1.5-mL centrifuge tubes were installed in the inner compartment of the assimilation chamber. Each tube was connected with a line to a 5-mL syringe outside of the chamber. Excised leaves (petioles recut under water), treated with 0 or 1 μg of chlorsulfuron, were placed with their petioles in the tubes containing a 5 mm phosphate buffer (pH 6.0) with 0.5 mM EDTA (10, 13, 16). The CO2 concentration in the chamber was maintained at 400 μL/L. 14CO2 was applied for 30 min at 6, 12, or 24 h after the herbicide treatment. The bathing solution was changed 1, 3, 5, 7, 9, 11, and 13 h after the start of the 14CO2 application. The solutions containing the exudates were put in culture tubes and weighed. A 700-μL aliquot was taken for the determination of 14C activity by LSS. The amount of sugar in the exudates was determined by an anthrone-based colorimetric method (22). The data were expressed as percentages of the appropriate controls.

**Exudation of Assimilates following Application of [3H]- and [14C]Sucrose**

The third leaf of field pennycress seedlings was treated with 0 or 1 μg of chlorsulfuron. The treatments were applied to a 1-cm2 oval-shaped area on the adaxial surface of the leaves. Twelve or 24 h later, the treated leaves were excised and placed with their petioles in 1.5-mL centrifuge tubes containing 0.5 μM phosphate buffer (pH 6.0) with 0.5 mM EDTA. [14C]-Sucrose was applied to the area of the leaf that had been treated with chlorsulfuron, and [3H]Sucrose was applied to the area of the leaf that had been treated with blank application solution. The bathing solution was changed 2, 4, 6, 8, 10, and 12 h after the application of the radiolabeled sucrose. Twelve h after application of the radiolabeled sucrose the leaves were washed with 10% ethanol to remove unabsorbed sucrose (5). The leaves were frozen with liquid nitrogen and stored at −20°C until they were combusted in a biological sample oxidizer.

**Incorporation of 14C into Excised Leaves**

Leaves were excised and exposed to 14CO2 (185 kBq), 24 h after treatment with 0 or 1 μg chlorsulfuron. Following a 30-min labeling period and a 330-min chase period the leaves were frozen with liquid nitrogen and stored at −20°C.

**Extraction of Leaf Tissue**

The various fractions were extracted according to a procedure adapted from Dickson (9). The tissue was homogenized in MCW. Following a phase separation, the upper water-alcohol phase was transferred to a boiling flask and reduced to dryness under vacuum. The residue was dissolved in 1 mL water and stored at −20°C.

**Fractionation of the Water-Alcohol Fraction**

The water-alcohol fraction was fractionated using an ion exchange chromatography method (1). The whole fraction was loaded on a cation exchange column (4.5 mL; Dowex 50X8-400; hydrogen form) connected in series with an anion

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**Figure 4. Exudation profiles of excised field pennycress leaves treated with 0 or 1 μg chlorsulfuron, excised, and exposed to 14CO2 (30 min pulse) 6, 12, or 24 h later. Six to 8 h before herbicide application the seedlings were placed with their roots in half-strength Hoagland solution containing 1 mm L-valine, L-leucine, and L-isoleucine. The data, means, and standard errors are expressed as percentages of the cumulative total amount of 14C activity or sugars exuded by control leaves during the exudation period, and are the results of two runs with three leaves per herbicide dose.**
exchange column (4.5 mL; Dowex 1X8-400; formate form). The neutral fraction, containing the sugars, was eluted with 20 mL water. The columns then were disconnected and the amino acid fraction was eluted from the cation exchange column with 60 mL of 3 M HCl. The remaining sugars were eluted from the anion exchange column with an additional 10 mL water. All eluents were reduced to dryness at 40°C. The residues were dissolved in 1 mL water and stored at -20°C.

**Analysis of the Sugar Fraction**

The total amount of sugar in this fraction was determined colorimetrically (22) and total radioactivity was determined by LSS. The individual sugars were separated by HPLC (300 x 7.8 mm Aminex HPX-87H column; ambient temperature; 20-μL sample loop; 0.01 N H2SO4 mobile phase; 0.8 mL/min flow rate; refractive index detection). They were collected in scintillation vials and 14C activity in each was determined by LSS.

**Analysis of the Amino Acid Fraction**

The total amount of amino acids in this fraction was determined according to the method outlined by Moore (18). All samples were assayed in duplicate.

**RESULTS AND DISCUSSION**

**Fate of Chlorsulfuron**

Chlorsulfuron was absorbed and translocated slowly (Fig. 1). Twenty-four hours after application of 3.3 nmol of chlorsulfuron, 2.4 nmol of herbicide were recovered in the leaf washes and 0.8 nmol were recovered from the tissue. Only 0.05 nmol were translocated out of the treated leaf. Total recovery was 97%. Due to the low specific activity of the radiolabeled chlorsulfuron and the small amount of chlorsulfuron exported to the shoot apical tissue, the amount of herbicide present in that tissue could not be determined accurately.

Chlorsulfuron was metabolized very slowly. At 24 h after application of 14C-labeled chlorsulfuron, 90% of the extractable 14C activity was associated with unmetabolized chlorsulfuron. In the metabolism experiments, on average, 92% of the absorbed 14C activity was extracted, and 93% of the applied activity was recovered.

**Assimilate Transport**

Intact leaves of chlorsulfuron-treated seedlings exported only 60 to 70% of the amount of 14C assimilates exported by intact leaves of control plants 12 or 24 h after herbicide treatment (Fig. 2). No such effect was observed 6 h after...
herbicide treatment. Export of assimilate by leaves adjacent to the herbicide-treated leaves was decreased only at 24 h after herbicide treatment. Chlorsulfuron had no effect on the total amount of $^{14}$C$_2$ assimilated by the plants.

Excised leaves of chlorsulfuron-treated seedlings exuded less assimilate, both in terms of $^{14}$C activity and sugars, than excised leaves of control plants 12 or 24 h after herbicide treatment (Fig. 3; Table I). At 6 h after chlorsulfuron application, only a decrease in the total amount of sugars that was exuded was observed.

The decrease in the export of $^{14}$C activity by the chlorsulfuron-treated leaves following exposure to $^{14}$CO$_2$ confirms previous findings (7) that indicate that the herbicide has an effect on assimilate translocation. The agreement between the results obtained with intact seedlings and those with excised leaves occurred despite large differences in the amount of $^{14}$C activity translocated or exuded in the control plants or excised leaves. In intact seedlings, 16% of the total $^{14}$C activity assimilated was translocated out of the third leaf, 2 h after it had been exposed to $^{14}$CO$_2$. Excised leaves exuded only 4.6% of the total amount of $^{14}$C activity assimilated during the 13-h period following exposure to $^{14}$CO$_2$.

In intact seedlings treated with the branched-chain amino acids L-valine, L-leucine, and L-isoleucine, chlorsulfuron caused little or no decrease in assimilate transport (Fig. 4). This suggests that the decrease in assimilate transport is related directly to the mechanism of action of the herbicide. The details of how the inhibition of the biosynthesis of branched-chain amino acids, and as a consequence presumably that of proteins, is related to the decrease in the transport of assimilates out of the herbicide-treated leaf have not emerged in this study. One possibility is that the effect on assimilate translocation may be associated with the depletion of proteins involved in the transport of sucrose into the phloem.

The effect of chlorsulfuron on the assimilate transport system is restricted to a localized area close to where the herbicide is applied and enters the leaf. Chlorsulfuron decreased the amount of $^{14}$C activity and reducing sugars that was exuded by excised leaves following application of $[^{14}$C]-

<table>
<thead>
<tr>
<th>Sugar and Parameter</th>
<th>Units$^a$</th>
<th>Chlorsulfuron Blank</th>
<th>Chlorsulfuron 1 µg</th>
<th>Herbicide Effect as Percentage of Control$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose Total amount</td>
<td>nmol mg$^{-1}$</td>
<td>1.7</td>
<td>4.2</td>
<td>247</td>
</tr>
<tr>
<td>Specific activity</td>
<td>dpm nmol$^{-1}$</td>
<td>1.021</td>
<td>734</td>
<td>75</td>
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<td>Glucose Total amount</td>
<td>nmol mg$^{-1}$</td>
<td>11.9</td>
<td>16.0</td>
<td>144</td>
</tr>
<tr>
<td>Specific activity</td>
<td>dpm nmol$^{-1}$</td>
<td>369</td>
<td>408</td>
<td>102</td>
</tr>
<tr>
<td>Fructose Total amount</td>
<td>nmol mg$^{-1}$</td>
<td>5.7</td>
<td>7.4</td>
<td>140</td>
</tr>
<tr>
<td>Specific activity</td>
<td>dpm nmol$^{-1}$</td>
<td>557</td>
<td>739</td>
<td>127</td>
</tr>
</tbody>
</table>

$^a$ Weights (mg) refer to tissue fresh weights. $^b$ NS, not significant; * significant at P ≤ 0.05; ** significant at P ≤ 0.01.
once the sucrose has been loaded into the phloem tissue, it should be translocated, unless there is an effect on the unloading in the sink tissue, or there is an obstruction in the phloem between source and sink (6, 11). The fact that the chlorsulfuron-induced decrease in assimilate transport also occurs in excised leaves strongly suggests that the herbicide has an effect on the transport of assimilates into the phloem in the source tissue rather than an effect in the sink tissue.

The effect of chlorsulfuron on assimilate translocation appears to be mediated differently than the one caused by glyphosate, a herbicide also reported to decrease phloem transport (11). The decrease induced by glyphosate is due to a decrease in the net carbon exchange rate, which is attributed to a decrease in the concentration of ribulose bisphosphate (12, 19). The absence of an effect of chlorsulfuron on the total amount of $^{14}$CO$_2$ assimilated by the treated leaves suggests that in the time period studied (up to 24 h) in these experiments, the herbicide had no measurable effect on photosynthesis. This confirms earlier reports (14). The accumulation of sucrose in chlorsulfuron-treated leaf tissue suggests an effect analogous to that of $p$-chloromercuribenzenesulfonic acid, which inhibits the loading of sucrose into the phloem (17).

Concomitant with the increase in sugars in chlorsulfuron-treated leaf tissue is an increase in the concentration of amino acids. Both the size of the pools of the individual amino acids (or of groups of biosynthetically related amino acids), and the flux of carbon atoms through them are under metabolic control. We postulate that a decrease in the biosynthesis of one group of amino acids, i.e. the branched-chain amino acids, might not necessarily result in an immediate decrease in the total amount of amino acids in the leaf tissue. On the contrary, a decrease in the synthesis of the branched-chain amino acids might first of all result in a decrease in protein synthesis, due to a lack of availability of these amino acids. This decrease in protein synthesis might result in an accumulation of amino acids other than the branched-chain ones. The overall effect could be an increase in the total amount of amino acids in the tissue. In the absence of data on the composition of the amino acid fractions and on the total amount of $^{14}$C activity incorporated in each amino acid, the details of the effect of chlorsulfuron on amino acid metabolism in field pennycress leaves remain speculative.

One of the consequences of chlorsulfuron-induced decrease in the rate of assimilate transport out of the treated leaves is a decrease in the rate at which the herbicide molecules themselves are translocated out of the treated tissue (11). Devine et al. (6) have suggested that on the basis of its physical-chemical properties chlorsulfuron should be translocated more readily than it is in whole plants. Assuming that chlorsulfuron has no direct effect on the loading of herbicide molecules into the phloem tissue, i.e. an effect not mediated through the inhibition of branched-chain amino acid biosynthesis, supplying the treated plant with branched-chain amino acids should increase the rate of chlorsulfuron transport.

The decrease in the export of assimilates out of leaves of field pennycress seedlings in which the synthesis of branched-chain amino acids has been inhibited by the herbicide chlorsulfuron suggests a close link between the continued availability and/or synthesis of these amino acids and assimilate export. The nature of this link remains to be determined.

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