Effect of Chlorophenoxy Herbicides on Soluble Sugars and on Pathogenesis by *Drechslera sorokiniana* in Sequentially Senescent Leaves of *Poa pratensis*

James P. Madsen and Clinton F. Hodges

Postdoctoral research fellow, Department of Agronomy, University of Kentucky, Lexington 400546-0091; and professor of horticulture and of plant pathology, Department of Horticulture, Iowa State University, Ames 50011.

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ABSTRACT

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In studies of the four most recently formed visible leaves on shoots of *Poa pratensis*, severity of leaf spot of *Drechslera sorokiniana* increased on progressively older leaves of control, 2-(2-methyl-4-chlorophenoxy)-propionic acid (MCPP)-, and 2-(2,4,5-trichlorophenoxy)propionic acid (2,4,5-TP)-treated plants. Each progressively older leaf generally was more severely diseased on herbicide-treated plants than on control plants, except for leaf three. Total soluble sugar content of leaves of plants treated with either herbicide was less than that of controls; sucrose, glucose, and fructose constituted the loss. Inoculation of the two youngest leaves of control plants also decreased total soluble sugars; inoculation of leaf three of control plants increased sugars and had no effect on leaf four. No correlation existed between total sugar content of uninoculated control leaves and severity of leaf spot after inoculation. Inoculation of leaves of all ages on plants treated with MCPP or 2,4,5-TP induced an increase in soluble sugars, primarily in glucose and fructose. The decrease in total soluble sugars and sucrose in leaves of MCPP-treated plants correlated with increased leaf spot severity; no correlations existed for 2,4,5-TP-treated plants. Herbicide-induced changes in the soluble sugar content of sequentially developing and senescing leaves are discussed relative to the hypothesis that factors that promote senescence may enhance leaf spot severity.

Leaf spot caused in *Poa pratensis* L. by *Drechslera sorokiniana* (Sacc.) Subram. and Jain (=*Helminthosporium sativum* P. K. and B.) may be stimulated or inhibited by chlorophenoxy postemergence herbicides (7,8). Recent studies have shown that 10^{-4} M 2-(2-methyl-4-chlorophenoxy)propionic acid (MCPP) applied to the soil preceding inoculation of leaves increases the percentage of diseased leaf area, whereas applications of 10^{-4} M 2-(2,4,5-trichlorophenoxy)propionic acid (2,4,5-TP) have mixed effects on disease expression (7,8,17).

Poa pratensis subjected to mowing maintains three or four visible leaves per shoot that range from immature to nearly senescent (19). Leaf spot is progressively more severe on sequentially older leaves of *P. pratensis* (8–10). Application of 10^{-4} M MCPP to the soil before inoculation increases leaf spot severity on each older leaf (8). Application of 10^{-4} M 2,4,5-TP to the soil inhibits leaf spot on the two oldest leaves and has no effect on leaf spot development on the two youngest leaves (8).

Chlorophenoxy herbicides have well-documented effects on leaf sugar content in herbicide-sensitive plant species (1,22). Amounts of sugars in leaves of sensitive herbicide-treated plants generally are decreased due to increased respiration and the inhibition of photosynthesis (13,18,22,28). MCPP and 2,4,5-TP affect leaf sugars of herbicide-tolerant *P. pratensis* in a manner similar to the effects of the herbicides on herbicide-sensitive plants (17). Applications of 10^{-4} M MCPP or 2,4,5-TP decrease quantities of total soluble sugars in leaves of *P. pratensis* (17). The total soluble sugar decrease induced by the herbicides correlates with increased percentages of diseased leaf area on a whole-shoot basis (17). Increased respiration, the loss of chlorophyll, and decreased photosynthesis are components of leaf senescence processes that decrease leaf sugar levels (28). Decreased leaf sugar levels have been associated with subsequent increases in leaf spot severity (11,16). The decrease of leaf sugars by MCPP or 2,4,5-TP could be a factor in promoting senescence of leaves of *P. pratensis* that may predispose progressively older leaves of the plant to more severe development of leaf spot on *D. sorokiniana*.

The studies reported here were undertaken to evaluate the effect of 10^{-4} M MCPP and 2,4,5-TP on the soluble sugar content of sequentially developing and senescing leaves of *P. pratensis* and on the severity of leaf spot on leaves of different ages.

MATERIALS AND METHODS

Poa pratensis 'Newport' was vegetatively propagated in a steamed mixture of loam and peat 2:1 in 7.6-cm-diameter plastic pots. Plants were grown in the greenhouse under 16 hr of light daily (daylight supplemented with incandescent lights) for 60 days before treatment. Cultures of *D. sorokiniana* (Sacc.) Subram. and Jain were maintained on 20 ml of 1% Czapek Dox broth in 3% (v/v) Bacto-agar in 100 × 15-mm sterile plastic petri dishes. Only 20-day-old cultures started from hyphal tips of isolates from diseased leaf tissue of *P. pratensis* were used (6).

Plants were treated with 40 ml (20 ml each, 4 and 2 days before inoculation) of 10^{-4} M 2-(2-methyl-4-chlorophenoxy)propionic acid (MCPP) or 2-(2,4,5-trichlorophenoxy)propionic acid (2,4,5-TP) or with distilled water (control) applied to the soil. The four youngest visible leaves of one shoot were inoculated with 5–10 conidia suspended in 0.02 ml of sterile distilled water at five positions, 1 cm apart, along a 10-cm section of the leaf approximately 5 cm from the leaf tip in a special inoculation apparatus (23). Uninoculated plants received 0.02 ml of sterile distilled water as a control. The concentration of conidial suspensions was determined with a particle counter (High Accuracy Products Corp., Montclair, CA 91763). Each treatment was conducted on 17 individual shoots per replication and was replicated three times. Plants were incubated for 6 days at 24 C under continuous fluorescent light (80–90 $\mu E/m^2/sec$), and then leaf samples were collected and evaluated for disease severity before being prepared for leaf sugar analysis.

Sugar content was determined for both inoculated and uninoculated leaves. Disease severity was evaluated as the percentage of diseased leaf tissue per inoculated 10-cm leaf section (8). The values from 17 shoots of each treatment for each leaf age

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were averaged to determine the disease severity of each treatment replication. The data presented are the means of three replicate values for each treatment.

The amount of sucrose, glucose, fructose, and raffinose in leaves of plants of each treatment were determined by a method described previously (17). Data were analyzed as a $3 \times 2 \times 4$ factorial design for the mean percentage of diseased leaf tissue and for the quantity of individual sugars and their total of the four sequentially aged leaves of each treatment. Mean comparisons were made according to Duncan's multiple range test.

RESULTS

There was no difference in the severity of leaf spot of the two youngest leaves of herbicide-nontreated *P. pratensis* (Fig. 1). However, leaf spot severity increased from the second to the third to the fourth leaf of the control (Fig. 1). Soil applications of MCPP enhanced leaf spot development on all except the third leaf, and applications of 2,4,5-TP stimulated disease on the second and fourth leaves only (Fig. 1).

Total soluble sugars of uninoculated, herbicide-nontreated control leaves 1, 2, and 4 were not different, but the total soluble sugar content of leaf 3 was less than that of the other leaves (Fig. 2). Soil applications of MCPP or 2,4,5-TP resulted in decreased amounts of total soluble sugars in uninoculated leaves, with no differences among leaf ages or between the two herbicides (Fig. 2). Total soluble sugars of inoculated control plants decreased in leaves 1 and 2, increased in leaf 3, and did not change in leaf 4 compared with those of uninoculated control plants (Fig. 2). Inoculation of plants treated with MCPP or 2,4,5-TP increased the total soluble sugars of leaves of each age except the youngest leaf of 2,4,5-TP-treated *P. pratensis* (Fig. 2).

The amount of sucrose in uninoculated *P. pratensis* mostly

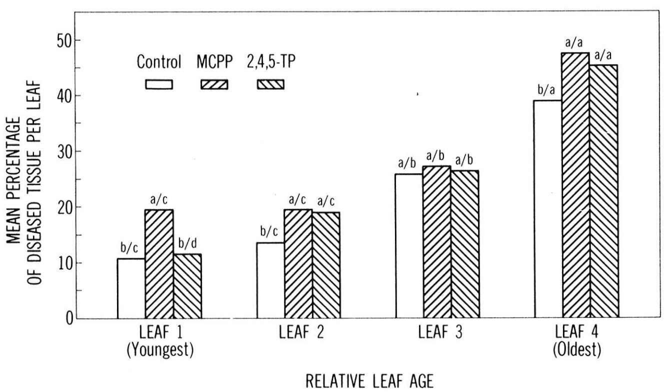

Fig. 1. Mean percentage of diseased leaf area of four sequentially aged leaves of control and herbicide-treated *Poa pratensis* inoculated with *Drechslera sorokiniana*. Values are the average of three replicates of 17 shoots each. Values with the same letter among control and herbicide treatments within relative leaf age (across a/) or among relative leaf age within control and herbicide treatments (across /a) are not significantly different according to Duncan's multiple range test ($P = 0.05$).

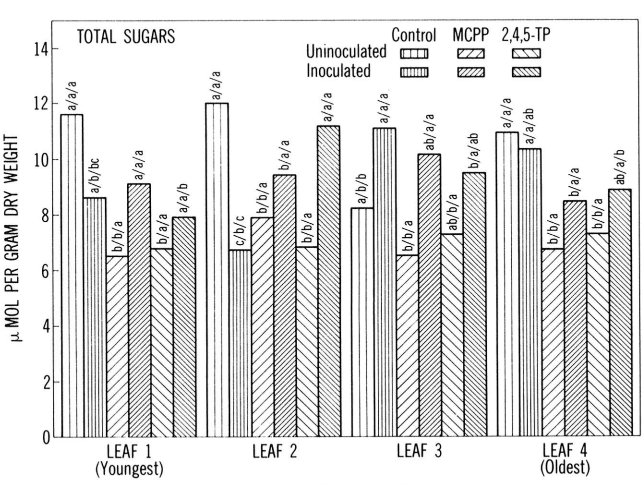

Fig. 2. Mean content of total soluble sugars of four sequentially aged leaves of control and herbicide-treated *Poa pratensis*, both uninoculated and inoculated with *Drechslera sorokiniana*. Values are the average of three replicates of leaf tissue of 17 shoots each. Values with the same letter among control and herbicide treatments within inoculation status and relative leaf age (across a/ /), between uninoculated and inoculated plants within control and herbicide treatments and relative leaf age (across /a/), or among relative leaf ages within control and herbicide treatments and inoculation status (across / /a/ /), are not significantly different according to Duncan's multiple range test ($P = 0.05$).

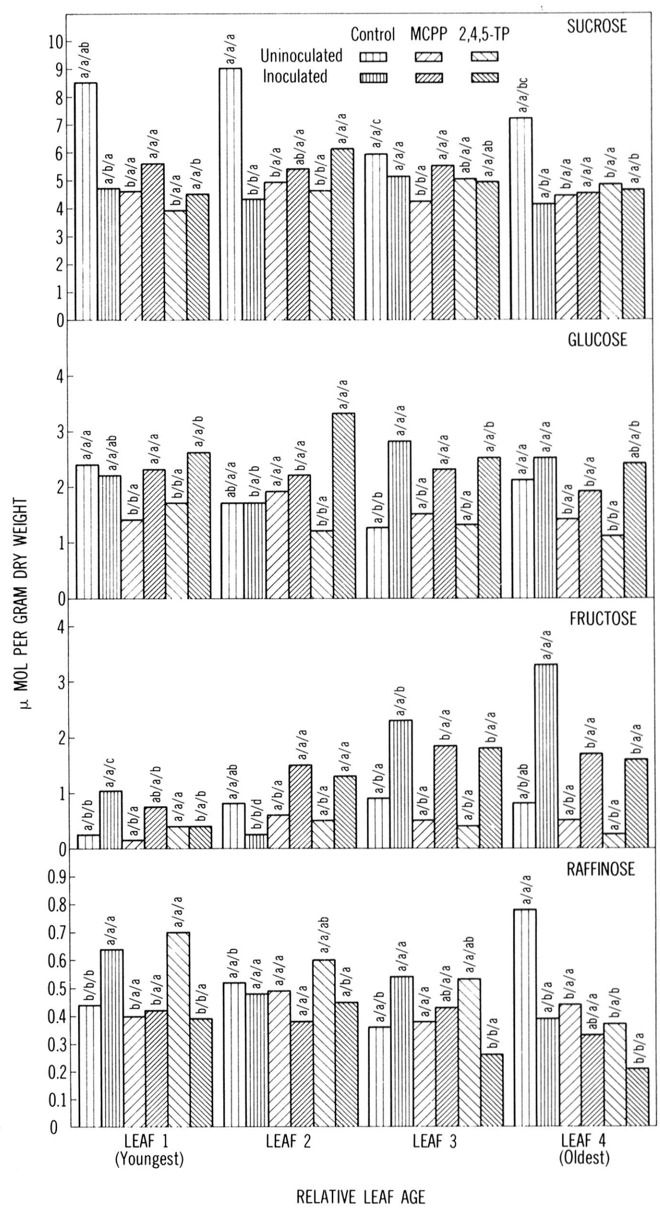

Fig. 3. Mean content of individual soluble sugars of four sequentially aged leaves of control and herbicide-treated *Poa pratensis*, both uninoculated and inoculated with *Drechslera sorokiniana*. Values are the average of three replicates of 17 shoots each. Values with the same letter among control and herbicide treatments within inoculation status and relative leaf age (across a/ /), between uninoculated and inoculated plants within control and herbicide treatments and relative leaf age (across /a/), or among relative leaf ages within control and herbicide treatments and inoculation status (across / /a) for each sugar are not significantly different according to Duncan's multiple range test ($P = 0.05$).

paralleled that of total sugars over the four leaf ages and both herbicide treatments (Fig. 3). However, inoculated control plants had decreased sucrose levels in leaves 1, 2, and 4 with no change in leaf 3, compared with uninoculated plants (Fig. 3). Inoculation of herbicide-treated plants had little influence on the sucrose content of leaves. Only leaf 3 of MCPP-treated plants and leaf 2 of 2,4,5-TP-treated plants showed an increase of sucrose after inoculation (Fig. 3).

The effects of leaf age and herbicide treatment on the glucose content of uninoculated control leaves of *P. pratensis* were consistent with those effects on total soluble sugars and sucrose except for a rise in glucose in leaf 2 of MCPP-treated plants (Fig. 3). The content of glucose in leaf 3 of controls increased after inoculation (Fig. 3). Inoculated plants had increased glucose in leaves 1 and 3 of MCPP-treated plants and all leaves of 2,4,5-TP-treated plants compared with uninoculated leaves (Fig. 3).

The amount of fructose in leaf 1 of uninoculated, herbicide-nontreated control plants was less than that of leaves 2, 3, and 4, which were not different (Fig. 3). Herbicides had no effect on the fructose content of uninoculated leaves of any age (Fig. 3). Inoculated control plants had increased fructose levels in leaves 1, 3, and 4 and had decreased fructose in leaf 2 compared with uninoculated controls (Fig. 3). Inoculated herbicide-treated plants had increased fructose in all leaves except leaf 1 of 2,4,5-TP-treated plants (Fig. 3).

The raffinose content of leaf 4 of uninoculated, herbicide-nontreated control plants was greater than that of the three younger leaves (Fig. 3). Herbicides had little effect on raffinose in uninoculated leaves. Application of 2,4,5-TP increased the raffinose content in leaf 1, and both MCPP and 2,4,5-TP decreased the amount of raffinose in leaf 4 (Fig. 3). Inoculated control plants had increased raffinose in leaf 1 and decreased raffinose in leaf 4 compared with uninoculated plants (Fig. 3). Inoculation of MCPP-treated plants had no significant effect on the raffinose content of leaves (Fig. 3), but inoculated 2,4,5-TP-treated plants had decreased raffinose in each leaf compared with uninoculated leaves of 2,4,5-TP-treated plants (Fig. 3).

Correlation coefficients were determined for the comparison of the percentage of diseased leaf tissue with the pre- and postinfection (uninoculated and inoculated) sugar content of sequentially older leaves of herbicide-nontreated control plants. The correlation for the preinfection (uninoculated) content of fructose vs diseased area of the four leaves of control plants was $r = +0.52$. The correlation for the postinfection (inoculated) quantities of fructose and raffinose vs diseased area of the four leaves of control plants were $r = +0.65$ and $r = -0.55$, respectively. Correlation coefficients for the comparison of the effects of MCPP and 2,4,5-TP on the content of leaf sugars with the effects of the herbicides on disease severity compared with the herbicide-nontreated controls were determined. The correlations for the preinfection quantities of sucrose and total soluble sugars in leaves of MCPP-treated plants vs the diseased leaf area of MCPP-treated, inoculated plants were $r = -0.62$ and $r = -0.52$, respectively. The correlation for the postinfection content of raffinose in leaves of 2,4,5-TP-treated plants vs the diseased leaf area of 2,4,5-TP-treated, inoculated plants was $r = +0.56$.

DISCUSSION

It has been hypothesized that sequential senescence of leaves of *P. pratensis* promotes the severity of leaf spot caused by *D. sorokiniana* (8–10). The results of this study support this hypothesis. The percentage of diseased leaf area of inoculated herbicide-nontreated control plants was greatest on the two oldest leaves (Fig. 1).

The application of 10^{-4} M MCPP to the soil before inoculation enhanced leaf spot on all except the third leaf, and 10^{-4} M 2,4,5-TP enhanced leaf spot on the second and fourth leaves (Fig. 1). It has been postulated that application of auxinlike postemergence herbicides to leaves may promote senescence and, thus, enhance leaf spot severity (8,9). Senescent leaves generally contain decreased amounts of sugars due to chlorophyll loss, reduced photosynthesis, and increased respiration (27). The third leaf of uninoculated, herbicide-nontreated control plants contained the least amount of sugar of the four leaves (Fig. 2). The oldest leaf (leaf 4) contained slightly less sugar than leaves 1 and 2 but more than leaf 3 (Fig. 2). The third leaf is fully expanded and is probably a major exporter of sugar within the plant. The rise in the quantities of sugars in the oldest leaf may be due to the hydrolysis of polysaccharides during the senescence process (27).

The preinfection (uninoculated) amount of total soluble sugars in the four sequentially older leaves of herbicide-nontreated control plants did not significantly correlate with the increasing severity of leaf spot on older leaves. A decrease in the amount of leaf sugars has been suggested to promote leaf spot of *D. sorokiniana* (11,16). Leaf spot of *P. pratensis* has been classified as a "low sugar" disease on the basis of correlations of low sugar levels with increased leaf spot severity (16). This direct relationship between leaf sugar content and leaf spot severity has been questioned (3,5,24). The "low sugar" concept does not seem to apply to the increased leaf spot severity on older leaves of control plants in this study.

Although no direct correlations existed between preinfection leaf sugar content and leaf spot severity of control plants, a relationship did exist between the influence of the herbicides on leaf sugars and leaf spot. MCPP and 2,4,5-TP decreased sugar amounts in leaves of herbicide-tolerant *P. pratensis* in a manner indicative of changes that occurred during leaf senescence (Fig. 2). The decrease in the preinfection content of sucrose and total soluble sugars in uninoculated leaves of MCPP-treated plants was significantly correlated with increased leaf spot of inoculated, MCPP-treated plants compared with the control (Fig. 2). High sugar levels inhibit the induction of cell-wall degrading enzymes in some host-parasite interactions (14,26). Conversely, the decrease in the content of leaf sugars after herbicide treatment possibly could stimulate the production of cell-wall-degrading enzymes after inoculation and facilitate early pathogenesis. The postinfection increase of leaf sugars in herbicide-treated plants could result from increased cell-wall degradation (Fig. 2).

The quantities of glucose and fructose in the two oldest leaves of herbicide-nontreated control plants increased after infection (Fig. 3). This increase of sugars corresponded with the most severely infected leaves (Fig. 1). Some pathogens are known to cause the formation of metabolic sinks at infection sites (12). The increased translocation of photosynthate to the most severely infected older leaves of control plants could explain the decrease in the amount of sugars in the two younger leaves after inoculation (Fig. 3). The potential facilitation of pathogenesis by the increased production of cell-wall degrading enzymes in all leaves of herbicide-treated plants with decreased sugar content at the time of inoculation also could result in greater sink activity. The presence of a metabolic sink at infection sites also might enhance the proposed herbicide-induced senescence, which would both stimulate leaf spot and increase sugars in leaves after infection (4,20).

The correlation coefficients indicate different effects of MCPP and 2,4,5-TP. MCPP has a significant influence on leaf sugars that was significantly correlated with the effect of the herbicide on leaf spot development on the four leaves. The effect of 2,4,5-TP on sugar content is similar to that of MCPP (Fig. 3), but no significant correlations existed relative to leaf spot. Application of 2,4,5-TP had no effect on leaf spot of the youngest leaf, whereas MCPP caused a rise in the percentage of diseased leaf area compared with that of the control (Fig. 1). The differences in activity of the two herbicides may in part be explained by differences in translocation and inactivation in *P. pratensis* of MCPP and 2,4,5-TP after application to the soil (2,15,25).

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