Madsen, James Patrick

INFLUENCE OF POSTEMERGENCE HERBICIDES ON THE PHYSIOLOGY OF PATHOGENESIS BY DRECHSLERA SOROKINIANA ON SEQUENTIALLY SENESCENT LEAVES OF POA PRATENSIS

Iowa State University PH.D. 1982

Influence of postemergence herbicides on the physiology of pathogenesis by *Drechslera sorokiniana* on sequentially senescent leaves of *Poa pratensis*

by

James Patrick Madsen

A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Departments: Horticulture
Plant Pathology, Seed and Weed Sciences
Co-majors: Horticulture
Plant Pathology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Departments

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1982

TABLE OF CONTENTS

GENERAL INTRODUCTION

Leaf spot on Poa pratensis L. caused by Drechslera sorokiniana (Sacc.) Subram. and Jain (= Helminthosporium sativum P. K. and B.) is a major disease in the north-central states. Leaf spot is one of several diseases caused by D. sorokiniana on grass species. Other diseases are leaf blight and seedling, crown and root rots (3, 4, 8, 16, 24, 39). These diseases may be chronic throughout the growing season.

Various cultural practices used in maintaining turf influence leaf spot. Irrigation (15) and nitrogen fertilization (6, 31) generally enhance leaf spot on P. pratensis. Mowing practices also may influence D. sorokiniana leaf spot (7, 11).

The use of postemergence herbicides to selectively eliminate weeds from turf with minimal toxicity to the grass species is an important cultural practice. The effects of postemergence herbicides on fungal pathogens and diseases have been reviewed extensively (1, 5, 9, 10, 18, 19, 20, 29, 36).

Phenoxy acid postemergence herbicides have various effects on D. sorokiniana leaf spot on P. pratensis. Many of the phenoxy herbicides enhance leaf spot particularly on older leaves where extensive chlorosis may occur (13). One phenoxy herbicide, 2,4,5-TP, inhibits leaf spot at certain concentrations (12, 13).

Leaf spot is influenced by light quality and photoperiod (26). Lighting conditions that tend to promote leaf senescence also promote leaf spot (14). This corresponds with increasing severity of leaf spot

on progressively older leaves of P. pratensis (13). A hypothesis has been proposed that select phenoxy herbicides may promote leaf senescence which enhances D. sorokiniana leaf spot (14).

Phenoxy acid herbicides are auxin-like compounds whose physiological effects on herbicide-susceptible plants have been the subject of several reviews (2, 21, 23). The physiology of these herbicides in herbicide-tolerant species is not well understood. Defining potential physiological effects is made difficult by limited absorption and translocation and by conjugation with plant constituents, side-chain degradation or lengthening, ring hydroxylation or cleavage and oxidation of phenoxy acids in herbicide-tolerant plants (23, 38). The herbicides are absorbed by tolerant plants and the herbicides or their metabolites could potentially influence the physiology of the plants (14).

An investigation of the effects of soil applied postemergence herbicides on nonstructural carbohydrate and free amino acid concentrations of leaves of P. pratensis could be indicative of an influence of the herbicides on intermediary metabolism in P. pratensis. Changes in concentrations of leaf sugars and amino acids are known to affect diseases caused by Drechslera sp. (17, 25, 33, 37).

Much work has been done relative to potential toxin(s) produced by D. sorokiniana (34). Cultures of D. sorokiniana contain many related sesquiterpenoid compounds that have inhibitory or stimulatory effects on plant growth (34). The role of the toxin(s) in pathogenesis by D. sorokiniana is unknown. Toxic substances produced by D. sorokiniana

could possibly interact with potentially herbicide-induced ethylene to produce the severe chlorosis associated with leaf spot of herbicide-treated P. pratensis (30).

An initial investigation of potential physiological effects of postemergence herbicides on herbicide-tolerant P. pratensis and the correlations of these effects with subsequent leaf spot severity is warranted. Research was conducted to study the influence of post-emergence herbicides on the physiology of pathogenesis by D. sorokiniana on leaves of P. pratensis.

EXPLANATION OF DISSERTATION FORMAT

The reader should be aware of the format of presentation of material in this dissertation. The format is approved by the Thesis Office at Iowa State University. The individual parts of the dissertation are written for submission to scientific journals. The general introduction and overall summary are intended to consolidate the individual papers into an integrated presentation of the various aspects of the research.

PART I. POSTEMERGENCE HERBICIDES AND THE PHYSIOLOGY OF PATHOGENESIS BY *DRECHSLERA SOROKINIANA* ON LEAVES OF *POA PRATENSIS*: WHOLE SHOOT NONSTRUCTURAL CARBOHYDRATES AND FREE AMINO ACIDS

INTRODUCTION

Leaf spot of Kentucky bluegrass (Poa pratensis L.) caused by Drechslera sorokiniana (Sacc.) Subram. and Jain (= Helminthosporium sativum P. K. and B.) is influenced by cultural practices such as nitrogen fertilization (5, 31) and mowing height (7, 11), and by such environmental factors as light (26) and temperature (15). Postemergence herbicides have recently been demonstrated to influence the severity of leaf spot (13). Postemergence herbicides generally stimulate leaf spot, but 2,4,5-TP selectively stimulates or inhibits leaf spot depending upon concentration of the herbicide and method of application (13).

Management of grasses used for turf includes applications of selective postemergence herbicides to herbicide-tolerant perennial grass species. The herbicides are absorbed and inactivated in tolerant plants by degradation, hydroxylation of the aromatic ring, or conjugation with plant constituents (25). The phenoxy acid herbicides generally function as auxin analogs and can influence carbohydrate and amino acid metabolism of treated susceptible plants (1, 18, 22, 24, 28, 37). The concentrations of leaf sugars are decreased as a result of increased respiration and the inhibition of photosynthesis (18, 24, 28, 37). Phenoxy herbicides decrease the protein and amino acid content in leaves of susceptible plants (1, 22). The effect of postemergence herbicides on the sugar and free amino acid content of leaves of a herbicide-tolerant species like P. pratensis is largely unknown.

Infection of leaves by fungi also affects leaf sugar and free amino acid concentrations. A metabolic sink may form at infection sites resulting in an increase in the concentration of sugars in infected leaves (9, 17). Fungal enzyme activity and the stimulation of host respiration also influence sugar concentrations in infected plants (4, 19). The concentration of free amino acids in leaves generally decreases initially with infection by Drechslera halodes, but later increases due to the activity of proteolytic enzymes of the pathogen (8).

The concentrations of sugars and amino acids in plants have been implied to have a direct influence on susceptibility to diseases by Drechslera sp. (16, 34, 36). Leaf spot of P. pratensis has been termed a "low sugar" disease based upon correlations of low leaf sugar concentrations with severe leaf spot (23). The direct effect of sugar concentration on Drechslera leaf spot has been questioned (6, 10, 31). However, the observed effects of postemergence herbicides on the severity of leaf spot of P. pratensis and the probable influence on sugar and free amino acid concentrations in the plant warrant an investigation into a possible correlation. Research was conducted to evaluate the effects of select postemergence herbicides on leaf spot severity and on leaf sugar and free amino acid concentrations of herbicide-tolerant P. pratensis as a preliminary step in determining the role of phenoxy acid herbicides in pathogenesis by D. sorokiniana.

MATERIALS AND METHODS

Poa pratensis 'Newport' was vegetatively propagated in a steamed 2:1 loam-peat mix in 7.6 cm plastic pots. Plants were grown in the greenhouse for 60 days prior to treatment under 16 hr of light (day light supplemented with incandescent lights). Cultures of Drechslera sorokiniana (Sacc.) Subram. and Jain were maintained on 20 ml of 1% Czapek Dox broth in 3% (v/v) Bacto-agar in 15x85 mm sterile plastic petri dishes. Culture virulence was maintained by isolating hyphal-tips from diseased leaf tissue of P. pratensis obtained by periodic inoculations. Only 20-day-old cultures were used in the study (12).

Plants were treated with 40 ml (20 ml each 4 and 2 days prior to incubation) 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 sterile distilled water at 5 positions 1 cm apart along a 10 cm section of the leaf approximately 5 cm from the leaf tip in a special inoculation apparatus (30). Uninoculated plants received 0.02 ml distilled water as a control. Conidia suspensions were prepared with an automatic particle counter (High Accuracy Products Corp., Montclair, CA 91763). Each treatment consisted of 17 individual shoots with 4 leaves each and was replicated 3 times. Plants were incubated for 6 days at 24 C under continuous fluorescent light (80-90 $\mu E\ M^{-2}\ sec^{-1}$) and then evaluated for disease severity followed by preparation for sugar and amino acid

extraction. Sugars and free amino acids were determined in leaf tissues from inoculated and uninoculated plants. Disease severity was evaluated on 10-cm lengths of leaf blades of inoculated plants by the method described by Hodges (14). Estimated lesion area was expressed as a percentage of the area of 4 leaves from one shoot. The values from the 17 shoots of each treatment were averaged to determine the disease severity of each treatment replication. The data presented are the means of these values for three replicates of each treatment.

The 10-cm leaf sections for each treatment replicate were collected, freeze-dried at -50 C for 48 hr and ground in a Wiley mill. This material was used for both sugar and amino acid analyses. Sugars were extracted in 80% ethanol for 4 hr. The extract was evaporated and chlorophyll was partitioned in 20 ml 1:1 ethyl ether-water. The aqueous phase was prepared for thin-layer chromatography (3). Sugars were absorbed onto Kieselguhr G (250 μm thick) buffered with 0.02 M sodium acetate. Sucrose, glucose, and fructose were separated with ethyl acetate and 65% isopropanol (65:35 v/v), and raffinose was separated with n-propanol, ethyl acetate, and water (40:50:10 v/v). All sugars were visualized with aniline-diphenylamine-phosphoric acid (21). Sugars were referenced to standard sugar concentrations, and relative concentrations were determined with a scanning diffuse reflectance densitometer (Kontes, Vineland, NJ 08360) and calculated by a computing integrator (Spectra-Physics, Santa Clara, CA 95051).

Free amino acids were extracted in 95% ethanol for 24 hr and in 3 aliquots of 80% ethanol for 24 hr each. Ethanol extracts were pooled and

air-dried. Chlorophyll was partitioned with 30 ml of 1:1 ethyl ether-water. The aqueous phase containing free amino acids was demineralized on a column of AG 50W-X8-H^+ ion exchange resin of 200-400 mesh (Bio-Rad Laboratories, Richmond, CA 94804) and eluted with 2 N ammonium hydroxide. The eluate was air-dried and analyzed on an amino acid analyzer. Data were analyzed as a 3x2 factorial design for the mean percentage of diseased leaf tissue, individual and total sugars, and individual and total free amino acids of leaves of one shoot of each treatment.

RESULTS

Application of 10^{-4}M MCPP to the soil prior to inoculation of the leaves with conidia of D. sorokiniana resulted in a significantly higher percentage of diseased leaf area than the inoculated control (Table 1.1). Soil drench applications of 10^{-4}M 2,4,5-TP resulted in a diseased leaf area percentage less than that of MCPP-treated plants and greater than that of control plants, but not significantly different in either case (Table 1.1).

Table 1.1. Mean percentage of diseased leaf area of control and herbicide-treated Poa pratensis inoculated with Drechslera sorokiniana[a]

	Herbicide treatment		
	Control	MCPP	2,4,5-TP
Mean percentage of diseased leaf area	22.3 b	28.4 a	25.6 ab

[a]Mean percentage of diseased leaf area is the average of 3 replications of 17 shoots with 4 leaves each. Numbers followed by the same letter are not significantly different according to Duncan's multiple range test (P = 0.05).

Both herbicides influenced the total sugar concentration in the leaves of uninoculated plants (Table 1.2). MCPP and 2,4,5-TP decreased total sugars below the amount in uninoculated controls (Table 1.2). Only sucrose of the individual sugars was decreased in leaves of uninoculated

Table 1.2. Mean concentration (μmol/g D. W.) of nonstructural carbohydrates of leaves of control and herbicide-treated *Poa pratensis* both uninoculated and inoculated with *Drechslera sorokiniana*[a]

	Uninoculated			Inoculated		
Carbohydrates	Control	MCPP	2,4,5-TP	Control	MCPP	2,4,5-TP
Sucrose	7.7 a/a	4.5 b/a	4.6 b/a	4.5 a/b	5.0 a/a	5.3 a/a
Glucose	1.8 a/a	1.5 a/b	1.3 a/b	2.3 a/a	2.2 a/a	2.7 a/a
Fructose	0.7 a/b	0.4 a/b	0.4 a/b	1.8 a/a	1.4 a/a	1.3 a/a
Raffinose	0.5 a/a	0.4 a/a	0.5 a/a	0.5 a/a	0.4 a/a	0.3 a/a
Total	10.7 a/a	6.9 b/b	6.8 b/b	9.2 a/a	9.3 a/a	9.3 a/a

[a]Mean concentration (μmol/g D. W.) of nonstructural carbohydrates is the average value of 3 replications from the analysis of tissue from 4 leaves of 17 shoots each. Numbers followed by the same letter among control and herbicide treatments within carbohydrate and uninoculated or inoculated plants (across a/) or between uninoculated and inoculated plants within carbohydrate and control and herbicide treatment (across /a) are not significantly different according to Duncan's multiple range test (P = 0.05).

plants due to applications of MCPP or 2,4,5-TP (Table 1.2). No differences in sugar concentrations existed among herbicide treatments within plants inoculated with D. sorokiniana (Table 1.2).

Differences in sugar concentrations existed between uninoculated and inoculated leaves (Table 1.2). Among control plants inoculated with D. sorokiniana, sucrose decreased and fructose increased relative to uninoculated control plants (Table 1.2). Inoculation of MCPP- or 2,4,5-TP-treated plants with D. sorokiniana increased glucose, fructose and total sugars in leaves compared to that in leaves of uninoculated plants treated with MCPP or 2,4,5-TP (Table 1.2). Raffinose remained unchanged in response to all treatments (Table 1.2).

MCPP and 2,4,5-TP had little effect on the free amino acid content of uninoculated leaves of P. pratensis (Table 1.3). MCPP decreased Ala, Lys, and His, and 2,4,5-TP decreased Lys compared to the uninoculated control (Table 1.3). Herbicide-treated plants inoculated with D. sorokiniana exhibited greater variation in free amino acid content (Table 1.3). Asp increased in MCPP-treated plants, and Ser, Gly, Ala, Val, Ile, Leu, Tyr, His, Arg and total amino acids decreased in 2,4,5-TP-treated plants compared to the inoculated control (Table 1.3).

The concentration of free amino acids in leaves of P. pratensis was influenced by infection by D. sorokiniana (Table 1.3). Inoculated leaves of control plants increased in Ser, Glu, Gly, Ala, Val, Ile, Leu, His and total amino acids compared to uninoculated controls, conversely, Lys decreased relative to uninoculated controls (Table 1.3). Inoculation

Table 1.3. Mean concentration (μmol/g D. W.) of free amino acids of leaves of control and herbicide-treated Poa pratensis both uninoculated and inoculated with Drechslera sorokiniana[a]

	Uninoculated			Inoculated		
Amino acids	Control	MCPP	2,4,5-TP	Control	MCPP	2,4,5-TP
Proline	1.02 a/a	0.46 a/b	0.47 a/a	1.36 a/a	1.13 a/a	0.99 a/a
Histidine	0.20 a/b	0.12 b/b	0.17 a/b	0.28 a/a	0.29 a/a	0.21 b/a
Lysine	0.39 a/a	0.33 b/a	0.30 b/a	0.32 ab/b	0.37 a/a	0.29 b/a
Arginine	0.34 a/a	0.27 a/b	0.33 a/a	0.40 a/a	0.34 ab/a	0.27 b/a
Glycine	0.31 a/b	0.29 a/b	0.31 a/a	0.37 a/a	0.35 a/a	0.30 b/a
Threonine	1.12 a/a	1.16 a/a	1.25 a/a	1.06 a/a	1.07 a/a	0.83 a/b
Alanine	3.39 a/b	2.91 b/b	3.49 a/a	4.14 a/a	3.86 a/a	2.88 b/b
Valine	0.71 a/b	0.70 a/b	0.78 a/a	0.87 a/a	0.91 a/a	0.67 b/a
Serine	2.75 a/b	3.03 a/b	3.04 a/a	3.67 a/a	3.78 a/a	2.64 b/a
Isoleucine	0.32 a/b	0.35 a/a	0.35 a/a	0.40 a/a	0.42 a/a	0.31 b/a
Leucine	0.24 a/b	0.25 a/a	0.24 a/a	0.29 a/a	0.29 a/a	0.21 b/a
Aspartic acid	0.27 a/a	0.30 a/b	0.27 a/a	0.30 b/a	0.41 a/a	0.30 b/a
Glutamic acid	1.31 a/b	1.56 a/a	1.35 a/a	1.66 ab/a	1.79 a/a	1.43 b/a
Phenylalanine	0.58 a/a	0.52 a/a	0.54 a/a	0.51 a/a	0.50 a/a	0.38 a/b
Tyrosine	0.38 a/a	0.33 a/a	0.37 a/a	0.36 a/a	0.33 a/a	0.22 b/b
Total	13.33 a/b	12.60 a/b	13.27 a/a	16.01 a/a	15.85 a/a	11.96 b/a

[a]Mean concentration (μmol/g D. W.) of free amino acids is the average value of 3 replications from the analysis of tissue from 4 leaves of 17 shoots each. Numbers followed by the same letter among control and herbicide treatments within amino acid and uninoculated or inoculated plants (across a/) or between uninoculated and inoculated plants within carbohydrate and control and herbicide treatments (across /a) are not significantly different according to Duncan's multiple range test (P = 0.05).

of MCPP-treated plants increased Asp, Ser, Pro, Gly, Ala, Val, His, Arg and total amino acids compared to uninoculated MCPP-treated plants (Table 1.3). Inoculation of 2,4,5-TP-treated plants decreased Thr, Ala, Tyr and Phe but increased His relative to uninoculated 2,4,5-TP-treated plants (Table 1.3).

Correlation coefficients were calculated for comparisons of sugar and free amino acid concentrations with the percentage of diseased leaf area of control and herbicide-treated plants. The pre-infection (uninoculated) concentrations of sucrose and total sugars in leaves compared to the percentage of diseased leaf area resulted in significant correlation coefficients of -0.776 and -0.679 ($P = 0.05$), respectively (Table 1.4). No significant correlations occurred between amino acids and disease at the 5% level (Table 1.5).

Table 1.4. Correlation coefficients for the comparison of nonstructural carbohydrate concentrations (μmol/g D. W.) of uninoculated and inoculated leaves of Poa pratensis with the mean percentage of diseased leaf area of herbicide-treated plants[a]

Carbohydrates	Uninoculated	Inoculated
Sucrose	-0.776**	+0.212
Glucose	-0.074	-0.031
Fructose	-0.255	-0.214
Raffinose	+0.055	-0.131
Total	-0.679**	-0.160

[a]Correlation coefficients were determined from values of 3 replicates of each treatment. Numbers followed by ** and significant at P = 0.05.

Table 1.5. Correlation coefficients for the comparison of free amino acid concentrations (μmol/g D. W.) of uninoculated and inoculated leaves of Poa pratensis with the mean percentage of diseased leaf area of herbicide-treated plants[a]

Amino acids	Uninoculated	Inoculated
Proline	-0.577*	+0.310
Histidine	-0.392	-0.065
Lysine	-0.315	+0.307
Arginine	-0.286	-0.392
Glycine	-0.143	-0.301
Threonine	+0.312	-0.028
Alanine	-0.204	-0.255
Valine	+0.192	-0.033
Serine	+0.201	+0.098
Isoleucine	+0.431	+0.015
Leucine	+0.337	-0.004
Aspartic acid	+0.122	+0.246
Glutamic acid	+0.426	-0.072
Phenylalanine	+0.045	-0.177
Tyrosine	-0.006	-0.127
Total	-0.064	-0.008

[a]Correlation coefficients were determined from values of 3 replicates of each treatment. Numbers followed by * are significant at P = 0.10.

DISCUSSION

The postemergence herbicides MCPP and 2,4,5-TP have opposite effects on the severity of D. sorokiniana leaf spot on P. pratensis (14). Soil application of 10^{-4}M MCPP stimulates leaf spot, whereas 10^{-4}M 2,4,5-TP lessens disease severity (14). The results presented support the stimulatory effect of MCPP, but the percentage diseased leaf area of 2,4,5-TP-treated plants was not different from that of the control (Table 1.1). The relatively high virulence of the isolate of D. sorokiniana used in this study (22% diseased leaf area of the control) could have negated the inhibitory influence of 2,4,5-TP.

Both MCPP and 2,4,5-TP caused a decrease in total sugars of uninoculated leaves of P. pratensis (Table 1.2). This corresponds with previous observations of sugar loss from leaves of plants susceptible to MCPP and 2,4,5-TP, and is probably associated with increased respiration and decreased photosynthesis (18, 24, 28, 37). Therefore, decreases in the sugar content of leaves of MCPP- and 2,4,5-TP-treated plants seem to be independent of susceptibility or tolerance to the herbicides. It also confirms that sugar loss is not the principal cause of death in herbicide-treated plants (35).

The concentrations of sucrose and total sugars of uninoculated leaves of herbicide-treated plants correlated negatively with the percentage diseased leaf area of respective inoculated plants. The effect of leaf sugar concentrations on D. sorokiniana leaf spot is probably not a direct relationship (6, 10, 31). The metabolism of

sugars within herbicide-treated, infected leaves is complex. Respiration, photosynthesis, translocation, enzyme activity, and the pathogen's utilization of sugars interact to influence sugar concentrations in such leaves (4, 18, 19, 24, 28, 37). Therefore, an interpretation of leaf sugar data is difficult. Treatments that decrease sugars prior to inoculation result in more severe leaf spot and a subsequent rise in sugar content following infection (Table 1.2). High sugar concentrations inhibit the induction of cell-wall degrading enzymes in some host-parasite interactions (20, 33). Therefore, decreased levels of sugars in leaves or herbicide-treated plants could stimulate the induction of cell-wall degrading enzymes following inoculation and facilitate pathogenesis.

Infection by D. sorokiniana results in a decrease of sucrose and an increase of fructose with no change in total sugars compared to the uninoculated control (Table 1.2). Increased respiration is common to fungal infection (4, 19). Sucrose may be hydrolyzed to glucose and fructose prior to entry into respiration pathways. Some pathogens alter translocation of low molecular weight metabolites like sugars to form metabolic sinks at infection sites (9, 17). The lack of a herbicide effect on total sugars of infected leaves may be due to the formation of a strong sink in the heavily infected leaves of herbicide-treated plants (Table 1.2).

Application of 10^{-4}M MCPP or 2,4,5-TP to P. pratensis had little effect on the free amino acid content of uninoculated leaves (Table 1.3).

The herbicides generally decrease free amino acids in leaves of susceptible plants (1, 22). Decreased rates of translocation of herbicides in tolerant species could delay herbicide-induced decreases in free amino acids in leaves (25). Measurement of free amino acids in select tissues of P. pratensis at time intervals after herbicide application would be informative.

Free amino acids in leaves of P. pratensis increased following inoculation with D. sorokiniana (Table 1.3). Similar increases have been noted in various host-parasite interactions (8). The increase could be due to proteolysis or to increased translocation of amino acids to the infected leaves (8, 9, 17, 29).

MCPP-treated, inoculated plants had greater concentrations of total amino acids in their leaves than did leaves of MCPP-treated, uninoculated plants (Table 1.3). However, 2,4,5-TP-treated, inoculated plants had total amino acid levels in their leaves not different from those in leaves of uninoculated 2,4,5-TP-treated plants (Table 1.3). This observation was the only difference between the two herbicides in effects on sugars or amino acids. Soil application of 2,4,5-TP at various concentrations results in erratic effects on leaf spot severity (14). The degree of inactivation of herbicides in P. pratensis could be responsible for the effects in this study (2, 22, 27, 32).

REFERENCES

1. Ashton, F. M., and D. E. Bayer. 1976. Effects on solute transport and plant constituents. Pages 219-253. In L. J. Audus, ed. Herbicides: physiology, biochemistry, ecology. Vol. 1, 2nd ed. Academic Press, New York. 608 p.

2. Ashton, F. M., and A. S. Crafts. 1981. Mode of action of herbicides. 2nd ed. John Wiley and Sons, New York. 525 p.

3. Brenner, M., A. Niederwieser, and G. Pataki. 1969. Amino acids and derivatives. Pages 730-786. In E. Stahl, ed. Thin-layer chromatography, a laboratory handbook. 2nd ed. Springer-Verlag, New York. 1,041 p.

4. Bushnell, W. R., and P. J. Allen. 1962. Respiratory changes in barley leaves produced by single colonies of powdery mildew. Plant Physiol. 37:751-758.

5. Cheesman, J. H., E. C. Roberts, and L. H. Tiffany. 1965. Effects of nitrogen level and osmotic pressure of the nutrient solution on incidence of Puccinia graminis and Helminthosporium sativum infection of Merion Kentucky bluegrass. Agron. J. 57:599-602.

6. Couch, H. B., and L. D. Moore. 1971. Influence of nutrition and total nonstructural carbohydrate content on Helminthosporium sativum-incited leaf spot on Kentucky bluegrass. Phytopathology 61:888.

7. Drechsler, C. 1930. Leaf spot and foot rot of Kentucky bluegrass caused by Helminthosporium vagans. J. Agr. Res. 40:447-456.

8. Dwivedi, K., and P. Shukla. 1980. Amino acid changes in mung foliage infested with Drechslera halodes (leaf spot disease). Indian J. Mycol. Plant Path. 10:192-193.

9. Farkas, G. L. 1978. Senescence and plant disease. Pages 391-412. In J. G. Horsfall and E. B. Cowling, ed. Plant disease: An advanced treatise. Vol. III. How plants suffer from disease. Academic Press, New York. 487 p.

10. Gibbs, A. F., and R. D. Wilcoxson. 1972. Effects of sugar content of Poa pratensis on Helminthosporium leaf disease. Physiol. Plant Pathol. 2:279-287.

11. Halisky, P. M., C. R. Funk, and R. E. Engel. 1966. Melting-out of Kentucky bluegrass varieties by Helminthosporium vagans as influenced by turf management practices. Plant Dis. Rep. 50:703-706.

12. Hodges, C. F. 1972. Influence of culture age and temperature on germination of Helminthosporium sorokinianum conidia and on pathogenicity to Poa pratensis. Phytopathology 62:1133-1137.

13. Hodges, C. F. 1978. Postemergent herbicides and the biology of Drechslera sorokiniana: Influence on severity of leaf spot on Poa pratensis. Phytopathology 68:1359-1363.

14. Hodges, C. F. 1980. Interaction of sequential leaf senescence of Poa pratensis and pathogenesis by Drechslera sorokiniana as influenced by postemergent herbicides. Phytopathology 70:628-630.

15. Hodges, C. F., and J. P. Madsen. 1978. The competitive and synergistic interactions of Drechslera sorokiniana and Curvularia geniculata on leaf spot development on Poa pratensis. Can. J. Bot. 56:1240-1247.

16. Horsfall, J. G., and A. E. Dimond. 1957. Interactions of tissue sugar, growth substances, and disease severity. Z. Pflanzenkrankh. 64:415-421.

17. Huber, D. M. 1978. Disturbed mineral nutrition. Pages 163-181. In J. G. Horsfall and E. B. Cowling, ed. Plant disease: An advanced treatise. Vol. III. How plants suffer from disease. Academic Press, New York. 487 p.

18. Kirkwood, R. C. 1976. Action on respiration and intermediary metabolism. Pages 443-492. In L. J. Audus, ed. Herbicides: physiology, biochemistry, ecology. Vol. 1, 2nd ed. Academic Press, New York. 608 p.

19. Krupka, L. R. 1959. Metabolism of oats susceptible to Helminthosporium victoriae and victorin. Phytopathology 49:587-594.

20. Kuc, J. 1978. Changes in intermediary metabolism caused by disease. Pages 349-374. In J. G. Horsfall and E. B. Cowling, ed. Plant disease: An advanced treatise. Vol. III. How plants suffer from disease. Academic Press, New York. 487 p.

21. Lewis, B. A., and F. Smith. 1969. Sugars and derivatives. Pages 807-837. In E. Stahl, ed. Thin-layer chromatography, a laboratory handbook. 2nd ed. Springer-Verlag, New York. 1,041 p.

22. Loos, M. A. 1975. Phenoxyalkanoic acids. Pages 1-128. In P. C. Kearney and D. D. Kaufman, ed. Herbicides: chemistry, degradation, and mode of action. Vol. 1, 2nd ed. Marcel Dekker, New York. 500 p.

23. Lukens, R. J. 1970. Melting-out of Kentucky bluegrass, a low-sugar disease. Phytopathology 60:1276-1278.

24. Moreland, D. E. 1967. Mechanisms of action of herbicides. Annu. Rev. Plant Physiol. 18:365-386.

25. Naylor, A. W. 1976. Herbicide metabolism in plants. Pages 397-426. In L. J. Audus, ed. Herbicides: physiology, biochemistry, ecology. Vol. 1, 2nd ed. Academic Press, New York. 608 p.

26. Nilsen, K. N., C. F. Hodges, and J. P. Madsen. 1979. Pathogenesis of Drechslera sorokiniana leaf spot on progressively old leaves of Poa pratensis as influenced by photoperiod and light quality. Physiol. Plant Pathol. 15:171-176.

27. Norris, L. A., and V. H. Freed. 1966. The absorption and translocation characteristics of several phenoxyalkyl herbicides in bigleaf maple. Weed Res. 6:203-211.

28. Penner, D., and E. M. Ashton. 1966. Biochemical metabolic changes in plants induced by chlorophenoxy herbicides. Residue Rev. 14:39-113.

29. Raghunathan, R., A. Madhadevan, and G. Rangaswami. 1966. Biochemical changes in the banana fruit-coat caused by Gleosporium musarum infection. Indian Phytopath. 19:162-167.

30. Robinson, P. W., and C. F. Hodges. 1976. An inoculation apparatus for evaluation of Bipolaris sorokiniana lesion development on progressively older leaves of Poa pratensis. Phytopathology 66:360-362.

31. Robinson, P. W., and C. F. Hodges. 1977. Effect of nitrogen fertilization on free amino acid and soluble sugar content of Poa pratensis and on infection by Drechslera sorokiniana. Phytopathology 67:1239-1244.

32. Sanad, A. J. 1971. Studies on the uptake and translocation of ^{14}C-labeled herbicides in Agrostemma githago and Tussilago farfara. Weed Res. 11:215-223.

33. Sands, D. C., and R. L. Lukens. 1974. Effect of glucose and adenosine phosphates on production of extracellular carbohydrates of Alternaria solani. Plant Physiol. 54:666-669.

34. Simmonds, P. M. 1960. Infection of wheat with Helminthosporium sativum in relation to the nitrogen content of the plant tissues. Can. J. Plant Sci. 40:139-145.

35. Smith, F. G., C. L. Hamner, and R. F. Carlson. 1947. Changes in food reserves and respiratory capacity of bindweed tissues accompanying herbicidal action of 2,4-dichlorophenoxyacetic acid. Plant Physiol. 22:58-65.

36. Vidhyase, P. 1978. Role of amino acids and amides in Helminthosporiose disease incidence in finger-millet. Indian Phytopathol. 30:41-46.

37. Wort, D. J. 1964. Effects of herbicides on plant composition and metabolism. Pages 291-334. In L. J. Audus, ed. The physiology and biochemistry of herbicides. Academic Press, New York. 555 p.

PART II. EFFECT OF POSTEMERGENCE HERBICIDES ON NONSTRUCTURAL CARBOHYDRATES IN SEQUENTIALLY SENESCENT LEAVES OF *POA PRATENSIS* AND ON PATHOGENESIS BY *DRECHSLERA SOROKINIANA*

INTRODUCTION

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

Poa pratensis subjected to mowing maintains 3 or 4 visible leaves per shoot that range from immature to near senescent (18). Sequentially older leaves of P. pratensis promote leaf spot development (8, 9, 10). Application of 10^{-4}M MCPP to the soil prior to inoculation results in increased leaf spot severity on each older leaf (8). Application of 10^{-4}M 2,4,5-TP to the soil inhibits leaf spot only on the two oldest leaves and has no effect on the two youngest leaves (8).

Postemergence herbicides have well-documented effects on leaf sugar concentrations in herbicide-susceptible plant species (1, 21). Sugar concentrations in leaves of susceptible herbicide-treated plants are generally decreased due to increased respiration and to the inhibition of photosynthesis (13, 17, 21, 28). Subtoxic applications of phenoxy herbicides increase sucrose in some plants (22). MCPP and 2,4,5-TP affect leaf sugars of herbicide-tolerant P. pratensis in a manner similar to herbicide-susceptible plants. Applications of 10^{-4}M MCPP or 2,4,5-TP decrease sugar concentrations in leaves of P. pratensis (see page 5).

The decrease of the total sugar concentration induced by the herbicides correlates with increased diseased leaf area percentages on a whole shoot basis (see page 5). Increased respiration, the loss of chlorophyll, and decreased photosynthesis are components of leaf senescence processes that decrease leaf sugar levels (27). Decreased leaf sugar concentrations 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 predisposes the plant to infection by D. sorokiniana. Studies were undertaken to evaluate the effects of soil applications of 10^{-4}M MCPP and 2,4,5-TP to P. pratensis on sugar concentrations and on leaf spot severity of sequentially developing and senescing leaves.

MATERIALS AND METHODS

Poa pratensis 'Newport' was vegetatively propagated in a steamed 2:1 loam-peat mix in 7.6 cm plastic pots. Plants were grown in the greenhouse under 16 hr of light (day light supplemented with incandescent lights) for 60 days prior to treatment. Cultures of Drechslera sorokiniana (Sacc.) Subram. and Jain were maintained on 20 ml or 1% Czapek Dox broth in 3% (v/v) Bacto-agar in 15x85 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 prior to incubation) 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 sterile distilled water at 5 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 sterile distilled water as a control. Conidia suspensions were prepared with an automatic particle counter (High Accuracy Products Corp., Montclair, CA 91763). Each treatment consisted of 17 individual shoots and was replicated 3 times. Plants were incubated 6 days at 24 C under continuous fluorescent light (80-90 $\mu E\ M^{-2}\ sec^{-1}$) and then evaluated for disease severity prior to preparation for leaf sugar analysis.

Sugar content was determined for both inoculated and uninoculated leaves. Disease severity was evaluated as the percentage diseased leaf tissue per inoculated 10 cm leaf section (8). The values from 17 shoots of each treatment for each leaf age were averaged to determine the disease severity of each treatment replication. The data presented are the means of replicate values for each treatment.

The concentrations of sucrose, glucose, fructose, and raffinose in leaves of plants of each treatment were determined by a method described previously (see page 5). Data were analyzed as a 3x2x4 factorial design for the mean percentage of diseased leaf tissue and for individual and total leaf sugar concentrations of the four sequentially aged leaves of shoots of each treatment.

RESULTS

There was no difference in the severity of disease on leaf tissue of the two youngest leaves of untreated P. pratensis (Figure 2.1). However, leaf spot severity increased from the second to the third to the fourth leaf of the control (Figure 2.1). Soil applications of MCPP enhanced leaf spot development on all but the third leaf, and applications of 2,4,5-TP stimulated disease on the second and fourth leaves only (Figure 2.1).

Total sugar concentrations of uninoculated, untreated P. pratensis leaves 1, 2, and 4 were not different, but the total sugar content of leaf 3 was less than that of the other leaves (Figure 2.2). Soil applications of MCPP or 2,4,5-TP resulted in decreased amounts of total sugar in uninoculated leaves with no differences among leaf ages or between the two herbicides (Figure 2.2). Inoculation of control plants with D. sorokiniana decreased total sugars in leaves 1 and 2, increased it in leaf 2, and caused no change in leaf 4 compared to uninoculated plants (Figure 2.2). Inoculation of plants treated with MCPP or 2,4,5-TP increased the total sugars of leaves of each age except the youngest leaf of 2,4,5-TP-treated P. pratensis (Figure 2.2).

The concentration of sucrose in uninoculated P. pratensis paralleled that of total sugars over the four leaf ages and both herbicide treatments (Figure 2.3). However, inoculation with D. sorokiniana of control plants decreased sucrose levels in leaves 1, 2, and 4 with no change in leaf 3 compared to uninoculated plants (Figure 2.3). Inoculation of herbicide-

Figure 2.1. Mean percentage of diseased leaf area of four sequentially aged leaves of control and herbicide-treated Poa pratensis inoculated with Drechslera sorokiniana

Mean percentage of diseased leaf area is the average of 3 replications of 17 shoots each. Numbers followed by 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).

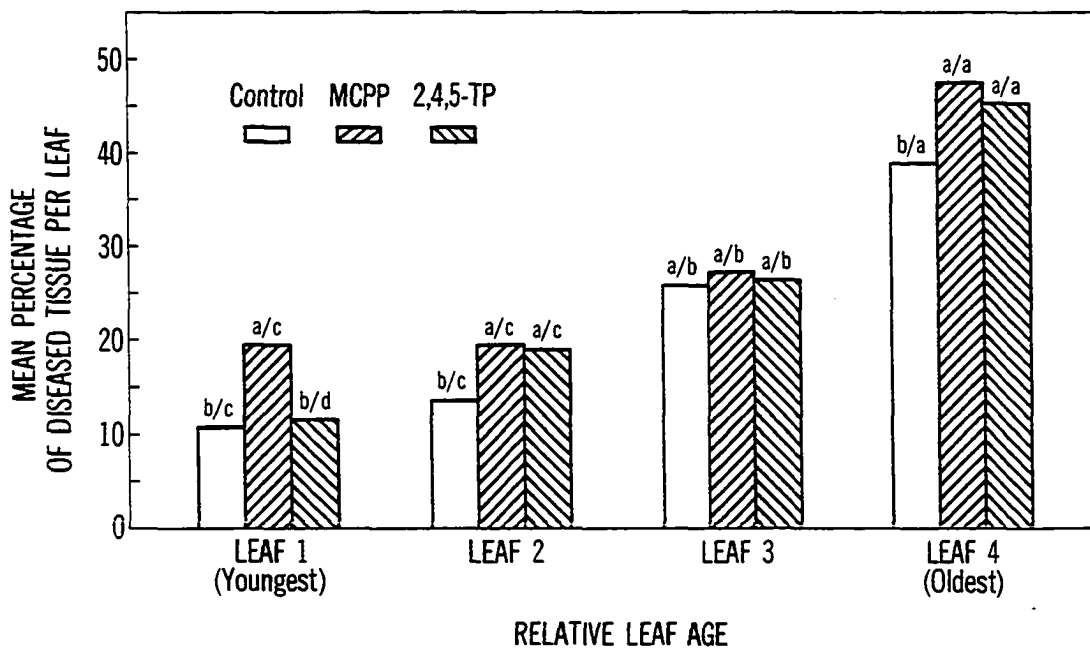
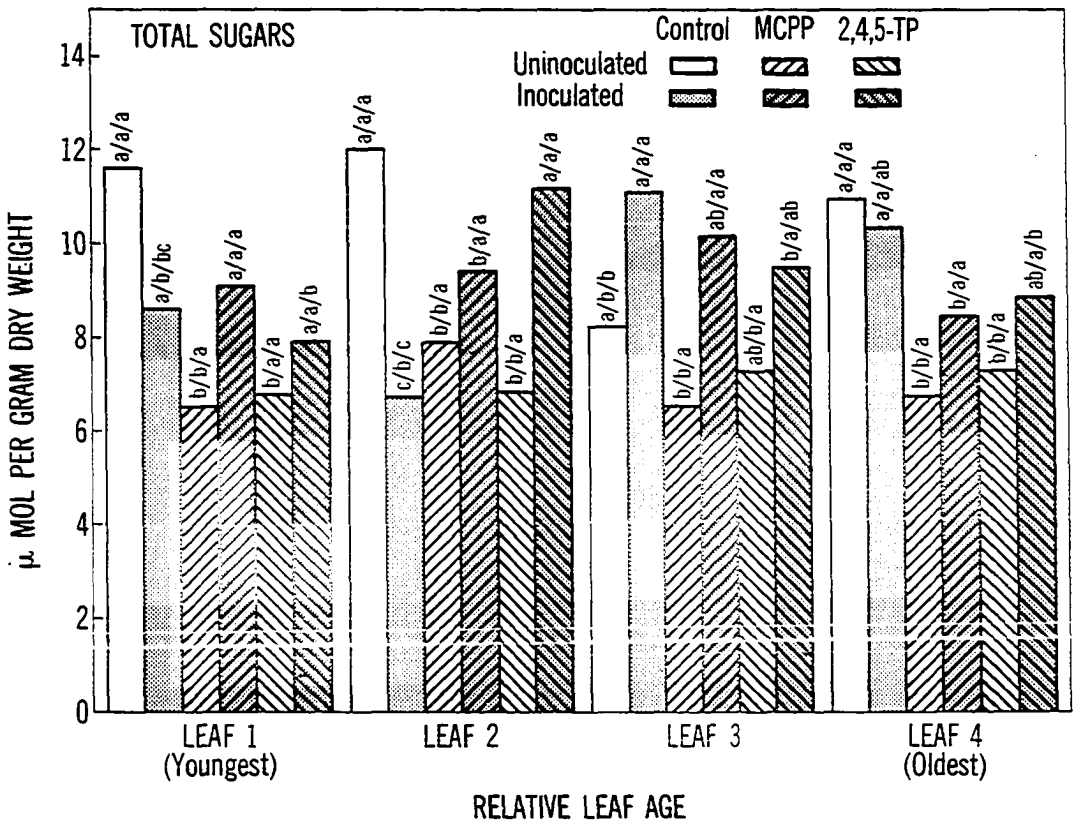

MEAN PERCENTAGE
OF DISEASED TISSUE PER LEAF
50
40
30
20
10
0
Control
MCPP
2,4,5-TP
b/c
a/c
b/d
b/c
a/c
a/c
a/b
a/b
a/b
b/a
a/a
a/a
LEAF 1
(Youngest)
LEAF 2
LEAF 3
LEAF 4
(Oldest)
RELATIVE LEAF AGE

Figure 2.2. Mean concentration (μmol/g D. W.) of total sugars of four sequentially aged leaves of control and herbicide-treated *Poa pratensis* both uninoculated and inoculated with *Drechslera sorokiniana*

Mean concentration (μmol/g D. W.) of total sugars is the average value of 3 replications from the analysis of leaf tissue of 17 shoots each. Numbers followed by 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).


TOTAL SUGARS
Control
MCPP
2,4,5-TP
Uninoculated
Inoculated
μ MOL PER GRAM DRY WEIGHT
0
2
4
6
8
10
12
14
a/a/a
a/b/bc
b/b/a
a/a/a
b/a/a
a/a/b
a/a/a
c/b/c
b/b/a
b/a/a
b/b/a
a/a/a
a/b/b
a/a/a
b/b/a
ab/a/a
ab/b/a
b/a/ab
a/a/a
a/a/ab
b/b/a
b/a/a
b/b/a
ab/a/b
LEAF 1
(Youngest)
LEAF 2
LEAF 3
LEAF 4
(Oldest)
RELATIVE LEAF AGE

treated plants had little influence on the sucrose concentration of leaves. Only leaf 3 of MCPP-treated and leaf 2 of 2,4,5-TP-treated plants showed an increase of sucrose following inoculation (Figure 2.3).

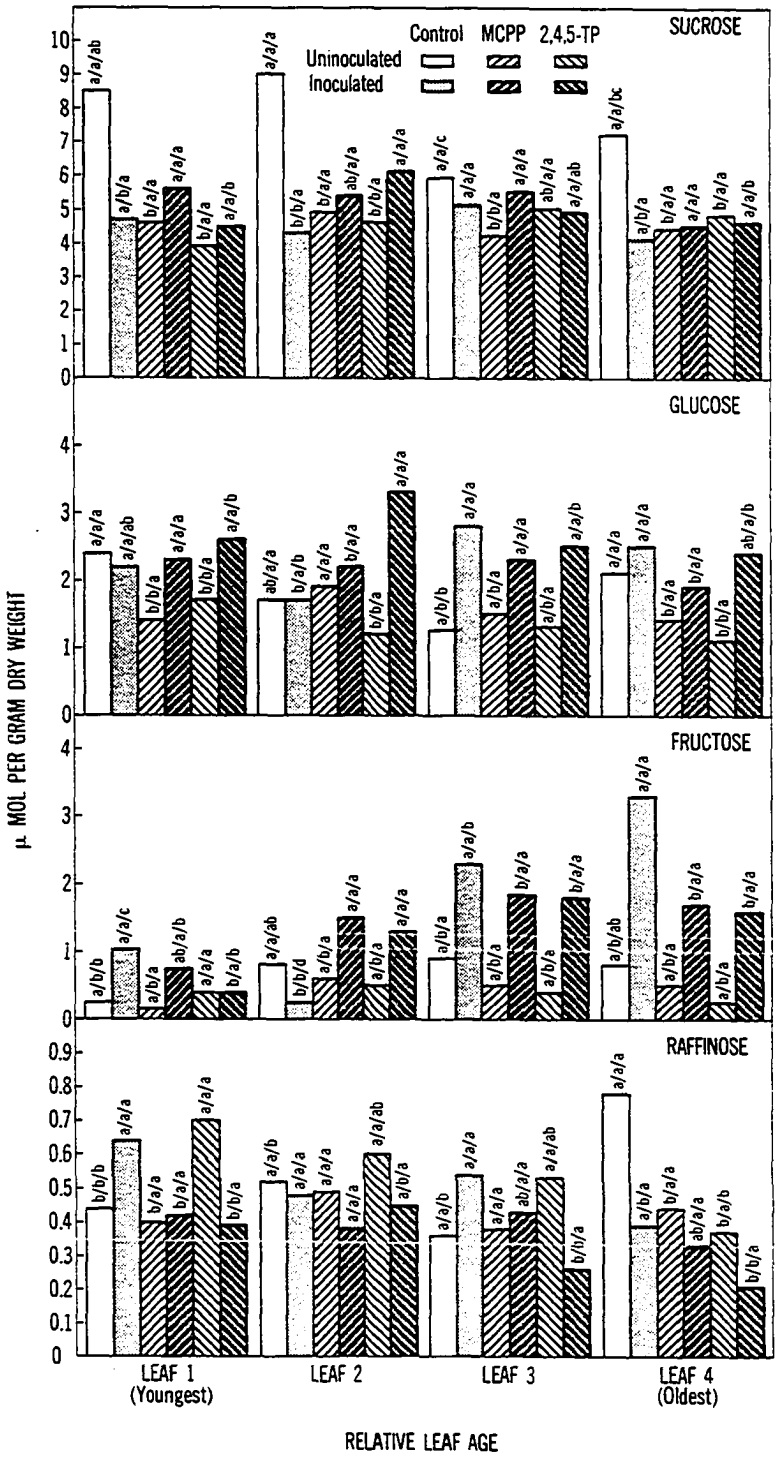
The effects of leaf age and herbicide treatment on the glucose content of uninoculated P. pratensis were consistent with those effects on total sugar and sucrose concentrations except for a rise in glucose in leaf 2 of MCPP-treated plants (Figure 2.3). The concentration of glucose of untreated P. pratensis increased in leaf 3 following inoculation (Figure 2.3). Inoculation with D. sorokiniana increased glucose in leaves 1 and 3 of MCPP-treated and all leaves of 2,4,5-TP-treated P. pratensis (Figure 2.3).

The concentration of fructose in leaf 1 of uninoculated, untreated P. pratensis was less than that of leaves 2, 3, and 4 which were not significantly different (Figure 2.3). Herbicides had no effect on the fructose concentration of uninoculated leaves of any age (Figure 2.3). Inoculation of control plants increased fructose levels in leaf 1, decreased fructose in leaf 2 and increased fructose nearly three-fold in leaves 3 and 4 (Figure 2.3). Inoculation of herbicide-treated plants increased fructose in all leaves except for leaf 1 of 2,4,5-TP-treated plants (Figure 2.3).

The raffinose content of leaf 4 of uninoculated control plants was greater than that of the 3 younger leaves (Figure 2.3). Herbicides had little effect on raffinose in uninoculated leaves. Application of 2,4,5-TP increased raffinose content in leaf 1, and both MCPP and 2,4,5-TP

Figure 2.3. Mean concentration (μmol/g D. W.) of nonstructural carbohydrates of four sequentially aged leaves of control and herbicide-treated Poa pratensis both uninoculated and inoculated with Drechslera sorokiniana

Mean concentration (μmol/g D. W.) of nonstructural carbohydrates is the average value of 3 replications from the analysis of leaf tissue of 17 shoots each. Numbers followed by 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 carbohydrate are not significantly different according to Duncan's multiple range test (P = 0.05).


SUCROSE
GLUCOSE
FRUCTOSE
RAFFINOSE
Control
MCPP
2,4,5-TP
Uninoculated
Inoculated
μ MOL PER GRAM DRY WEIGHT
LEAF 1 (Youngest)
LEAF 2
LEAF 3
LEAF 4 (Oldest)
RELATIVE LEAF AGE

decreased the amount of raffinose in leaf 4 (Figure 2.3). Inoculation of control plants increased raffinose in leaf 1 and decreased raffinose in leaf 4 compared to uninoculated plants (Figure 2.3). Inoculation of MCPP-treated plants had no significant effect on the raffinose content of leaves (Figure 2.3). However, inoculation of 2,4,5-TP-treated plants decreased raffinose in each leaf (Figure 2.3).

Correlation coefficients were determined for the comparison of the percentage of diseased leaf tissue with pre- and post-infection (uninoculated and inoculated) concentrations of sugars in sequentially aged leaves of control plants (Table 2.1). The pre-infection (uninoculated) concentration of fructose correlated positively with increasing disease severity over the four leaves of control plants (Table 2.1). The post-infection (inoculated) concentrations of fructose and raffinose correlated positively and negatively respectively with increasing disease severity (Table 2.1). Correlation coefficients for the comparison of the effects of MCPP and 2,4,5-TP on the concentrations of leaf sugars with the effects of the herbicides on disease severity as compared to the control were determined (Table 2.2). The decrease of the pre-infection concentration of sucrose and total sugars in leaves of MCPP-treated plants correlated with the increased percent of diseased leaf area of MCPP-treated, inoculated plants compared to inoculated control plants (Table 2.2). The effect of 2,4,5-TP on the post-infection concentration of raffinose correlated positively with increased percent diseased leaf area of 2,4,5-TP-treated, inoculated plants compared to inoculated control plants (Table 2.2).

Table 2.1. Correlation coefficients for the comparison of nonstructural carbohydrate concentrations (μmol/g D. W.) of four sequentially aged leaves of Poa pratensis with the mean percentage of diseased leaf area of control plants[a]

Carbohydrates	Uninoculated	Inoculated
Sucrose	-0.430	-0.080
Glucose	-0.120	+0.360
Fructose	+0.520*	+0.650**
Raffinose	+0.040	-0.550*
Total sugars	-0.310	+0.450

[a]Correlation coefficients were determined from values of 3 replicates of each treatment. Numbers followed by ** are significant at P = 0.05 and by * are significant at P = 0.10.

Table 2.2. Correlation coefficients for the comparison of effects of 10^{-4} M MCPP and 2,4,5-TP on the concentration (µmol/g D. W.) of nonstructural carbohydrates of four sequentially aged leaves of Poa pratensis with effects on the mean[a] percentage of diseased leaf area as compared to the control

	Uninoculated		Inoculated	
Carbohydrates	MCPP	2,4,5-TP	MCPP	2,4,5-TP
Sucrose	-0.618**	-0.246	+0.394	+0.328
Glucose	-0.154	-0.454	+0.224	+0.180
Fructose	+0.349	-0.370	+0.003	+0.047
Raffinose	+0.065	-0.351	+0.278	+0.564*
Total sugars	-0.520*	-0.383	+0.154	+0.058

[a]Correlation coefficients were determined for the difference of herbicide treatments and control treatments from values of 3 replications for each treatment. Numbers followed by ** are significant at P = 0.05 and by * are significant at P = 0.10.

DISCUSSION

Sequential senescence of leaves of P. pratensis has been suggested as promotive of D. sorokiniana leaf spot severity (8, 9, 10). The results of this study support this hypothesis. The percentage diseased leaf area of untreated plants was greatest on the two oldest leaves (Figure 2.1).

The application of 10^{-4}M MCPP to the soil prior to inoculation enhanced leaf spot on all but the third leaf, and 10^{-4}M 2,4,5-TP enhanced leaf spot on the second and fourth leaves (Figure 2.1). Auxin-like postemergence herbicides have been implied to induce conditions in leaves promotive of 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, untreated P. pratensis contained the least amount of sugar of the four leaves of one shoot (Figure 2.2). The oldest leaf (leaf 4) contained slightly less sugar than leaves 1 and 2 but more than leaf 3 (Figure 2.2). The third leaf is fully expanded and is a major exporter of sugar within the plant. The rise in the concentration of sugars in the oldest leaf may be due to the hydrolysis of polysaccharides as part of the senescence process (27).

The pre-infection (uninoculated) concentration of total sugars in the four sequentially aged leaves of control plants did not correlate with the increasing severity of leaf spot on older leaves (Table 2.1).

A decrease in the concentration of leaf sugars has been suggested as promotive of D. sorokiniana leaf spot (11, 16). Leaf spot of P. pratensis has been classified as a "low sugar" disease based upon correlations of low sugar concentrations with increased leaf spot severity (16). This direct relationship between leaf sugar concentration and leaf spot severity has been questioned (3, 5, 24). The "low sugar" concept does not apply to the increase in leaf spot on older leaves of P. pratensis in this study.

Although no direct correlations existed between pre-infection leaf sugar concentrations and leaf spot severity of control plants, a relationship does exist between the influence of the herbicides on leaf sugars and leaf spot. MCPP and 2,4,5-TP decreased sugar concentrations in leaves of herbicide-tolerant P. pratensis in a manner indicative of changes occurring during leaf senescence (Figure 2.2). The decrease in the pre-infection concentration of sucrose and total sugars in leaves of MCPP-treated plants correlated with increased leaf spot compared to the control (Table 2.2). High sugar concentrations inhibit the induction of cell-wall degrading enzymes in some host-parasite interactions (14, 26). Conversely, the decrease in the concentration of leaf sugars following herbicide treatment could induce cell-wall degrading enzymes following inoculation and thus facilitate pathogenesis. The increase of leaf sugars in herbicide-treated plants following inoculation could be the result of increased cell-wall degradation (Figure 2.2).

The concentrations of glucose and fructose in the two oldest leaves of control plants increased following infection (Figure 2.3). This

increase of sugars corresponded with the most severely infected leaves (Figure 2.1). Some pathogens are known to form 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 concentration of sugars in the two younger leaves following inoculation (Figure 2.3). The facilitation of pathogenesis by the potential low sugar induction of cell-wall degrading enzymes in all leaves of herbicide-treated plants also could result in greater sink activity. The presence of a metabolic sink at infection sites also might enhance herbicide-induced senescence which would both stimulate leaf spot and increase sugars in leaves following infection (4, 19).

The correlation coefficients indicate different effects of MCPP and 2,4,5-TP (Table 2.2). MCPP has a significant influence on leaf sugars that correlates with the herbicides' effect on leaf spot development on the four leaves (Table 2.2). The effect of 2,4,5-TP on the concentration of sugars is similar to that of MCPP (Figure 2.3), but no correlations exist between these effects and those effects on leaf spot (Table 2.2). Application of 2,4,5-TP had no effect on leaf spot of the youngest leaf where MCPP caused a dramatic rise in the percent diseased leaf area compared to the control (Figure 2.1). 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 following application to the soil (2, 15, 20, 25).

REFERENCES

1. Ashton, F. M., and D. E. Bayer. 1976. Effects on solute transport and plant constituents. Pages 219-253. In L. J. Audus, ed. Herbicides: physiology, biochemistry, ecology. Vol. 1, 2nd ed. Academic Press, New York. 608 p.

2. Ashton, F. M., and A. S. Crafts. 1981. Mode of action of herbicides. 2nd ed. John Wiley and Sons, New York. 525 p.

3. Couch, H. B., and L. D. Moore. 1971. Influence of nutrition and total nonstructural carbohydrate content on Helminthosporium sativum-incited leaf spot on Kentucky bluegrass. Phytopathology 61:888.

4. Farkas, G. L. 1978. Senescence and plant disease. Pages 391-412. In J. G. Horsfall and E. B. Cowling, ed. Plant disease: An advanced treatise. Vol. III. How plants suffer from disease. Academic Press, New York. 487 p.

5. Gibbs, A. F., and R. D. Wilcoxson. 1972. Effects of sugar content of Poa pratensis on Helminthosporium leaf disease. Physiol. Plant Pathol 2:279-287.

6. Hodges, C. F. 1972. Influence of culture age and temperature on germination of Helminthosporium sorokinianum conidia and on pathogenicity to Poa pratensis. Phytopathology 62:1133-1137.

7. Hodges, C. F. 1978. Postemergent herbicides and the biology of Drechslera sorokiniana: Influence on severity of leaf spot on Poa pratensis. Phytopathology 68:1359-1363.

8. Hodges, C. F. 1980. Interaction of sequential leaf senescence of Poa pratensis and pathogenesis by Drechslera sorokiniana as influenced by postemergent herbicides. Phytopathology 70:628-630.

9. Hodges, C. F. 1980. Postemergent herbicides and pathogenesis by Drechslera sorokiniana on leaves of Poa pratensis. Pages 101-112. In P. O. Larsen and B. G. Joyner, ed. Advances in turfgrass pathology. Harcourt Brace Jovanovich, Inc., Duluth. 197 p.

10. Hodges, C. F., and J. P. Madsen. 1979. Leaf senescence as a factor in the competitive and synergistic interactions of Drechslera sorokiniana and Curvularia geniculata on Poa pratensis. Can. J. Bot. 57:1706-1711.

11. Horsfall, J. G., and A. E. Dimond. 1957. Interactions of tissue sugar, growth substances, and disease severity. Z. Pfanzenkrankh 64:415-421.

12. Huber, D. M. 1978. Disturbed mineral nutrition. Pages 163-181. In J. G. Horsfall and E. B. Cowling, ed. Plant disease: An advanced treatise. Vol. III. How plants suffer from disease. Academic Press, New York. 487 p.

13. Kirkwood, R. C. 1976. Action on respiration and intermediary metabolism. Pages 443-492. In L. J. Audus, ed. Herbicides: physiology, biochemistry, ecology. Vol. 1, 2nd ed. Academic Press, New York. 608 p.

14. Kuc, J. 1978. Changes in intermediary metabolism caused by disease. Pages 349-374. In J. G. Horsfall and E. B. Cowling, ed. Plants disease: An advanced treatise. Vol. III. How plants suffer from disease. Academic Press, New York. 487 p.

15. Loos, M. A. 1975. Phenoxyalkanoic acids. Pages 1-128. In P. C. Kearney and D. D. Kaufman, ed. Herbicides: chemistry, degradation, and mode of action. Vol. 1, 2nd ed. Marcel Dekker, New York. 500 p.

16. Lukens, R. J. 1970. Melting-out of Kentucky bluegrass, a low-sugar disease. Phytopathology 60:1276-1278.

17. Moreland, D. E. 1967. Mechanisms of action of herbicides. Annu. Rev. Plant Physiol. 18:365-386.

18. Nilsen, K. N., and C. F. Hodges. 1980. Photomorphogenically defined light and resistance of Poa pratensis to Drechslera sorokiniana. Plant Physiol. 65:569-573.

19. Nooden, L. D. 1980. Senescence in the whole plant. Pages 219-244. In K. V. Thimann, ed. Senescence in plants. CRC Press, Boca Raton, Fl. 276 p.

20. Norris, L. A., and V. H. Freed. 1966. The absorption and translocation characteristics of several phenoxyalkyl herbicides in bigleaf maple. Weed Res. 6:203-211.

21. Penner, D., and E. M. Ashton. 1966. Biochemical and metabolic changes in plants induced by chlorophenoxy herbicides. Residue Rev. 14:39-113.

22. Ries, S. K. 1976. Subtoxic effects on plants. Pages 313-344. In L. J. Audus, ed. Herbicides: physiology, biochemistry, ecology. Vol. II, 2nd ed. Academic Press, New York. 564 p.

23. Robinson, P. W., and C. F. Hodges. 1976. An inoculation apparatus for evaluation of Bipolaris sorokiniana lesion development on progressively older leaves of Poa pratensis. Phytopathology 66:360-362.

24. Robinson, P. W., and C. F. Hodges. 1977. Effect of nitrogen fertilization on free amino acid and soluble sugar content of Poa pratensis and on infection by Drechslera sorokiniana. Phytopathology 67:1239-1244.

25. Sanad, A. J. 1971. Studies on the uptake and translocation of ^{14}C-labeled herbicides in Agrostemma githago and Tussilago farfara. Weed Res. 11:215-223.

26. Sands, D. C., and R. L. Lukens. 1974. Effect of glucose and adenosine phosphates on production of extracellular carbohydrates of Alternaria solani. Plant Physiol. 54:666-669.

27. Thimann, K. V. 1980. The senescence of leaves. Pages 85-115. In K. V. Thimann, ed. Senescence in plants. CRC Press, Boca Raton, Fl. 276 p.

28. Wort, D. J. 1964. Effects of herbicides on plant composition and metabolism. Pages 291-334. In L. J. Audus, ed. The physiology and biochemistry of herbicides. Academic Press, New York. 555 p.

PART III. EFFECT OF POSTEMERGENCE HERBICIDES ON FREE AMINO ACIDS IN SEQUENTIALLY SENESCENT LEAVES OF *POA PRATENSIS* AND ON PATHOGENESIS BY *DRECHSLERA SOROKINIANA*

INTRODUCTION

The severity of leaf spot caused by Drechslera sorokiniana (Sacc.) Subram. and Jain (= Helminthosporium sativum P. K. and B.) on Poa pratensis L. is directly related to the age of the leaves of P. pratensis (9, 10, 11, see page 25). Shoots of P. pratensis generally maintain 3-4 visible leaves under mowing during the growing season. Leaf spot is minimal on the two youngest leaves and is most severe on the two oldest leaves (9, see page 25). The symptoms on older leaves are large necrotic lesions with chlorotic halos and some chlorotic streaking (10). A hypothesis that factors promoting leaf senescence in P. pratensis enhance leaf spot severity was proposed on the basis of similar results of two independent studies on the effects of phenoxy acid herbicides, light quality and photoperiod on leaf spot (10). The auxin-like phenoxy herbicides generally stimulate leaf spot of P. pratensis, especially on older leaves (9). Leaf spot symptoms of herbicide-treated P. pratensis are necrotic lesions surrounded by large chlorotic areas which in older leaves may extend throughout the whole leaf (9). Similar results occur under blue + far red light spectra and short photoperiods (16, 17). Both phenoxy herbicides and light conditions may promote conditions in older leaves typical of leaf senescence which result in enhanced leaf spot severity.

The hydrolysis of protein is typical of senescing leaves (26). Free amino acids are utilized as respiratory substrate (26) or are translocated out of leaves to other plant parts (26) resulting in a net loss of amino

acids in senescing leaves. Application of phenoxy herbicides to susceptible plants typically results in a decrease of amino acid concentrations in leaves and an increase in stems and roots (2, 6, 15, 22). Application of phenoxy herbicides to herbicide-tolerant P. pratensis influences individual free amino acid concentrations in leaves on a whole plant basis (see page 5). Pre-infection (uninoculated) levels of amino acids do not correlate with subsequent leaf spot severity on a whole shoot basis after infection (see page 5). Infection sites may act as a sink, resulting in an influx of amino acids to infected leaves (12, 21, 27), and some amino acids may be utilized by the pathogen as substrate (1, 27). The possibility of a phenoxy herbicide-amino acid-senescence relationship and the possible influence of leaf senescence on leaf spot severity forms the basis for an investigation of how phenoxy herbicides influence free amino acids in sequentially older leaves of P. pratensis and the subsequent effects on leaf spot severity.

MATERIALS AND METHODS

Poa pratensis L. 'Newport' was vegetatively propagated and grown in the greenhouse as described previously (see page 5). Cultures of Drechslera sorokiniana (Sacc.) Subram. and Jain were grown and maintained as described earlier (see page 5).

Herbicide treatments consisted of 40 ml (20 ml each 4 and 2 days prior to incubation) 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 sterile distilled water at 5 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 sterile distilled water as a control. Conidia suspensions were prepared with an automatic particle counter (High Accuracy Products Corp., Montclair, CA 91763). Each treatment consisted of 17 individual shoots and was replicated 3 times. Plants were incubated at 24 C under continuous fluorescent light (80-90 $\mu E\ M^{-2}\ sec^{-1}$) for 6 days and then evaluated for disease severity (see page 25).

Leaf tissue was prepared for, and free amino acid concentrations were determined on a leaf age basis by the method described earlier (see page 5). Data were analyzed as a 3x2x4 factorial design for the mean percentage of diseased leaf tissue and for individual and total free amino acid concentrations of the four sequentially aged leaves of shoots of each treatment. Individual amino acids are grouped according

to chemical structure. The groups are presented in order of decreasing relative water solubility.

Correlations for the comparison of the amino acid concentrations in the four leaves of control shoots just prior to inoculation (uninoculated) with leaf spot severity 6 days after inoculation were calculated. Correlations for the comparison of leaf spot severity of four leaves of control shoots with amino acid concentrations in infected leaves (inoculated) 6 days after inoculation also were calculated. The effect of either herbicide on amino acid concentrations in four uninoculated leaves of P. pratensis compared to uninoculated control leaves was correlated with the effect of either herbicide of leaf spot severity 6 days after inoculation compared to inoculated control leaves. The effect of either herbicide on leaf spot severity 6 days after inoculation compared to inoculated control leaves was correlated with the effect of either herbicide on amino acid concentrations in infected leaves 6 days after inoculation compared to infected control leaves.

RESULTS

Disease Severity

The percentage of diseased tissue of the two youngest leaves of control plants was not different (Figure 3.1). However, the percentage of diseased tissue increased from leaf 2 to leaf 3 to leaf 4 of control plants (Figure 3.1). Soil applications of 10^{-4}M MCPP enhanced leaf spot on all but the third leaf, whereas applications of 2,4,5-TP stimulated disease of leaves 2 and 4 only (Figure 3.1).

Total Free Amino Acids

The concentration of total amino acids in leaves of uninoculated control plants was greatest in leaf 1 and least in leaf 3, leaves 2 and 4 were not different (Figure 3.2). Application of either herbicide to uninoculated plants had no effect on total amino acids of leaves of any age compared to the control, but the concentration of total amino acids in leaf 1 of MCPP-treated plants was greater than that of 2,4,5-TP-treated plants (Figure 3.2).

Inoculation of control plants increased total amino acids in all but the second leaf when compared to uninoculated controls (Figure 3.2). Inoculation of MCPP-treated plants increased total amino acids in all leaves, but inoculation of 2,4,5-TP-treated plants resulted in no change in total amino acids in leaves 2, 3 and 4 and a decrease in the youngest leaf compared to their uninoculated counterparts (Figure 3.2).

Figure 3.1. Mean percentage of diseased leaf area of four sequentially aged leaves of control and herbicide-treated Poa pratensis inoculated with Drechslera sorokiniana

Mean percentage of diseased leaf area is the average of 3 replications of 17 shoots each. Numbers followed by 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).

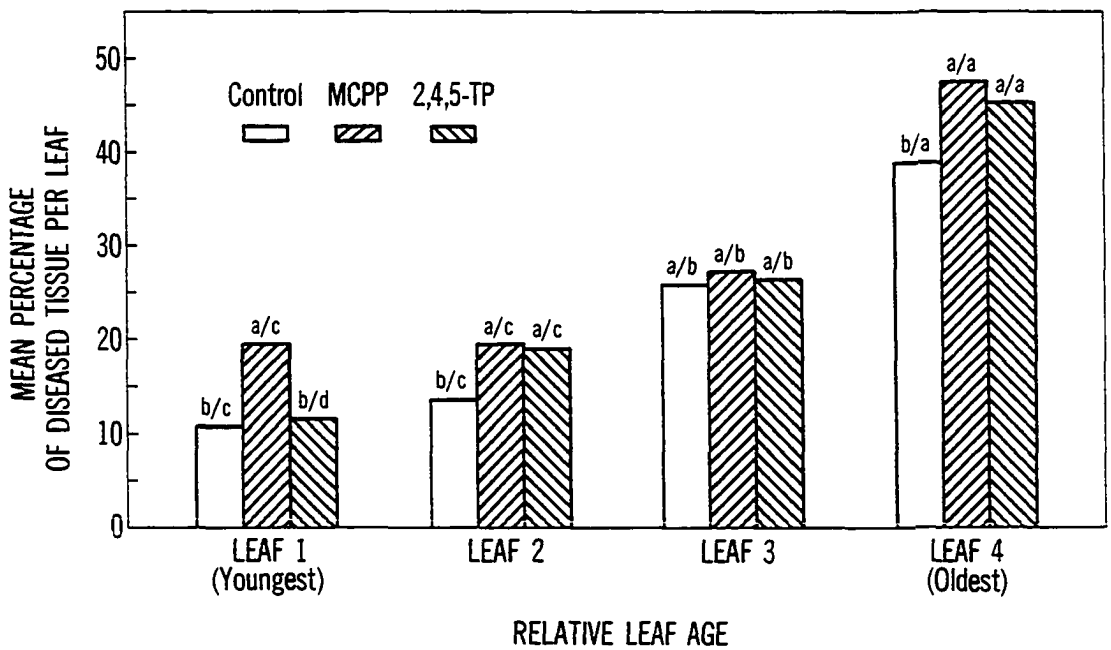
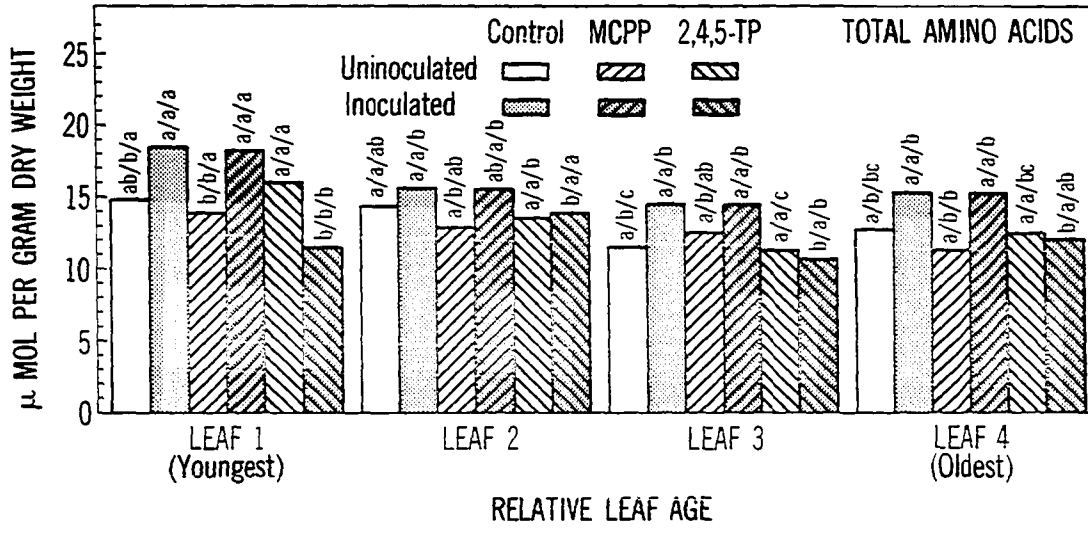

MEAN PERCENTAGE
OF DISEASED TISSUE PER LEAF
50
40
30
20
10
0
Control
MCPP
2,4,5-TP
b/c
a/c
b/d
b/c
a/c
a/c
a/b
a/b
a/b
b/a
a/a
a/a
LEAF 1
(Youngest)
LEAF 2
LEAF 3
LEAF 4
(Oldest)
RELATIVE LEAF AGE

Figure 3.2. Mean concentration (μmol/g D. W.) of total amino acids of four sequentially aged leaves of control and herbicide-treated Poa pratensis both uninoculated and inoculated with Drechslera sorokiniana

Mean concentration (μmol/g D. W.) of total amino acids is the average value of 3 replications from the analysis of leaf tissue of 17 shoots each. Numbers followed by 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).


Control MCPP 2,4,5-TP
TOTAL AMINO ACIDS
Uninoculated
Inoculated
μ MOL PER GRAM DRY WEIGHT
0
5
10
15
20
25
ab/b/a
a/a/a
b/b/a
a/a/a
a/a/a
b/b/b
a/a/ab
a/a/b
a/b/ab
ab/a/b
a/a/b
b/a/a
a/b/c
a/a/b
a/b/ab
a/a/b
a/a/c
b/a/b
a/b/bc
a/a/b
a/b/b
a/a/b
a/a/bc
b/a/ab
LEAF 1
(Youngest)
LEAF 2
LEAF 3
LEAF 4
(Oldest)
RELATIVE LEAF AGE

Heterocyclic Amino Acids

The concentration of Pro in leaves 2 and 4 of uninoculated control plants was greater than that in leaves 1 and 3 (Figure 3.3). The concentration of His in uninoculated control plants decreased from the youngest to the oldest leaf (Figure 3.3). Application of MCPP to uninoculated plants decreased Pro in leaves 2 and 4 and His in all leaves compared to the control (Figure 3.3). The concentrations of Pro in leaves 2 and 4 and His in leaf 3 of uninoculated plants were decreased following application of 2,4,5-TP (Figure 3.3).

Inoculation of control plants increased Pro in leaves 1 and 3 and His in leaves 1, 2 and 4 compared to the uninoculated control (Figure 3.3). Inoculation of MCPP-treated plants increased Pro in leaves 1, 2 and 4 and His in all leaves, and inoculation of 2,4,5-TP-treated plants increased Pro in leaf 2 and His in leaves 2, 3 and 4 compared to uninoculated herbicide-treated plants (Figure 3.3).

Diamino-monocarboxylic Acids

The concentrations of Lys and Arg in leaves of uninoculated control plants were greatest in leaf 1 and least in leaf 4 (Figure 3.4). The concentration of Lys in all leaves and Arg in leaves 1, 2 and 4 decreased in MCPP-treated uninoculated plants compared to the uninoculated control (Figure 3.4). Application of 2,4,5-TP to uninoculated plants increased Arg in leaf 1 and decreased Lys in all leaves compared to the control (Figure 3.4).

Inoculation of control plants decreased Lys in all leaves and increased Arg in leaves 1, 2 and 3 compared to the uninoculated control

Figure 3.3. Mean concentration (μmol/g D. W.) of heterocyclic amino acids of four sequentially aged leaves of control and herbicide-treated Poa pratensis both uninoculated and inoculated with Drechslera sorokiniana

Mean concentration (μmol/g D. W.) of heterocyclic amino acids is the average value of 3 replications from the analysis of leaf tissue of 17 shoots each. Numbers followed by 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 amino acid are not significantly different according to Duncan's multiple range test (P = 0.05).

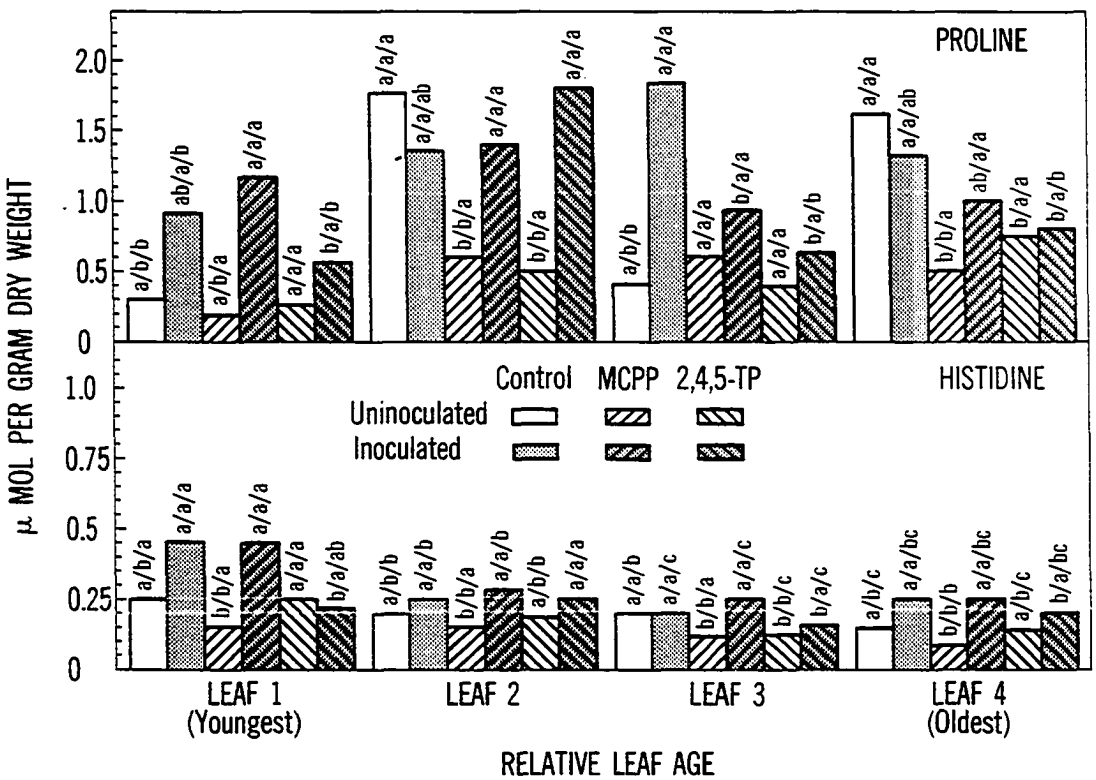
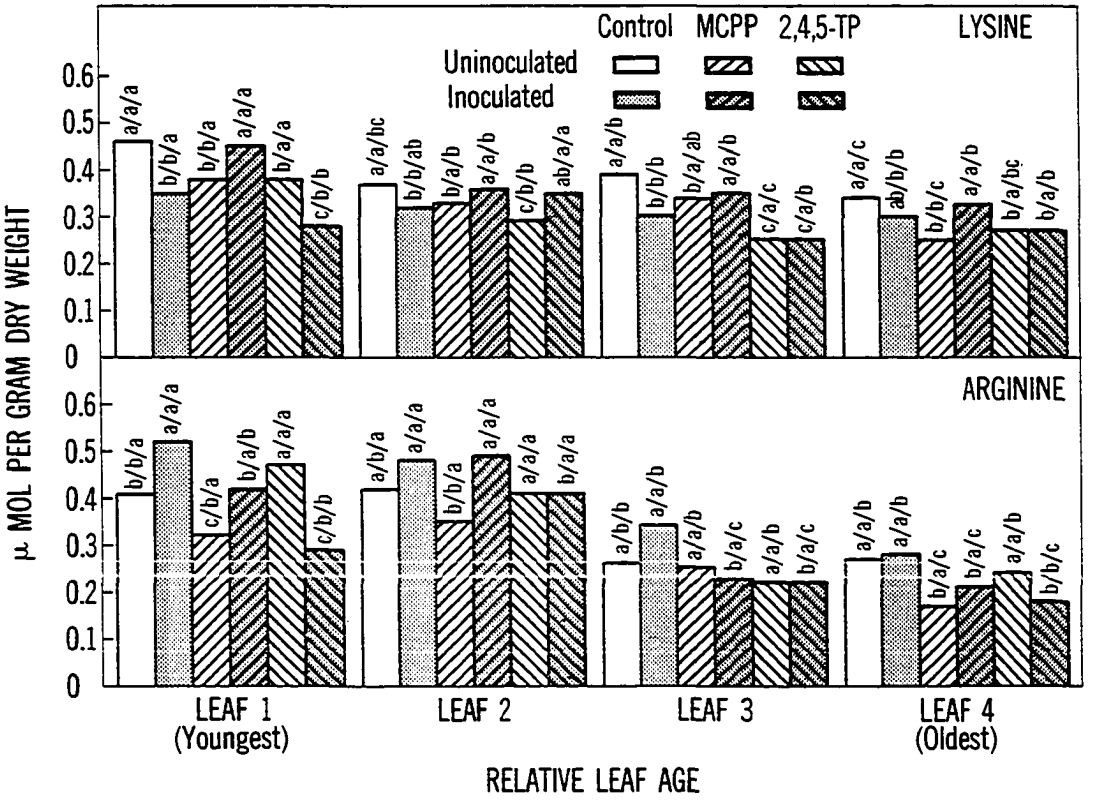

PROLINE
HISTIDINE
Control
MCPP
2,4,5-TP
Uninoculated
Inoculated
μ MOL PER GRAM DRY WEIGHT
RELATIVE LEAF AGE
LEAF 1 (Youngest)
LEAF 2
LEAF 3
LEAF 4 (Oldest)

Figure 3.4. Mean concentration (μmol/g D. W.) of diamino-monocarboxylic acids of four sequentially aged leaves of control and herbicide-treated Poa pratensis both uninoculated and inoculated with Drechslera sorokiniana

Mean concentration (μmol/g D. W.) of diamino-monocarboxylic acids is the average value of 3 replications from the analysis of leaf tissue of 17 shoots each. Numbers followed by 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 amino acid are not significantly different according to Duncan's multiple range test (P = 0.05).


Control
MCPP
2,4,5-TP
Uninoculated
Inoculated
LYSINE
ARGININE
μ MOL PER GRAM DRY WEIGHT
0.6
0.5
0.4
0.3
0.2
0.1
0
a/a/a b/b/a b/b/a a/a/a b/a/a c/b/b
a/a/bc b/b/ab b/a/b a/a/b c/b/b ab/a/a
a/a/b b/b/b b/a/ab a/a/b c/a/c c/a/b
a/a/c ab/b/b b/b/c a/a/b b/a/bc b/a/b
b/b/a a/a/a c/b/a b/a/b a/a/a c/b/b
a/b/a a/a/a b/b/a a/a/a a/a/a b/a/a
a/b/b a/a/b a/a/b b/a/c a/a/b b/a/c
a/a/b a/a/b b/a/c b/a/c a/a/b b/b/c
LEAF 1
(Youngest)
LEAF 2
LEAF 3
LEAF 4
(Oldest)
RELATIVE LEAF AGE

(Figure 3.4). Inoculation of MCPP-treated plants increased Lys in leaves 1 and 4 and Arg in leaves 1 and 2 compared to uninoculated MCPP-treated plants (Figure 3.4). Inoculation of 2,4,5-TP-treated plants increased Lys in leaf 2 and decreased Lys in leaf 1 and Arg in leaves 1 and 4 compared to uninoculated 2,4,5-TP-treated plants (Figure 3.4).

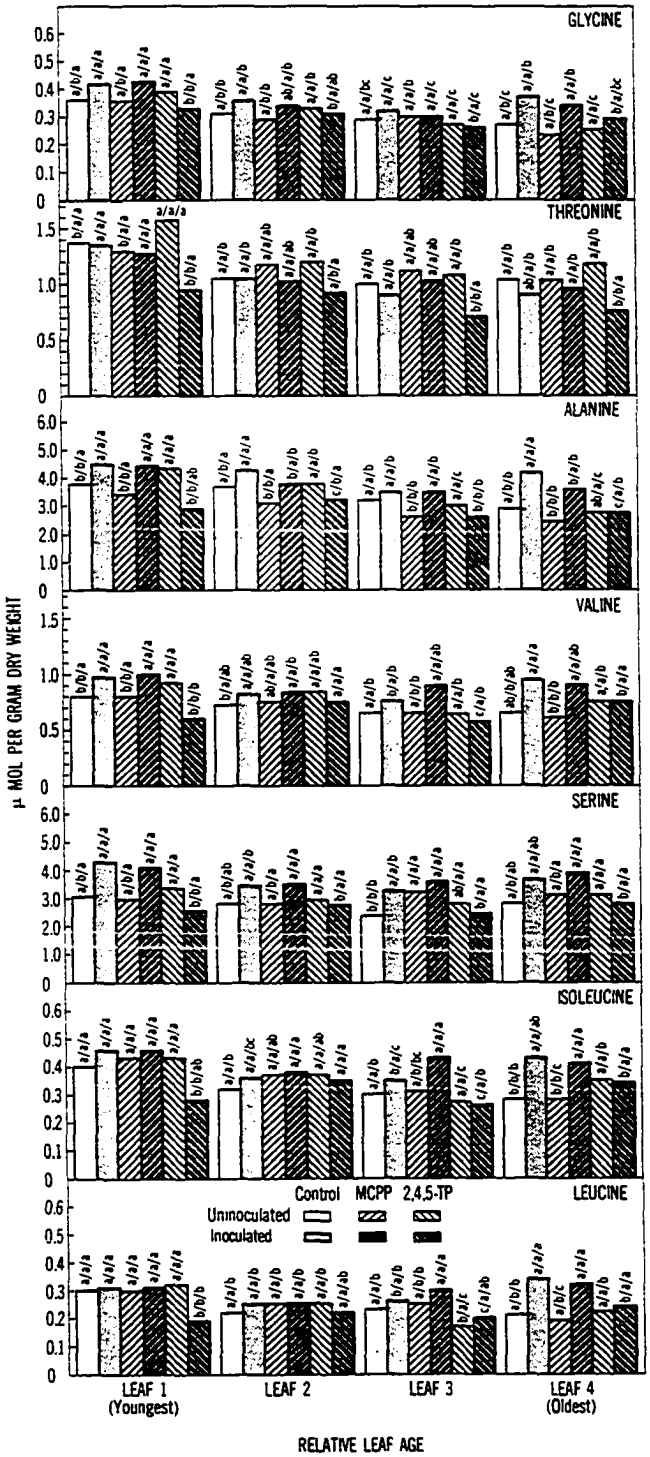
Monoamino-monocarboxylic Acids

The concentrations of Gly, Thr, Ala, Val, Ser, Ile and Leu generally decreased from the youngest to the oldest leaves of uninoculated control plants (Figure 3.5). Ala in leaves 2, 3 and 4 decreased and Ser in leaf 3 increased in uninoculated plants following MCPP application (Figure 3.5). Application of 2,4,5-TP to uninoculated plants increased Thr and Ala in leaf 1, Val in leaves 1 and 2 and Ile in leaf 4 and decreased Leu in leaf 3 compared to the uninoculated control (Figure 3.5).

Inoculation of control plants increased Gly and Ala in leaves 1, 2 and 4, Val in leaves 1 and 4, Ser in all leaves and Ile and Leu in leaf 4 compared to the uninoculated control (Figure 3.5). Inoculation of MCPP-treated plants increased the concentration of Gly and Ser in leaves 1, 2 and 4, Ala in all leaves, Val in leaves 1, 3 and 4 and Ile and Leu in leaves 3 and 4 compared to uninoculated MCPP-treated plants (Figure 3.5). Inoculation of 2,4,5-TP-treated plants increased Thr in all leaves, Ala in leaves 1, 2 and 3 and Gly, Ser, Val, Ile and Leu in leaf 1 compared to uninoculated 2,4,5-TP-treated plants (Figure 3.5).

Figure 3.5. Mean concentration (μmol/g D. W.) of monoamino-monocarboxylic acids of four sequentially aged leaves of control and herbicide-treated Poa pratensis both uninoculated and inoculated with Drechslera sorokiniana

Mean concentration (μmol/g D. W.) of monoamino-monocarboxylic acids is the average value of 3 replications from the analysis of leaf tissue of 17 shoots each. Numbers followed by 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 amino acid are not significantly different according to Duncan's multiple range test (P = 0.05).


GLYCINE
THREONINE
ALANINE
VALINE
SERINE
ISOLEUCINE
LEUCINE
μ MOL PER GRAM DRY WEIGHT
Control
MCPP
2,4,5-TP
Uninoculated
Inoculated
LEAF 1 (Youngest)
LEAF 2
LEAF 3
LEAF 4 (Oldest)
RELATIVE LEAF AGE

Monoamino-dicarboxylic Acids

The concentrations of Asp and Glu in leaf 1 of uninoculated control plants were greater than that in the three older leaves (Figure 3.6). Applications of MCPP to uninoculated plants increased Asp in leaf 2 and Glu in leaves 3 and 4 compared to the control while application of 2,4,5-TP to uninoculated plants increased Glu in the fourth leaf compared to the control (Figure 3.6).

Inoculation of control plants increased Glu in all leaves, but no change occurred in Asp compared to the uninoculated control (Figure 3.6). The concentrations of Asp and Glu increased in leaves 1, 2 and 4 following inoculation of MCPP-treated plants compared to uninoculated MCPP-treated plants (Figure 3.6). Inoculation of 2,4,5-TP-treated plants increased Asp in leaves 3 and 4 and Glu in leaf 4 and decreased Asp and Glu in leaf 1 compared to uninoculated 2,4,5-TP-treated plants (Figure 3.6).

Aromatic Amino Acids

The concentration of Phe in leaves 3 and 4 of uninoculated control plants was less than that of leaves 1 and 2 (Figure 3.7). The concentration of Tyr in leaves of uninoculated control plants decreased from the youngest to the oldest leaf (Figure 3.7). The concentration of Phe in leaves 2 and 4 of uninoculated plants was less after treatment with MCPP than in the uninoculated control plants (Figure 3.7). Application of 2,4,5-TP to uninoculated plants decreased Tyr in leaf 3 compared to the uninoculated control (Figure 3.7).

Figure 3.6. Mean concentration (μmol/g D. W.) of monoamino-dicarboxylic acids of four sequentially aged leaves of control and herbicide-treated Poa pratensis both uninoculated and inoculated with Drechslera sorokiniana

Mean concentration (μmol/g D. W.) of monoamino-dicarboxylic acids is the average value of 3 replications from the analysis of leaf tissue of 17 shoots each. Numbers followed by 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 amino acid are not significantly different according to Duncan's multiple range test (P = 0.05).

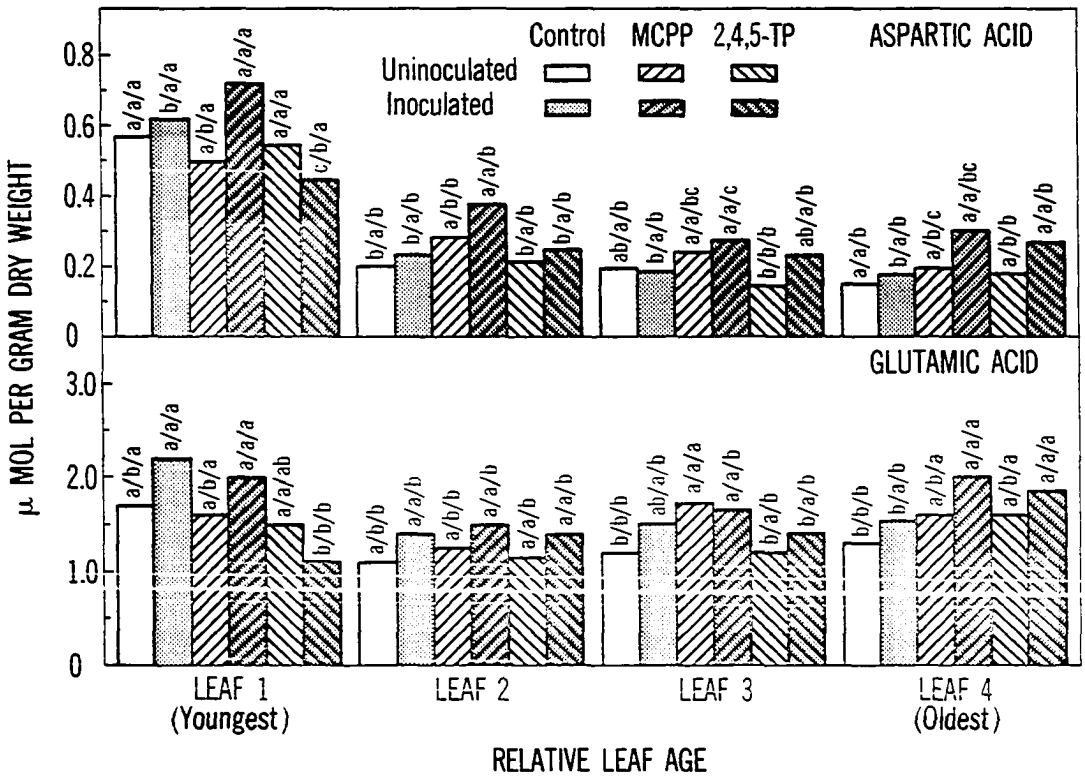
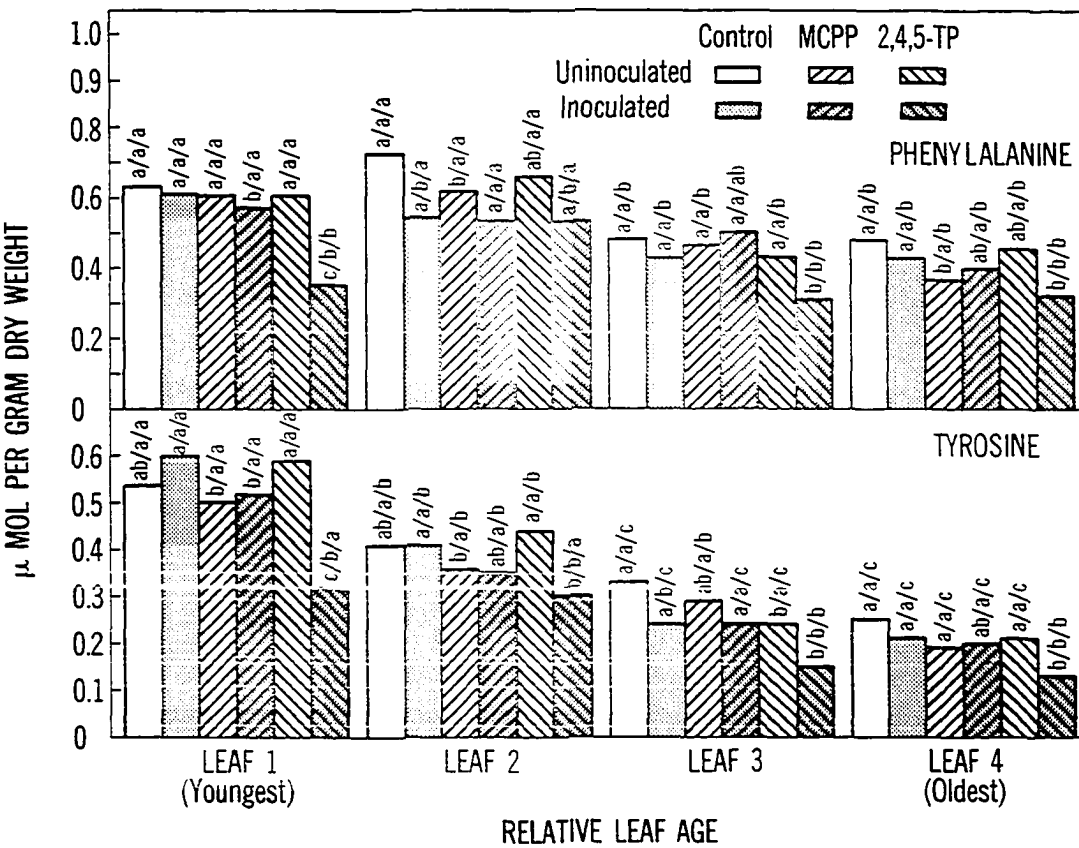

Control
MCPP
2,4,5-TP
Uninoculated
Inoculated
ASPARTIC ACID
GLUTAMIC ACID
μ MOL PER GRAM DRY WEIGHT
0.8
0.6
0.4
0.2
0
3.0
2.0
1.0
0
a/a/a b/a/a a/b/a a/a/a a/a/a c/b/a
b/a/b b/a/b a/b/b a/a/b b/a/b b/a/b
ab/a/b b/a/b a/a/bc a/a/c b/b/b ab/a/b
a/a/b b/a/b a/b/c a/a/bc a/b/b a/a/b
a/b/a a/a/a a/b/a a/a/a a/a/ab b/b/b
a/b/b a/a/b a/b/b a/a/b a/a/b a/a/b
b/b/b ab/a/b a/a/a a/a/b b/a/b b/a/b
b/b/b b/a/b a/b/a a/a/a a/b/a a/a/a
LEAF 1 (Youngest)
LEAF 2
LEAF 3
LEAF 4 (Oldest)
RELATIVE LEAF AGE

Figure 3.7. Mean concentration (μmol/g D. W.) of aromatic amino acids of four sequentially aged leaves of control and herbicide-treated Poa pratensis both uninoculated and inoculated with Drechslera sorokiniana

Mean concentration (μmol/g D. W.) of aromatic amino acids is the average value of 3 replications from the analysis of leaf tissue of 17 shoots each. Numbers followed by 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 amino acid are not significantly different according to Duncan's multiple range test (P = 0.05).


Control
MCPP
2,4,5-TP
Uninoculated
Inoculated
PHENYLALANINE
TYROSINE
μ MOL PER GRAM DRY WEIGHT
1.0
0.9
0.8
0.6
0.4
0.2
0
0.6
0.5
0.4
0.3
0.2
0.1
0
a/a/a
a/a/a
a/a/a
b/a/a
a/a/a
c/b/b
a/a/a
a/b/a
b/a/a
a/a/a
ab/a/a
a/b/a
a/a/b
a/a/b
a/a/b
a/a/ab
a/a/b
b/b/b
a/a/b
a/a/b
b/a/b
ab/a/b
ab/a/b
b/b/b
ab/a/b
a/a/b
b/a/b
ab/a/b
a/a/b
b/b/a
a/a/c
a/b/c
ab/a/b
a/a/c
b/a/c
b/b/b
a/a/c
a/a/c
a/a/c
ab/a/c
a/a/c
b/b/b
LEAF 1
(Youngest)
LEAF 2
LEAF 3
LEAF 4
(Oldest)
RELATIVE LEAF AGE

Inoculation of control plants decreased Phe in leaf 2 and Tyr in leaf 3 compared to the uninoculated control (Figure 3.7). Inoculation of herbicide-treated plants resulted in distinct herbicide effects. Inoculation of MCPP-treated plants resulted in no change in either Phe or Tyr in any leaf while inoculation of 2,4,5-TP-treated plants decreased both Phe and Tyr in all leaves compared to uninoculated herbicide-treated plants (Figure 3.7).

Correlations

Correlation coefficients for comparisons of the percentage of diseased leaf tissue with pre- and post-infection (uninoculated and inoculated, respectively) concentrations of amino acids in sequentially aged leaves were calculated for controls (Table 3.1). The pre-infection concentrations of Ala, Asp, and Tyr in leaves of control plants correlated negatively with the increase of leaf spot severity from the youngest to the oldest leaves of inoculated control plants (Table 3.1). The post-infection concentrations of Arg, Thr, Asp, Phe, Tyr in leaves of control plants correlated negatively and His correlated positively with leaf spot severity of control plants (Table 3.1).

Correlation coefficients for the comparison of the effects of MCPP and 2,4,5-TP on the concentrations of leaf free amino acids with the effects of the herbicides on leaf spot severity as compared to the control were determined (Table 3.2). The decrease in the pre-infection concentrations of Lys, Thr, Ser, Asp, Glu and total free amino acids in leaves of MCPP-treated plants correlated with the increased percent

of diseased leaf area of MCPP-treated, inoculated plants (Table 3.2). The decrease of the pre-infection concentration of Pro in leaves of 2,4,5-TP-treated plants correlated with the increased percent of diseased leaf area of 2,4,5-TP-treated, inoculated plants (Table 3.2). Increased percentage of diseased leaf area of MCPP-treated, inoculated plants correlated with increased Pro and decreased Phe post-infection concentrations in leaves of MCPP-treated plants (Table 3.2). Increased percentage of diseased leaf area of 2,4,5-TP-treated, inoculated plants correlated with increased post-infection concentrations of Arg, Thr, Ser, Ile, Leu, Phe, Tyr and total free amino acids in leaves of 2,4,5-TP-treated plants (Table 3.2).

Table 3.1. Correlation coefficients for the comparison of amino acid concentrations (μmol/g D. W.) of four sequentially aged leaves of Poa pratensis both uninoculated and inoculated with Drechslera sorokiniana with the mean percentage of diseased leaf area of control plants[a]

Amino acids	Uninoculated	Inoculated
Proline	+0.300	+0.145
Histidine	-0.487	+0.528*
Lysine	-0.373	-0.312
Arginine	-0.424	-0.870**
Glycine	-0.439	-0.207
Threonine	-0.377	-0.742**
Alanine	-0.522*	-0.200
Valine	-0.267	+0.088
Serine	-0.188	-0.279
Isoleucine	-0.327	+0.061
Leucine	-0.210	+0.436
Aspartic acid	-0.548*	-0.643**
Glutamic acid	-0.258	-0.358
Phenylalanine	-0.350	-0.656**
Tyrosine	-0.532*	-0.834**
Total	-0.307	-0.433

[a]Correlation coefficients were determined from values of 3 replicates of each treatment. Numbers followed by ** are significant at P = 0.05 and by * are significant at P = 0.10.

Table 3.2. Correlation coefficients for the comparison of effects of 10^{-4}M MCPP and 2,4,5-TP on the concentration (μmol/g D. W.) of amino acids of four sequentially aged leaves of Poa pratensis both uninoculated and inoculated with Drechslera sorokiniana with effects on the mean percentage of diseased leaf area as compared to the control[a]

	Uninoculated		Inoculated	
	MCPP	2,4,5-TP	MCPP	2,4,5-TP
Proline	-0.207	-0.764**	+0.887**	+0.223
Histidine	+0.413	-0.197	-0.323	+0.474
Lysine	-0.543*	-0.099	+0.227	+0.445
Arginine	-0.142	-0.437	-0.324	+0.600**
Glycine	-0.413	-0.059	+0.362	+0.290
Threonine	-0.533*	-0.249	-0.395	+0.642**
Alanine	+0.096	-0.409	-0.453	+0.172
Valine	-0.202	-0.189	-0.122	+0.444
Serine	-0.623**	-0.363	-0.250	+0.518*
Isoleucine	-0.126	-0.098	-0.101	+0.584**
Leucine	-0.319	-0.081	+0.087	+0.586**
Aspartic acid	-0.594**	-0.113	-0.086	+0.441
Glutamic acid	-0.641**	-0.021	-0.221	+0.463
Phenylalanine	-0.003	-0.321	-0.665**	+0.619**
Tyrosine	+0.229	-0.192	-0.350	+0.523*
Total	-0.544*	-0.400	+0.212	+0.559*

[a]Correlation coefficients were determined for the difference of herbicide treatments and control treatments from values of 3 replications for each treatment. Numbers followed by ** are significant at P = 0.05 and by * are significant at P = 0.10.

DISCUSSION

Leaf spot of Poa pratensis caused by Drechslera sorokiniana is enhanced by conditions that promote leaf senescence (10). Leaf spot is most severe on older near-senescent leaves of a shoot and is typified by more extensive chlorosis (16). The results of the percentage diseased leaf area of sequentially senescent leaves of plants in this study support this hypothesis (Figure 3.1). Decreasing photoperiod, blue + far red light and auxin-like herbicides enhance leaf spot severity (16, 17). Decreasing photoperiod and blue-biased light were believed to promote premature leaf senescence and enhance leaf spot (16).

The senescence process in leaves involves proteolysis, the loss of chlorophyll, decreased photosynthesis, increased respiration and a decline in RNA synthesis (26). The correlation of the concentration of leaf sugars with leaf spot severity in relation to leaf senescence has been investigated (see page 25). The concentration of amino acids in leaves of uninoculated control plants generally declined from the youngest to the oldest leaves (Figures 3.2-3.7). The concentration of amino acids in leaves may be associated with leaf spot severity (see page 5).

The auxin-like postemergence herbicides, MCPP and 2,4,5-TP, affect the physiology of susceptible plants in a manner similar to the senescence process and decrease the concentration of amino acids in leaves due to proteolysis and translocation of amino acids to the stem and growing point (2, 6, 22). Application of MCPP or 2,4,5-TP influenced the concentrations of amino acids in uninoculated leaves of herbicide-tolerant P. pratensis (Figures 3.2-3.7). The more water soluble amino

acids Pro, His, Lys and Arg are generally decreased by herbicide treatment while the less water soluble amino acids Asp and Glu are increased (Figures 3.2-3.7). The more water soluble amino acids are probably more readily translocated out of the leaves following proteolysis. The influence of MCPP on amino acid concentration is evident in all four leaves of one shoot in most cases whereas the influence of 2,4,5-TP is predominantly in the younger leaves (Figures 3.2-3.7).

Infection of leaves by D. sorokiniana increased the concentration of most amino acids in leaves of control plants (Figures 3.2-3.7). Infection sites may act as a sink for amino acids from proteolysis (12, 21, 27). The establishment of a nutrient sink at infection sites may enhance senescence of the plant and facilitate subsequent infection by D. sorokiniana (5, 18). Lys and Phe, however, decreased in all leaves following infection (Figures 3.4 and 3.7). High concentrations of Lys have been associated with the inhibition of proteolysis in Avena leaves (28). The Phe pool serves as a precursor in synthesis of phenol compounds which may be enhanced in response to fungal infection and decrease the concentration of Phe (7).

The interactions of the herbicides with infection by D. sorokiniana on amino acid concentrations reveal differences in activity of the two herbicides. Inoculation of plants treated with MCPP resulted in increases of Pro, His, Lys, Arg, Gly, Ala, Val and Ser in both young and old leaves and Ile and Leu in old leaves only (Figures 3.3-3.5). Inoculation of plants treated with 2,4,5-TP resulted in increases of the most water

soluble amino acids Pro and His in both young and old leaves, but decreases in most other amino acids in the youngest leaf compared to uninoculated 2,4,5-TP-treated plants (Figures 3.3-3.7). A difference in activity of the two herbicides also is evident in correlations of pre-infection (uninoculated) amino acid concentrations with subsequent disease severity (Table 3.1). The decrease of many amino acids from the youngest to the oldest leaf following application of MCPP correlated with the enhancement of leaf spot on each older leaf by MCPP (Table 3.1). Only decreased pre-infection Pro concentrations in leaves of 2,4,5-TP-treated plants correlated with increased disease severity (Table 3.1).

The difference between the herbicides' effects on amino acid concentrations may be explained by differences in translocation of the herbicides within P. pratensis (13). Monochlorinated MCPP is absorbed by the roots more readily than is 2,4,5-TP and is more easily translocated to leaves of herbicide-susceptible plants after absorption by the roots (19, 24). The less mobile 2,4,5-TP accumulates in root tips with limited translocation to the rest of the shoot after uptake by the roots of plants susceptible to the herbicide (3, 14). A previous paper reported decreasing leaf spot severity with increasing concentration of 2,4,5-TP (8). The percentage diseased leaf tissue of the youngest leaf is less following 2,4,5-TP treatment compared to MCPP treatment (Figure 3.1). It seems that the concentration of MCPP in leaves of any age stimulates proteolysis and enhances leaf spot. The concentration of 2,4,5-TP in leaves 2, 3 and 4 may be minimal and may have a limited influence on proteolysis and less enhancement of leaf spot than does MCPP.

Phenoxy acid herbicides, especially 2,4,5-TP which accumulates in the roots, could potentially influence root physiology and subsequently the whole shoot. The movement of root-produced cytokinins in the xylem is well known (18). The growth retardant CCC (chlormequat) induces root swelling due to increased cell division after application to roots of some plants (25). The root swelling has been attributed to increased cytokinin production in the roots which also increased the cytokinin concentration in the xylem (25). The phenoxy acid herbicides inhibit root elongation and stimulate cell division in some grass species (29). 2,4,5-TP is particularly injurious to roots of grasses (4). Increased cytokinin concentration in the xylem could influence the metabolism of the youngest leaf of 2,4,5-TP-treated plants. Cytokinins inhibit leaf senescence by increasing protein synthesis, decreasing respiration and inhibiting chlorophyll loss (29). The stimulation of protein synthesis could result in an influx of amino acids and an inhibition of leaf spot in the youngest leaf of 2,4,5-TP-treated plants compared to MCPP-treated plants (20).

REFERENCES

1. Arjunan, G., P. Vidhyasekaran, and T. K. Kandaswamy. 1976. Changes in amino acids and amides content in jowar leaves infected by Helminthosporium turcicum. Cur. Sci. 45:229-230.

2. Ashton, F. M., and D. E. Bayer. 1976. Effects on solute transport and plant constituents. Pages 219-253. In L. J. Audus, ed. Herbicides: physiology, biochemistry, ecology. Vol. 1, 2nd ed. Academic Press, New York. 608 p.

3. Ashton, F. M., and A. S. Crafts. 1981. Mode of action of herbicides. 2nd ed. John Wiley and Sons, New York. 525 p.

4. Callahan, L. M., and R. E. Engel. 1965. The effects of phenoxy herbicides on the physiology and survival of turfgrass. U. S. G. A. Green Sect. Rec. 3(1):1-5.

5. Farkas, G. L. 1978. Senescence and plant disease. Pages 391-412. In J. G. Horsfall and E. B. Cowling, ed. Plant disease: An advanced treatise. Vol. III. How plants suffer from disease. Academic Press, New York. 487 p.

6. Freiburg, S. R., and H. E. Clark. 1952. Effects of 2,4,-dichlorophenoxyacetic acid upon the nitrogen metabolism and water relations of soybean plants grown at different nitrogen levels. Bot. Gaz. 113:322-333.

7. Goodman, R. N., Z. Kiraly, and M. Zaitlin. 1967. The biochemistry and physiology of infections plant disease. D. Van Nostrand Co., Princeton, New Jersey. 354 p.

8. Hodges, C. F. 1978. Postemergent herbicides and the biology of Drechslera sorokiniana: Influence on severity of leaf spot on Poa pratensis. Phytopathology 68:1359-1363.

9. Hodges, C. F. 1980. Interaction of sequential leaf senescence of Poa pratensis and pathogenesis by Drechslera sorokiniana as influenced by postemergent herbicides. Phytopathology 70:628-630.

10. Hodges, C. F. 1980. Postemergent herbicides and pathogenesis by Drechslera sorokiniana on leaves of Poa pratensis. Pages 101-112. In P. O. Larsen and B. G. Joyner, ed. Advances in turfgrass pathology. Harcourt Brace Jovanovich, Inc., Duluth. 197 p.

11. Hodges, C. F., and J. P. Madsen. 1979. Leaf senescence as a factor in the competitive and synergistic interactions of Drechslera sorokiniana and Curvularia geniculata on Poa pratensis. Can. J. Bot. 57:1706-1711.

12. Huber, D. M. 1978. Disturbed mineral nutrition. Pages 163-181. In J. G. Horsfall and E. B. Cowling, ed. Plant disease: An advanced treatise. Vol. III. How plants suffer from disease. Academic Press, New York. 487 p.

13. Leopold, A. C., and P. E. Kriedemann. 1975. Plant growth and development. 2nd ed. McGraw-Hill Co., New York. 545 p.

14. Loos, M. A. 1975. Phenoxyalkanoic acids. Pages 1-128. In P. C. Kearney and D. D. Kaufman, ed. Herbicides: chemistry, degradation, and mode of action. Vol. 1, 2nd ed. Marcel Dekker, New York. 500 p.

15. Moreland, D. E. 1967. Mechanisms of action of herbicides. Annu. Rev. Plant Physiol. 18:365-386.

16. Nilsen, K. N., and C. F. Hodges. 1980. Photomorphogenically defined light and resistance of Poa pratensis to Drechslera sorokiniana. Plant Physiol. 65:569-573.

17. Nilsen, K. N., C. F. Hodges, and J. P. Madsen. 1979. Pathogenesis of Drechslera sorokiniana leaf spot on progressively old leaves of Poa pratensis as influenced by photoperiod and light quality. Physiol. Plant Pathol. 15:171-176.

18. Nooden, L. D. 1980. Senescence in the whole plant. Pages 219-244. In K. V. Thimann, ed. Senescence in plants. CRC Press, Boca Raton, Fl. 276 p.

19. Norris, L. A., and V. H. Freed. 1966. The absorption and translocation characteristics of several phenoxyalkyl herbicides in bigleaf maple. Weed Res. 6:203-211.

20. Osborne, D. J., and M. Hallaway. 1964. The auxin 2,4-D as a regulator of protein synthesis and senescence in detached leaves of Prunus. New Phytol. 63:334-346.

21. Raghunathan, R., A. Madhadevan, and G. Rangaswami. 1966. Biochemical changes in the banana fruit-coat caused by Gleosporium musarum infection. Indian Phytopath. 19:162-167.

22. Ries, S. K. 1976. Subtoxic effects on plants. Pages 313-344. In L. J. Audus, ed. Herbicides: physiology, biochemistry, ecology. Vol. II, 2nd ed. Academic Press, New York. 564 p.

23. Robinson, P. W., and C. F. Hodges. 1976. An inoculation apparatus for evaluation of Bipolaris sorokiniana lesion development on progressively older leaves of Poa pratensis. Phytopathology 66:360-362.

24. Sanad, A. J. 1971. Studies on the uptake and translocation of ^{14}C-labeled herbicides in Agrostemma githago and Tussilago farfara. Weed Res. 11:215-223.

25. Skene, K. G. M. 1970. The relationship between the effects of CCC on root growth and cytokinin levels in the bleeding sap of Vitus vinifera L. J. Exp. Bot. 21:418-431.

26. Thimann, K. V. 1980. The senescence of leaves. Pages 85-115. In K. V. Thimann, ed. Senescence in plants. CRC Press, Boca Raton, Fl. 276 p.

27. Van Andel, D. M. 1966. Amino acids and plant diseases. Ann. Rev. Phytopathol. 4:349-368.

28. Von Abrams, G. J. 1974. An effect of ornithine on degradation of chlorophyll and protein in excised leaf tissue. Z. Pflanzenphysiol. 72:410-421.

29. Weaver, R. J. 1972. Plant growth substances in agriculture. W. H. Freeman and Co., San Francisco. 594 p.

PART IV. INFLUENCE OF POSTEMERGENCE HERBICIDES ON ENDOGENOUS ETHYLENE AND ON CHLOROSIS OF LEAVES OF *POA PRATENSIS* INDUCED BY A TOXIC EXTRACT FROM *DRECHSLERA SOROKINIANA*

INTRODUCTION

Leaf spot on Poa pratensis L. caused by Drechslera sorokiniana (Sacc.) Subram. and Jain (= Helminthosporium sativum P. K. and B.) is characterized by necrotic lesions with chlorotic halos (17). Symptoms are more pronounced on older, near senescent leaves (16, see page 25). Application of select phenoxy acid postemergence herbicides to the soil prior to inoculation of leaves of P. pratensis with conidia of D. sorokiniana enhances subsequent leaf spot development primarily on older leaves by causing enlarged chlorotic halos and chlorotic streaking between lesions (16, 17, see page 25). Similar symptoms occur following incubation of inoculated plants under a blue + far red light spectrum and short (10 hr) photoperiods (23). The auxin-like postemergence herbicides, light quality, and short photoperiods have been suggested to promote senescence in older leaves and enhance leaf spot and the associated chlorosis (17).

The chlorotic halos associated with D. sorokiniana leaf spot are typical of chlorosis induced by fungal toxins (33). Culture filtrates of D. sorokiniana contain many related sesquiterpenoid compounds that have various effects on plant growth ranging from phytotoxicity to plant-growth stimulation (6). Much work has been done in trying to determine the nature of the compounds with phytotoxic activity, but many questions remain.

The first phytotoxic compound to be isolated from culture filtrates of D. sorokiniana was helminthosporal (10). Helminthosporal produced

symptoms similar to those caused by D. sorokiniana (31). However, it was later found to be an artefact of the extraction procedure (31). A precursor of helminthosporal, prehelminthosporal, was isolated and has demonstrated growth-inhibiting properties (31). A number of analogs of these compounds have been synthesized that have mixed effects on plant growth depending upon the bioassay and the experimental procedures (6). It seems plausible to assume based upon the body of literature regarding this subject, that D. sorokiniana may produce a non-host specific toxin(s) in culture.

Some question remains about the nature of the role of the toxin(s) in D. sorokiniana leaf spot. Toxic substances from D. sorokiniana were suggested to predispose barley to seedling blight caused by D. sorokiniana (21). Such predisposition has been questioned (33). A culture filtrate of D. sorokiniana caused typical leaf spot symptoms on both susceptible and resistant varieties of barley (13). The toxin(s) probably play(s) an indirect role in complex intractions affecting pathogenesis by D. sorokiniana.

The extensive chlorosis associated with D. sorokiniana leaf spot on postemergence herbicide-treated P. pratensis is indicative of a possible involvement of ethylene. Ethylene causes accelerated chlorophyll degradation in leaves of a number of plants (1, 2, 3). The hypothesis that leaf senescence promotes D. sorokiniana leaf spot also suggests a possible ethylene role (17). The auxin-like phenoxy herbicides generally stimulate ethylene production in leaves of herbicide-susceptible plants

(1) and have been shown to promote ethylene production in some herbicide-tolerant monocot species (1). Infection by fungal organisms also may increase the rate of ethylene production in the infected plant (1). Ethylene plays a major role in promoting senescence of certain plant parts and has a minor influence on leaf senescence (25, 32).

The interaction of potentially herbicide-induced ethylene and a toxin(s) from D. sorokiniana may be of significance in pathogenesis by D. sorokiniana on P. pratensis. Phenoxy acid herbicides damage chloroplasts, tonoplasts, plasmalemma and mitochondrial membranes of cells of some plants (5). Helminthosporal alters the permeability of the plasmalemma and tonoplasts of beet root cells (34). An interaction of ethylene and toxin in necrosis has been proposed where the generation of ethylene predisposes plant membranes to the toxin (26). Studies were undertaken to examine the interactions of phenoxy acid herbicides and a toxic extract from D. sorokiniana on endogenous ethylene concentrations and chlorophyll content of leaves of herbicide-tolerant P. pratensis.

MATERIALS AND METHODS

Plant Materials

Poa pratensis L. 'Newport' was used for all studies. Plants were propagated vegetatively in a steamed 2:1 loam-peat mix in 7.6 cm plastic pots. Plants were grown in the greenhouse under 16 hr of light (day light supplemented with incandescent lights) for 60 days prior to treatment. Cultures of Drechslera sorokiniana (Sacc.) Subram. and Jain were maintained on 20 ml of 1% Czapek Dox broth in 3% (v/v) Bacto-agar in 15x85 mm sterile plastic petri dishes. Culture virulence was maintained by isolating hyphal tips from isolates from diseased leaf tissue of P. pratensis obtained by periodic inoculations. Only 20-day-old cultures were used to seed media used in extracting toxic substances (14).

Extraction of Toxic Substances

Four 10-mm discs from the agar-grown cultures of D. sorokiniana were used to seed 200 ml of Czapek Dox broth (8.75 g/1000 ml H_2O). These broth cultures were grown for 6 days at 25 C under constant light, then the broth was filtered under vacuum through Whatman No. 1 filter paper to remove mycelia. The culture filtrate was then passed through a 0.45 μm Millipore filter under vacuum, then a 150 ml aliquot was treated 3 times with half-volumes of ethyl ether in a separatory funnel. The pooled ether extracts were dried with anhydrous Na_2SO_4 and evaporated under vacuum at 38 C (29). The residue was dissolved in 10 ml ethanol and re-evaporated. A 7 ml aliquot of sterile distilled

water was added to the residue, and placed on a shaker for 20 hr at 4 C in darkness. The toxic extract was stored in the dark at 4 C until it was applied to the leaves. Controls for the medium were made by extracting from unseeded Czapek Dox broth. A water control also was used.

Treatments

Because of variation in temperatures and light conditions in the greenhouse during the study, all plants were preconditioned prior to receiving treatment. Preconditioning consisted of incubation at 20 C under a 10 hr photoperiod for 4 days. Plants were treated with 40 ml (20 ml each, 4 and 2 days prior to treatment with the toxic extract) of 10^{-4}M 2-(2-methyl-4-chlorophenoxy)propionic acid (MCPP), 10^{-4}M 2-(2,4,5-trichlorophenoxy)propionic acid (2,4,5-TP) or with distilled water (herbicide control) applied to the soil. The four youngest visible leaves of 1 shoot received 0.02 ml of the toxic extract, medium control or H_2O control at 5 positions 1 cm apart along a 10 cm section of the leaf approximately 5 cm from the leaf tip in a special inoculation apparatus (27). Each treatment consisted of two shoots with 4 leaves each and was replicated 3 times.

Treated plants were incubated at 24 C under fluorescent light (80-90 $\mu E\ M^{-2}\ min^{-1}$) with a 10 hr photoperiod under atmospheric (1000 mbar) and hypobaric (233 mbar) pressures (24). Oxygen (100%) was flowed at a rate of 177 cc min^{-1} and CO_2 in O_2 (30,000 ppm) was flowed at a rate of 9 cc min^{-1} for a total flow rate of 186 cc min^{-1} at 233 mbar. Relative humidity was maintained near 100%.

Chlorophyll Determination

Chlorophyll concentrations were determined for the four leaves of H_2O control-, medium control-, and toxic extract-treated leaves of herbicide-treated or control plants at the time of incubation and at 24, 48 and 72 hr after incubation at 1000 mbar or 233 mbar. Leaves were harvested as 10-cm sections from the inoculation apparatus and cut into 2- or 3-mm segments. The leaf segments from 1 shoot were placed in a 50 ml Erlenmeyer flask and covered with 30 ml 95% ethanol. The stoppered flasks were kept in the dark. After 24 hr, the ethanol was decanted into a 250 ml Erlenmeyer flask and a second 30 ml aliquot of 95% ethanol was added to the flask containing the leaf segments. Both flasks were stoppered and stored in darkness. This process was repeated at 48 and 72 hr from leaf harvest. After 72 hr, the ethanol-chlorophyll solution was brought to 200 ml with ethanol. The leaf tissue was freeze-dried at -50 C for 48 hr and then weighed. The concentration of total chlorophyll was calculated and expressed as a percent of the chlorophyll of the medium control (18). The values from the leaves of two plants of each treatment were averaged. The means of 3 replicates of each treatment were analyzed as a 3x3x2 factorial.

Ethylene Determination

Endogenous ethylene was determined for medium control- and toxic extract-treated leaves of herbicide-treated or control plants at the time of incubation and at 24, 48 and 72 hr after incubation at 1000 mbar.

The internal gases of the four leaves of 1 shoot were extracted using a modification of the method described by Beyer and Morgan (7). The leaves were harvested in 20-cm sections and immersed in saturated $(NH_4)_2SO_4$. Internal gases were released and collected at 120 mbar for 3 min. A 1.0 cc gas sample was injected into a Varian 3700 GC with a FID. The injector and detector temperatures were 250 C, the oven temperature was 110 C and the carrier gas (He) flow rate was 30 cc min^{-1}. The FID signal was fed through a Cary Model 401 electrometer to a Spectra-Physics 4100 computing integrator. Data were expressed as $\mu l\ l^{-1}$ ethylene. The values from the leaves of two plants of each treatment were averaged. The means of 3 replicates of each treatment were analyzed as a 3x2 factorial.

RESULTS

The extract from Czapek Dox broth-grown cultures of D. sorokiniana (toxic extract) produced chlorosis of leaves of P. pratensis (Figure 4.4). The Czapek Dox medium control resulted in leaf chlorophyll contents (μg chl./mg D. W.) nearly equal to that of leaves of water control plants (±6%). Therefore, chlorophyll data are expressed as a percentage of the Czapek Dox medium control throughout the study.

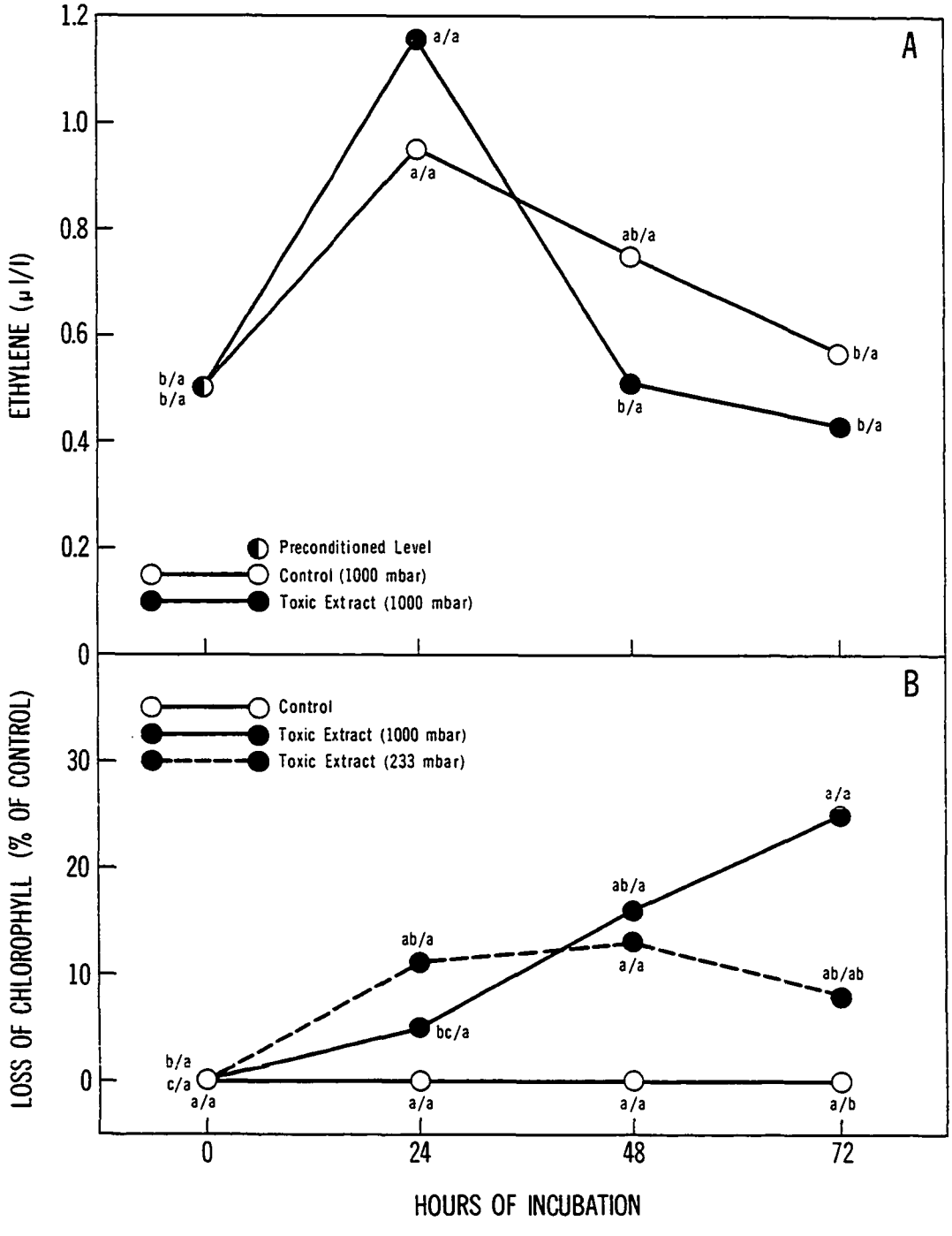
Effects of Toxic Extract on Endogenous Ethylene and Chlorophyll Content of Plants not Exposed to Herbicides

The concentration of endogenous ethylene in Czapek Dox medium control-treated leaves of plants receiving no herbicide rose from the level of preconditioned plants during the first 24 hr of incubation and then declined to a concentration not different from the preconditioned level (Figure 4.1A). The toxic extract caused a slightly higher rise of ethylene in leaves of plants receiving no herbicide after 24 hr incubation and a more severe decline after 48 and 72 hr than in the control (Figure 4.1A).

The loss of chlorophyll from leaves of toxic extract-treated leaves of plants receiving no herbicide and incubated at 1000 mbar increased from 0 to 72 hr incubation where the loss was greater than that of the control (Figure 4.1B). The chlorotic leaves frequently had a green to dark green area within the yellowed tissue (Figure 4.6). Incubation at 233 mbar resulted in a leveling off of the chlorophyll loss of toxic extract-treated leaves of plants receiving no herbicide after 24 hr

Figure 4.1. Effect of a toxic extract from cultures of Drechslera sorokiniana on endogenous ethylene and chlorophyll content of leaves of Poa pratensis plants not exposed to herbicides and incubated at 1000 and 233 mbar

Ethylene (µl/l) is the mean concentration in internal gases of 4 leaves of 2 shoots per treatment replicated 3 times. Controls are medium control-treated leaves. The loss of chlorophyll is the mean percentage of chlorophyll (µg/mg D. W.) in 4 leaves of 2 shoots per treatment replicated 3 times compared to medium control-treated leaves. Numbers followed by the same letter among hours of incubation within control and toxic extract treatments (across a/) or among control and toxic extract treatments within hours of incubation (down /a) are not significantly different according to Duncan's multiple range test (P = 0.05).


A
ETHYLENE (µl/l)
1.2
1.0
0.8
0.6
0.4
0.2
0
a/a
a/a
ab/a
b/a
b/a
b/a
b/a
Preconditioned Level
Control (1000 mbar)
Toxic Extract (1000 mbar)
B
LOSS OF CHLOROPHYLL (% OF CONTROL)
30
20
10
0
Control
Toxic Extract (1000 mbar)
Toxic Extract (233 mbar)
a/a
ab/a
ab/a
a/a
ab/ab
bc/a
b/a
c/a
a/a
a/a
a/a
a/a
a/b
0
24
48
72
HOURS OF INCUBATION

that remained below that of plants incubated at 1000 mbar and not different from the control through 72 hr incubation (Figures 4.1B and 4.5).

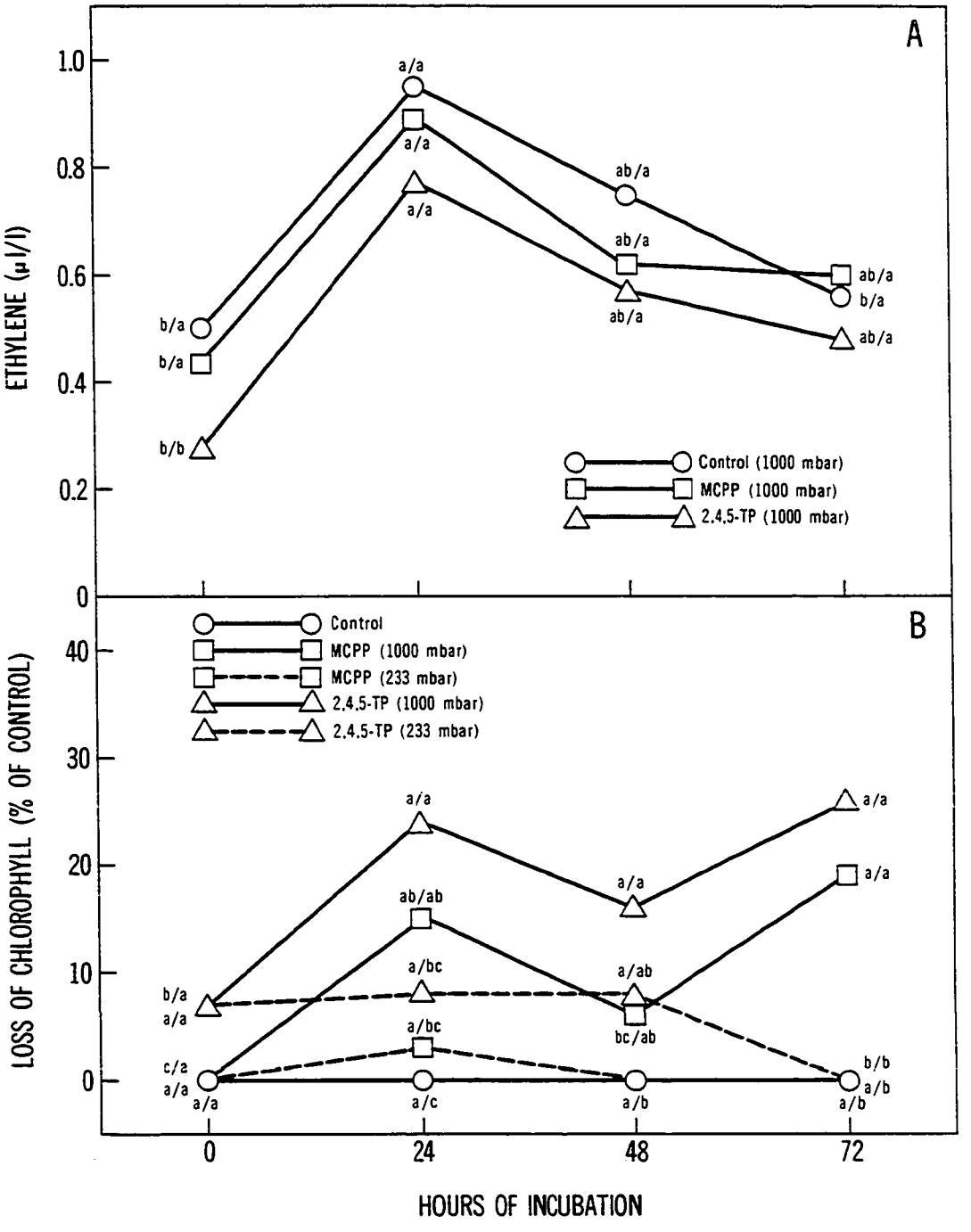
Effects of Postemergence Herbicides on Endogenous Ethylene and Chlorophyll Content of Leaves

The application of 10^{-4}M MCPP or 2,4,5-TP to the soil influenced ethylene and chlorophyll concentrations in Czapek Dox medium control-treated leaves of P. pratensis (Figures 4.2A and B). The concentration of ethylene in leaves of 2,4,5-TP-treated plants at 0 hr incubation (48 hr after the second herbicide application) was less than that of the control or MCPP-treated plants (Figure 4.2A). Ethylene determinations at 3 and 5 hr after the second herbicide application showed the same relationship. No differences in ethylene concentrations existed among leaves of herbicide-treated and control plants at 24, 48 and 72 hr incubation (Figure 4.2A). The concentration of ethylene in leaves of MCPP- or 2,4,5-TP-treated plants rose during the first 24 hr incubation and then declined as in the control (Figure 4.2A).

Application of MCPP or 2,4,5-TP to the soil prior to incubation caused chlorophyll loss from medium control-treated leaves of plants incubated at 1000 mbar for 24 hr (72 hr after second herbicide application) (Figure 4.2B). The loss of chlorophyll was greatest after 72 hr incubation at 1000 mbar (Figure 4.2B). Incubation of MCPP- or 2,4,5-TP-treated plants at 233 mbar resulted in chlorophyll loss of medium control-treated leaves not different from the herbicide control after 0, 24, 48 and 72 hr incubation (Figure 4.2B).

Figure 4.2. Effect of soil applied phenoxy acid herbicides on endogenous ethylene and chlorophyll content of Czapek Dox medium control-treated leaves of Poa pratensis incubated at 1000 and 233 mbar

Ethylene (μl/l) is the mean concentration in internal gases of 4 leaves of 2 shoots per treatment replicated 3 times. Control soils received H_2O. The loss of chlorophyll is the mean percentage of chlorophyll (μg/mg D. W.) in 4 leaves of 2 shoots per treatment replicated 3 times compared to medium control-treated leaves in soil receiving H_2O. The 0 hours of incubation is 48 hr after the second herbicide application. Numbers followed by the same letter among hours of incubation within herbicide and control treatments (across a/) or among herbicide and control treatments within hours of incubation (down /a) are not different according to Duncan's multiple range test (P = 0.05).


A
ETHYLENE (µl/l)
1.0
0.8
0.6
0.4
0.2
0
a/a
a/a
a/a
ab/a
ab/a
ab/a
b/a
b/a
b/b
b/a
ab/a
ab/a
Control (1000 mbar)
MCPP (1000 mbar)
2,4,5-TP (1000 mbar)
B
LOSS OF CHLOROPHYLL (% OF CONTROL)
40
30
20
10
0
Control
MCPP (1000 mbar)
MCPP (233 mbar)
2,4,5-TP (1000 mbar)
2,4,5-TP (233 mbar)
a/a
a/a
a/a
a/a
ab/ab
a/ab
a/bc
b/a
a/a
a/bc
c/a
a/a
bc/ab
b/b
a/b
a/a
a/c
a/b
a/b
0
24
48
72
HOURS OF INCUBATION

Interactions of Toxic Extract and Postemergence Herbicide-Treated Plants

The application of the toxic extract to leaves of herbicide-treated plants resulted in a rise in the ethylene concentration after 24 hr incubation as in Czapek Dox medium control-treated leaves of herbicide control plants (Figure 4.3A). The ethylene concentration declined from 24 to 48 to 72 hr of incubation at 1000 mbar (Figure 4.3A).

The loss of chlorophyll from toxic extract-treated leaves of MCPP- or 2,4,5-TP-treated plants after 24 and 48 hr incubation at 1000 mbar was not different from that of medium control-treated leaves of herbicide control plants (Figure 4.3B). After 72 hr incubation at 1000 mbar, the chlorophyll loss from toxic extract-treated leaves of 2,4,5-TP-treated plants was greater than the control whereas chlorophyll loss from toxic extract-treated leaves of MCPP-treated plants was not different from the control (Figure 4.3B). The chlorophyll loss from toxic extract-treated leaves of herbicide-treated plants incubated at 233 mbar was not different from similarly treated plants incubated at 1000 mbar, but the chlorophyll loss from toxic extract-treated leaves of MCPP-treated plants incubated at 233 mbar for 48 hr was greater than the control (Figure 4.3B).

Figure 4.3. Effect of soil applied phenoxy acid herbicides on endogenous ethylene and chlorophyll content of Drechslera sorokiniana toxic extract-treated leaves of Poa pratensis incubated at 1000 and 233 mbar

Ethylene (µl/l) is the mean concentration in internal gases of 4 leaves of 2 shoots per treatment replicated 3 times. Control leaves were treated with medium control and the plants were grown in soils receiving H_2O. The loss of chlorophyll is the mean percentage of chlorophyll (µg/mg D. W.) in 4 leaves of 2 shoots per treatment replicated 3 times compared to medium control-treated leaves of plants in soils receiving H_2O. The 0 hours of incubation is 48 hr after the second herbicide application. Numbers followed by the same letter among hours of incubation within herbicide and toxic extract treatments (across a/) or among herbicide and toxic extract treatments within hours of incubation (down /a) are not different according to Duncan's multiple range test (P = 0.05).

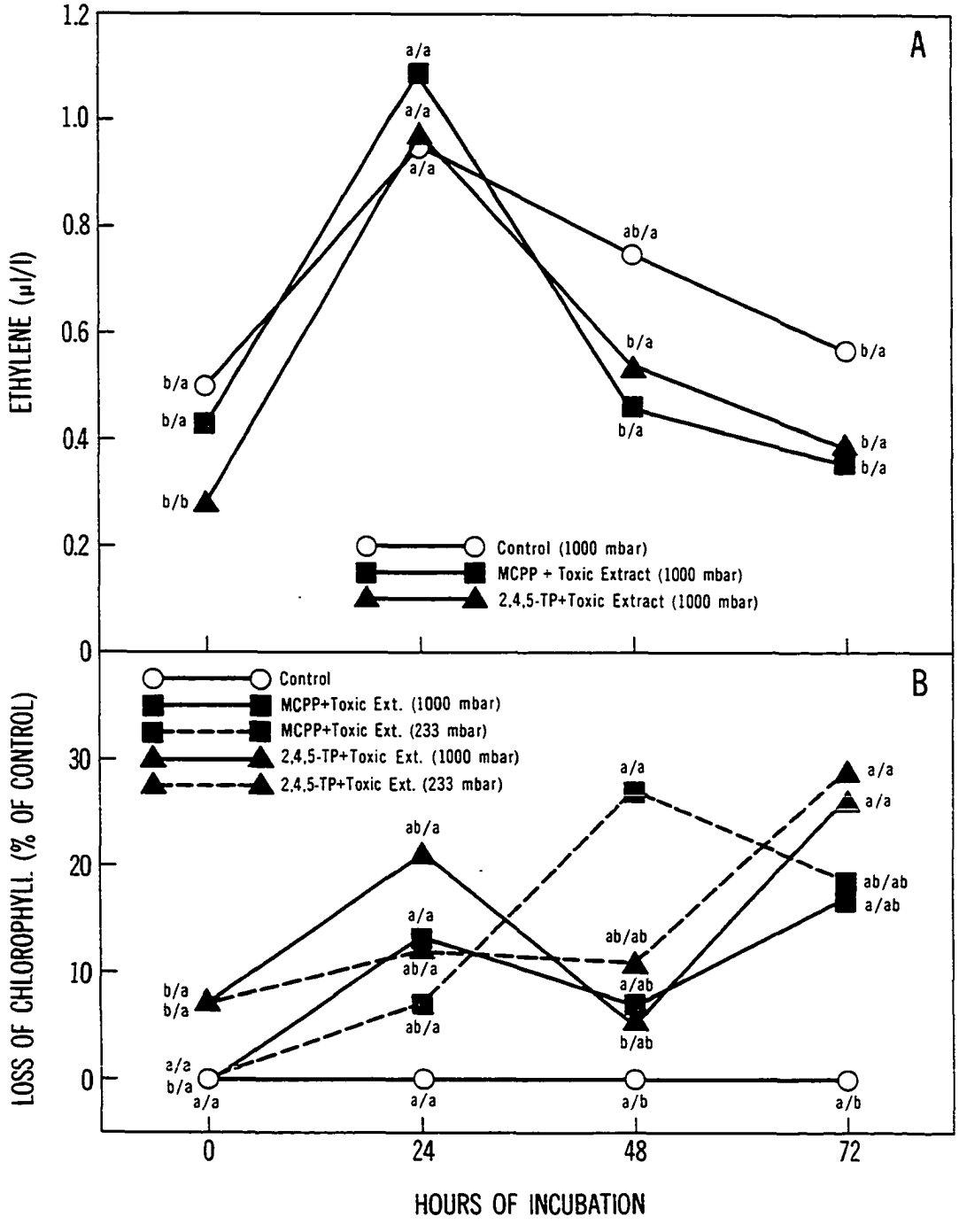
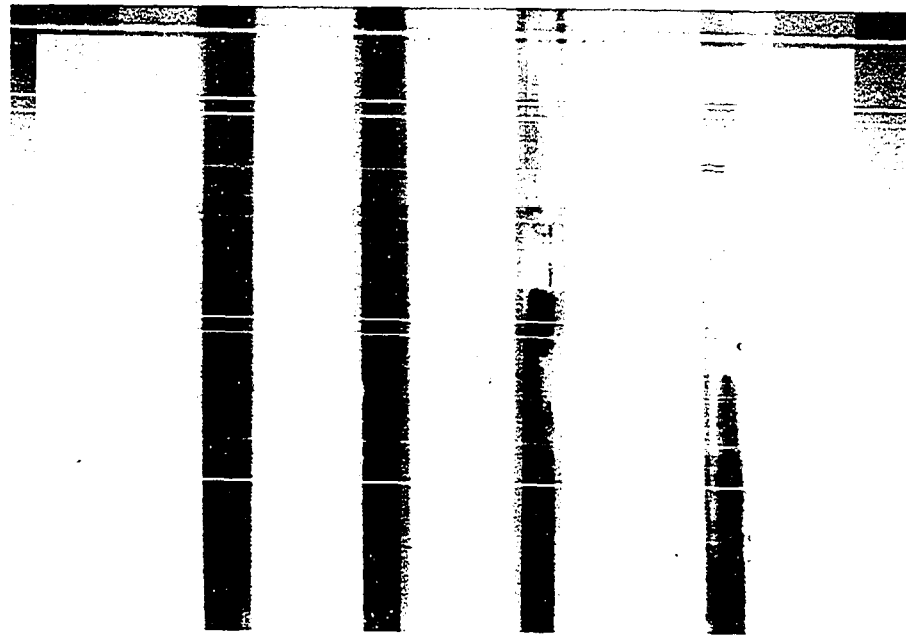

A
B
ETHYLENE (µl/l)
LOSS OF CHLOROPHYLL (% OF CONTROL)
HOURS OF INCUBATION
Control (1000 mbar)
MCPP + Toxic Extract (1000 mbar)
2,4,5-TP+Toxic Extract (1000 mbar)
Control
MCPP+Toxic Ext. (1000 mbar)
MCPP+Toxic Ext. (233 mbar)
2,4,5-TP+Toxic Ext. (1000 mbar)
2,4,5-TP+Toxic Ext. (233 mbar)

Figure 4.4. Typical chlorosis induced by a toxic extract from cultures or Drechslera sorokiniana of leaves of Poa pratensis incubated at 1000 mbar for 72 hr

Figure 4.5. Example of the alleviation of toxic extract-induced chlorosis of leaves of Poa pratensis by incubation of treated plants at 233 mbar for 72 hr



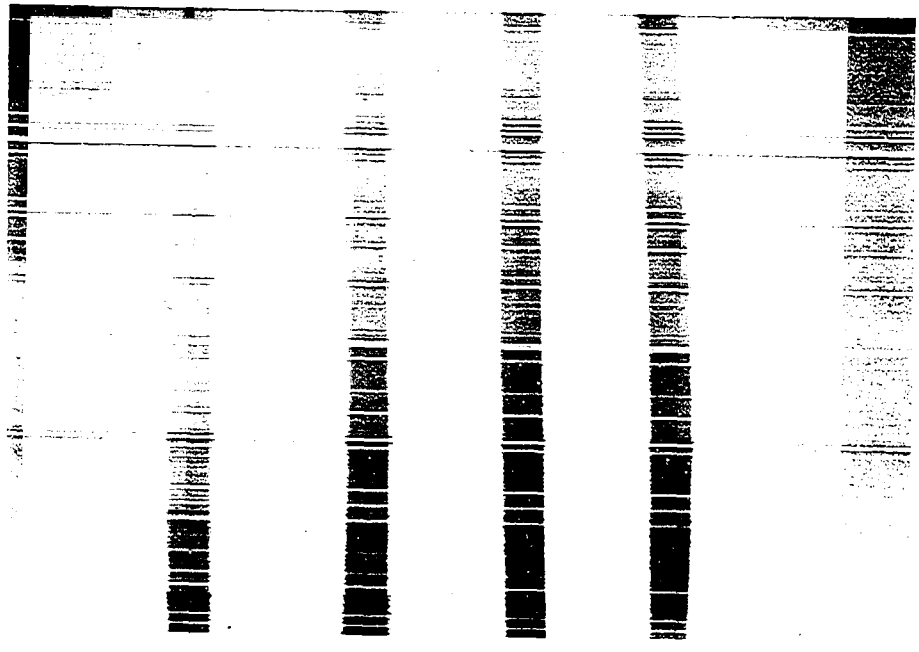
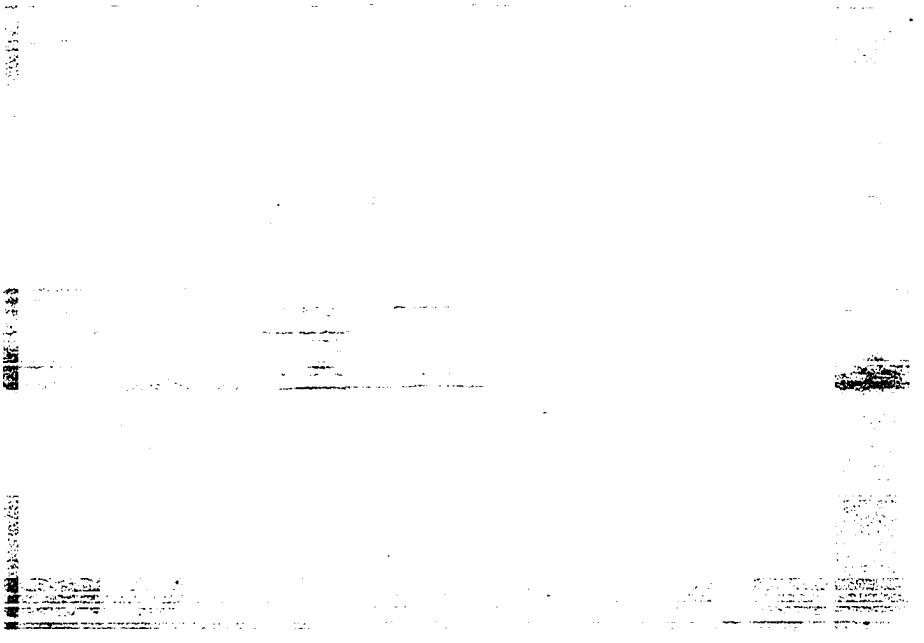


Figure 4.6. Example of a darkened "green island" within typical toxic extract-induced chlorotic area of a leaf of Poa pratensis incubated at 1000 mbar for 72 hr



DISCUSSION

The rise of endogenous ethylene in leaves of plants above the preconditioned level during the first 24 hr incubation corresponds with a previous response of plants to growth chamber environments measured by Nilsen and Hodges (unpublished). They have suggested that growth promotion of preconditioned plants by the environment of the growth chamber may promote elevated IAA and cytokinin levels that may increase ethylene biosynthesis. Ethylene levels declined from 24 to 48 to 72 hr incubation possibly suggesting an acclimation to the chamber conditions.

The toxic extract from cultures of D. sorokiniana had negligible effects on the ethylene concentrations in leaves of P. pratensis compared to the control. However, the pattern of ethylene levels in toxic extract-treated leaves rising above the control after 24 hr incubation and then falling below the control after 48 and 72 hr is similar to an ethylene response in virus-infected tobacco leaves (9). The virus-induced ethylene biosynthesis was regulated by the concentration of 1-amino-cyclopropane-1-carboxylic acid (ACC). A similar rise of ethylene followed by a decline over a 4 day period occurs with D. sorokiniana inoculations of P. pratensis (Nilsen and Hodges, unpublished). The toxin produced by Helminthosporium victoriae (HV-toxin) also stimulates ethylene production in oat leaf tissue (28).

The rise in ethylene in toxic extract-treated leaves precedes the increase in chlorophyll loss of leaves of plants incubated at 1000 mbar

indicating a potential involvement of ethylene in the chlorophyll loss associated with the toxic extract (Figure 4.1). The alleviation of this loss of chlorophyll by incubation at 233 mbar also indicates a possible ethylene involvement. Hypobaric systems have been used to decrease ethylene concentrations in post-harvest physiology experiments and for storage purposes (8, 11). The system used in this study provided an environment suitable for studying ethylene physiology of intact growing plants (24). Therefore, the results of the effects of hypobaric incubation on chlorophyll loss provide good evidence for implicating ethylene as one factor in toxic extract-induced chlorosis.

Membranes have been implied as the site of action for some toxins causing chlorosis. Tentoxin from Alternaria tenuis is a species selective toxin that causes chlorosis in seedlings of certain dicots. The toxin binds to chloroplast lamellae and inhibits photophosphorylation (12, 30). A hypothesis has been proposed implicating ethylene to predispose membranes to toxin-induced membrane damage (26). The mechanism of ethylene's predisposing effect is unknown.

The decline of endogenous ethylene concentrations from a peak at 24 hr incubation to below the control after 48 and 72 hr in toxic extract-treated leaves which corresponds with increasing chlorophyll loss is suggestive of reduced biosynthesis or increased metabolism of ethylene or an inhibition of binding by ethylene. Evidence exists for ethylene metabolism in tissue showing physiological responses to ethylene (19), but it is not known if metabolism is essential for ethylene activity.

The toxic extract may increase the sensitivity of tissue to ethylene-induced chlorosis. This question is beyond the scope of the work presented.

Application of MCPP or 2,4,5-TP to the soil had little effect on endogenous ethylene in medium control-treated leaves of P. pratensis compared to the herbicide control (Figure 4.2). The nature of the effects of phenoxy acid herbicides on herbicide-tolerant plants is not well understood. The auxin-like herbicides stimulate ethylene production in many herbicide-susceptible plants (4, 20). Ethylene levels in leaves of herbicide-tolerant species have been shown to be inhibited, unaffected or enhanced in response to phenoxy herbicides (4, 5, 20). The difference in ethylene production among species in response to phenoxy herbicides is not believed to be the determining factor in susceptibility or tolerance to the herbicides (22). Ethylene produced in response to the application of phenoxy herbicides may be a component of toxicity, but it is not considered to be singularly lethal (20).

The loss of chlorophyll from leaves of herbicide-treated P. pratensis compared to the control after 72 hr incubation at 1000 mbar indicates a potentially detrimental effect of phenoxy acid herbicides on the herbicide-tolerant species. Application of 2,4-D to barley leaves caused chloroplast damage (5). Rupturing and disintegration of the tonoplast, plasmalemma and mitochondria membranes of some plants has been caused by 2,4-D (5). The mechanism of these actions is unknown. The complete alleviation of chlorophyll loss in leaves of herbicide-treated plants by incubation at 233 mbar suggests an involvement of

ethylene in the herbicide-induced chlorophyll loss. The possibility exists that even naturally low levels of ethylene may predispose chloroplast membranes to damage by phenoxy acid herbicides.

The interaction of phenoxy herbicides and the toxic extract presents a more complex relationship. Ethylene levels were not affected by application of the toxic extract to leaves of herbicide-treated plants as it was in plants treated with either toxic extract or herbicide singularly (Figure 4.3A). Hypobaric incubation of herbicide-treated plants with toxic extract-treated leaves did not alleviate chlorophyll loss after 72 hr (Figure 4.3B). The appearance of the toxic extract-treated leaves of herbicide-treated plants incubated at 233 mbar resembled medium control-treated leaves of herbicide-treated plants incubated at 1000 mbar. Evidently, the herbicides together with the toxic extract cause chlorosis regardless of the concentration of ethylene.

The postemergence herbicides cause a loss of chlorophyll without visible yellowing in the presence of ethylene or in the presence of the toxic extract with lowered ethylene concentrations. The toxic extract causes leaf yellowing only if ethylene is present and does so regardless of whether plants are treated with either herbicide.

Differences exist between the effects of MCPP and 2,4,5-TP on pathogenesis by D. sorokiniana on P. pratensis. Application of MCPP to the soil generally stimulates lesion size on leaves infected from conidia of D. sorokiniana, and 2,4,5-TP has little effect or inhibits lesion development depending upon the concentration of the herbicide (15).

The results of this study indicate that 2,4,5-TP has no effect on toxic extract-induced chlorosis and that MCPP may have an inhibitory influence on the toxic extract after 72 hr incubation at 1000 mbar (Figures 4.1B and 4.3B). This suggests that the primary stimulation of D. sorokiniana leaf spot by MCPP may be to increase the area of necrotic tissue and not the chlorotic halo associated with the lesion. Likewise the inhibition of leaf spot by 2,4,5-TP does not seem to involve the limiting of chlorosis, but it may limit necrosis.

REFERENCES

1. Abeles, F. B. 1973. Ethylene in plant biology. Academic Press, New York. 302 p.

2. Aharoni, N., J. D. Anderson, and M. Lieberman. 1979. Feedback relationship between the biosynthesis and action of ethylene in senescing leaf disks. Plant Physiol. Supp. 63:92.

3. Aharoni, N., and M. Lieberman. 1979. Ethylene as a regulator of senescence in tobacco leaf discs. Plant Physiol. 64:801-804.

4. Ashton, F. M., and D. E. Bayer. 1976. Effects on solute transport and plant constituents. Pages 219-253. In L. J. Audus, ed. Herbicides: physiology, biochemistry, ecology. Vol. 1, 2nd ed. Academic Press, New York. 608 p.

5. Ashton, F. M., and A. S. Crafts. 1981. Mode of action of herbicides. 2nd ed. John Wiley and Sons, New York. 525 p.

6. Ballio, A. 1981. Structure-activity relationships. Pages 395-441. In R. D. Durbin, ed. Toxins in plant disease. Academic Press, New York. 515 p.

7. Beyer, E. M., Jr., and P. W. Morgan. 1970. A method for determining the concentration of ethylene in the gas phase of vegetative plant tissue. Plant Physiol. 46:352-354.

8. Burg, S. P., and E. A. Burg. 1966. Fruit storage at subatmospheric pressures. Science 153:314-315.

9. de Laat, A. M. M., and L. C. van Loon. 1982. Regulation of ethylene biosynthesis in virus-infected tobacco leaves. II. Time course of levels of intermediates and in vivo conversion rates. Plant Physiol. 69:240-245.

10. de Mayo, P., E. Y. Spencer, and R. W. White. 1961. Helminthosporal, the toxin from Helminthosporium sativum. I. Isolation and characterization. Can. J. Chem. 39:1608-1612.

11. Dilley, D. R., W. J. Carpenter, and S. P. Burg. 1975. Principles an application of hypobaric storage of cut flowers. Acta. Hort. 41:249-262.

12. Halloin, J. M., G. A. De Zoeten, G. Gaard, and J. C. Walker. 1970. The effects of tentoxin on chloroplast synthesis and plastid structure in cucumber and cabbage. Plant Physiol. 45:310-314.

13. Gayed, S. K. 1961. Production of symptoms of barley leaf spot disease by culture filtrate of Helminthosporium sativum. Nature 191:725-726.

14. Hodges, C. F. 1972. Influence of culture age and temperature on germination of Helminthosporium sorokinianum conidia and on pathogenicity to Poa pratensis. Phytopathology 62:1133-1137.

15. Hodges, C. F. 1978. Postemergent herbicides and the biology of Drechslera sorokiniana: Influence on severity of leaf spot on Poa pratensis. Phytopathology 68:1359-1363.

16. Hodges, C. F. 1980. Interaction of sequential leaf senescence of Poa pratensis and pathogenesis by Drechslera sorokiniana as influenced by postemergent herbicides. Phytopathology 70:628-630.

17. Hodges, C. F. 1980. Postemergent herbicides and pathogenesis by Drechslera sorokiniana on leaves of Poa pratensis. Pages 101-112. In P. O. Larsen and B. G. Joyner, ed. Advances in turfgrass pathology. Harcourt Brace Jovanovich, Inc., Duluth. 197 p.

18. Knudson, L. L., T. W. Tibbitts, G. E. Edwards. 1977. Measurement of ozone injury by determination of leaf chlorophyll concentration. Plant Physiol. 60:606-608.

19. Libermann, M. 1979. Biosynthesis and action of ethylene. Ann. Rev. Plant Physiol. 30:533-591.

20. Loos, M. A. 1975. Phenoxyalkanoic acids. Pages 1-128. In P. C. Kearney and D. D. Kaufman, ed. Herbicides: chemistry, degradation, and mode of action. Vol. 1, 2nd ed. Marcel Dekker, New York. 500 p.

21. Ludwig, R. A. 1960. Toxins. Pages 315-357. In J. G. Horsfall and A. E. Dimond, eds. Plant Pathology. Academic Press, New York.

22. Morgan, P. W. 1976. Effects on ethylene physiology. Pages 255-280. In L. J. Audus, ed. Herbicides: physiology, biochemistry, ecology. Vol. 1, 2nd ed. Academic Press, New York. 608 p.

23. Nilsen, K. N., C. F. Hodges, and J. P. Madsen. 1979. Pathogenesis of Drechslera sorokiniana leaf spot on progressively old leaves of Poa pratensis as influenced by photoperiod and light quality. Physiol. Plant Pathol. 15:171-176.

24. Nilsen, K. N., and C. F. Hodges. 1980. A hypobaric system for intact plant examination of stress ethylene-related leaf senescence. HortScience 15:429 Abstr.

25. Nooden, L. D. 1980. Senescence in the whole plant. Pages 219-244. In K. V. Thimann, ed. Senescence in plants. CRC Press, Boca Raton, Fl. 276 p.

26. Pegg, G. F. 1981. The role of nonspecific toxins and hormone changes in disease severity. Pages 13-44. In R. C. Staples and G. H. Toenniessen, eds. Plant disease control. John Wiley and Sons, New York. 339 p.

27. Robinson, P. W., and C. F. Hodges. 1976. An inoculation apparatus for evaluation of Bipolaris sorokiniana lesion development on progressively older leaves of Poa pratensis. Phytopathology 66:360-362.

28. Shain, L., and H. Wheeler. 1975. Production of ethylene by oats resistant and susceptible to victorin. Phytopathology 65:88-89.

29. Sommereyns, G., and J. L. Closset. 1978. Preparation of helminthosporal by extraction with diethyl ether from culture filtrates of Helminthosporium sativum P. K. and B. Phytopath. Z. 92:202-210.

30. Steele, J. A., T. F. Uchytil, R. D. Durbin, P. Bhatnagar, and D. H. Rich. 1976. Chloroplast coupling factor 1: A species specific receptor for tentoxin. Proc. Natl. Acad. Sci. USA 73:2245-2283.

31. Stoessl, A. 1981. Structure and biogenetic relations: fungal nonhost-specific. Pages 109-219. In R. D. Durbin, ed. Toxins in plant disease. Academic Press, New York. 515 p.

32. Thimann, K. V. 1980. The senescence of leaves. Pages 85-115. In K. V. Thimann, ed. Senescence in plants. CRC Press, Boca Raton, Fl. 276 p.

33. Wheeler, H. 1981. Role in pathogenesis. Pages 477-494. In R. D. Durbin, ed. Toxins in plant disease. Academic Press, New York. 515 p.

34. White, G. A., and E. Taniguchi. 1972. The mode of action of helminthosporal. II. Effect on permeability of plant cell membranes. Can. J. Bot. 50:1415-1420.

OVERALL SUMMARY

The stimulation of D. sorokiniana leaf spot on P. pratensis by MCPP and by increasing leaf age (Figure 2.1) confirms earlier work (12, 13). The effects of 2,4,5-TP on leaf spot are less consistent with previous work (Figure 2.1). This may be due to the uncertain fate of phenoxy herbicides in herbicide-tolerant plants (2, 23, 38).

Both herbicides influenced concentrations of sugars and amino acids in leaves of P. pratensis. The correlation of decreasing sugar concentrations in leaves of herbicide-treated plants with increasing leaf spot (Table 2.2) is suggestive of enhanced cell wall degrading enzymes induced by the decrease in leaf sugars (22). The increase of sugars in infected leaves is likely due to sink activity at the infection site (Figure 2.3). Decreasing concentrations of select amino acids in leaves of herbicide-treated P. pratensis also correlated with increasing leaf spot (Table 3.2). Decreased concentrations of amino acids in leaves is a component of leaf senescence (35). The effects of herbicide-induced changes in sugar and amino acid concentrations in leaves of P. pratensis on subsequent leaf spot severity are consistent with the hypothesis that leaf senescence promotes D. sorokiniana leaf spot (14).

The two phenoxy herbicides used in this study had different effects on intermediary metabolism of four progressively older leaves of shoots of P. pratensis. The differences may be explained by differences in uptake and translocation of MCPP and 2,4,5-TP by P. pratensis. MCPP is generally more mobile in plants than is 2,4,5-TP (28, 32) and influenced

sugars and amino acids in leaves of any age. The less mobile 2,4,5-TP probably concentrates in the roots (2, 23). It is probable that this concentration in the roots may influence root physiology and thus alter hormonal balances within the plant. This may partially explain the different effects of 2,4,5-TP on sugars and amino acids in young and old leaves of P. pratensis.

The toxic extract from cultures of D. sorokiniana induced chlorosis in leaves of P. pratensis that appeared similar to chlorosis associated with leaf spot (Figure 4.4). Characterization of the chemical nature of the toxic extract would be informative. A system to obtain the toxic material from infected leaves would help define the role of the toxin(s) in pathogenesis (40).

The evidence presented on the interactions of ethylene and the toxic extract is important as a preliminary step toward understanding the relationship of fungal toxins and plant hormones. The use of chlorophyll measurements as an indication of toxic extract-induced chlorosis was successful and would be useful in future studies on leaf spot. Chlorophyll measurements also allowed a quantification of the effects of postemergence herbicides on leaves of P. pratensis and showed a nonvisible loss of chlorophyll (Figure 4.2).

The relationship of the toxic extract and ethylene on chlorosis of leaves of P. pratensis was partially characterized by the use of a hypobaric system (27). The toxic extract caused chlorosis of leaves with naturally low levels of endogenous ethylene (Figure 4.1). The

alleviation of chlorosis by negative pressure (233 mbar) (Figure 4.1) is supportive of a proposed system where ethylene predisposes membranes to toxin-induced damage (30). However, a system where the toxic extract increases sensitivity of the tissue to ethylene-induced chlorosis also should be considered. The role of ethylene in D. sorokiniana leaf spot warrants further investigation.

REFERENCES FOR GENERAL INTRODUCTION AND OVERALL SUMMARY

1. Altman, J., and C. L. Campbell. 1977. Effect of herbicides on plant disease. Ann. Rev. Phytopathol 15:361-385.

2. Ashton, F. M., and A. S. Crafts. 1981. Mode of action of herbicides. 2nd ed. John Wiley and Sons, New York. 525 p.

3. Bean, G. A., and R. D. Wilcoxson. 1964. Helminthosporium leaf spot of bluegrass. Phytopathology 54:1065-1070.

4. Bean, G. A., and R. D. Wilcoxson. 1964. Pathogenicity of three species of Helminthosporium on roots of bluegrass. Phytopathology 54:1084-1085.

5. Bollen, W. B. 1961. Interactions between pesticides and soil microorganisms. Ann. Rev. Plant Physiol. 5:69-92.

6. Cheesman, J. H., E. C. Roberts, and L. H. Tiffany. 1965. Effects of nitrogen level and osmotic pressure of the nutrient solution on incidence of Puccinia graminis and Helminthosporium sativum infection of Merion Kentucky bluegrass. Agron. J. 57:599-602.

7. Drechsler, C. 1930. Leaf spot and foot rot of Kentucky bluegrass caused by Helminthosporium vagans. J. Agr. Res. 40:447-456.

8. Endo, R. M. 1961. Turfgrass disease in southern California. Plant Dis. Rep. 45:869-873.

9. Fletcher, W. W. 1960. The effect of herbicides on soil microorganisms. Pages 20-62. In E. K. Woodford and G. R. Sagar, ed. Herbicides and the soil. Blackwell Sci. Pub., Oxford. 88 p.

10. Gruen, H. E. 1959. Auxins and fungi. Ann. Rev. Plant Physiol. 10:405-440.

11. Halisky, P. M., C. R. Funk, and R. E. Engel. 1966. Melting-out of Kentucky bluegrass varieties by Helminthosporium vagans as influenced by turf management practices. Plant Dis. Rep. 50:703-706.

12. Hodges, C. F. 1978. Postemergent herbicides and the biology of Drechslera sorokiniana: Influence on severity of leaf spot on Poa pratensis. Phytopathology 68:1359-1363.

13. Hodges, C. F. 1980. Interaction of sequential leaf senescence of Poa pratensis and pathogenesis by Drechslera sorokiniana as influenced by postemergent herbicides. Phytopathology 70:628-630.

14. Hodges, C. F. 1980. Postemergent herbicides and pathogenesis by Drechslera sorokiniana on leaves of Poa pratensis. Pages 101-112. In P. O. Larsen and B. G. Joyner, ed. Advances in turfgrass pathology. Harcourt Brace Jovanovich, Inc., Duluth. 197 p.

15. Hodges, C. F., and W. M. Blaine. 1974. Midsummer occurrence of Helminthosporium sorokinianum leaf spot of selected cultivars of Poa pratensis. Plant Dis. Rep. 58:448-450.

16. Hodges, C. F., and G. A. Watschke. 1975. Pathogenicity of soil-borne Bipolaris sorokiniana on seed and roots of three perennial grasses. Phytopathology 65:398-400.

17. Horsfall, J. G., and A. E. Dimond. 1957. Interactions of tissue sugar, growth substances, and disease severity. Z. Pflanzenkrankh. 64:415-421.

18. Katan, J., and Y. Eshel. 1973. Interactions between herbicides and plant pathogens. Residue Rev. 45:145-177.

19. Kavanagh, T. 1969. The influence of herbicides on plant disease. I. Temperate fruit and hops. Sci. Proc. R. Dublin Soc. B. 2:179-189.

20. Kavanagh, T. 1974. The influence of herbicides on plant disease. II. Vegetables, root crops, and potatoes. Sci. Proc. R. Dublin Soc. Ser. b. 3:251-265.

21. Kirkwood, R. C. 1976. Action on respiration and intermediary metabolism. Pages 443-492. In L. J. Audus, ed. Herbicides: physiology, biochemistry, ecology. Vol. 1, 2nd ed. Academic Press, New York. 608 p.

22. Kuc, J. 1978. Changes in intermediary metabolism caused by disease. Pages 349-374. In J. G. Horsfall and E. B. Cowling, ed. Plant disease: An advanced treatise. Vol. III. How plants suffer from disease. Academic Press, New York. 487 p.

23. Loos, M. A. 1975. Phenoxyalkanoic acids. Pages 1-128. In P. C. Kearney and D. D. Kaufman, ed. Herbicides: chemistry, degradation, and mode of action. Vol. 1, 2nd ed. Marcel Dekker, New York. 500 p.

24. Ludwig, R. A., R. V. Clark, J. B. Julien, and D. B. Robinson. 1956. Studies on the seedling disease of barley caused by Helminthosporium sativum P. K. & B. Can. J. Bot. 34:653-673.

25. Lukens, R. J. 1970. Melting-out of Kentucky bluegrass, a low-sugar disease. Phytopathology 60:1276-1278.

26. Nilsen, K. N., C. F. Hodges, and J. P. Madsen. 1979. Pathogenesis of Drechslera sorokiniana leaf spot on progressively old leaves of Poa pratensis as influenced by photoperiod and light quality. Physiol. Plant Pathol. 15:171-176.

27. Nilsen, K. N., and C. F. Hodges. 1980. A hypobaric system for intact plant examination of stress ethylene-related leaf senescence. HortScience 15:429 Abstr.

28. Norris, L. A., and V. H. Freed. 1966. The absorption and translocation characteristics of several phenoxyalkyl herbicides in big leaf maple. Weed Res. 6:203-211.

29. Oka, I. N., and D. Pimentel. 1976. Herbicide (2,4-D) increases insect and pathogen pests on corn. Science 193:239-240.

30. Pegg, G. F. 1981. The role of nonspecific toxins and hormone changes in disease severity. Pages 13-44. In R. C. Staples and G. H. Toenniessen, eds. Plant disease control. John Wiley ans Sons, New York. 339 p.

31. Robinson, P. W., and C. F. Hodges. 1977. Effect of nitrogen fertilization on free amino acid and soluble sugar content of Poa pratensis and on infection by Drechslera sorokiniana. Phytopathology 67:1239-1244.

32. Sanad, A. J. 1971. Studies on the uptake and translocation of ^{14}C-labeled herbicides in Agrostemma githago and Tussilago farfara. Weed Res. 11:215-223.

33. Simmonds, P. M. 1960. Infection of wheat with Helminthosporium sativum in relation to the nitrogen content of the plant tissues. Can. J. Plant Sci. 40:139-145.

34. Stoessl, A. 1981. Structure and biogenetic relations: fungal nonhost-specific. Pages 109-219. In R. D. Durbin, ed. Toxins in plant disease. Academic Press, New York. 515 p.

35. Thimann, K. V. 1980. The senescence of leaves. Pages 85-115. In K. V. Thimann, ed. Senescence in plants. CRC Press, Boca Raton, Fl. 276 p.

36. Van Der Zweep, W. 1970. Effects of herbicides on susceptibility of plants and pests and diseases. Proc. 10th Brit. Weed Control Conf., Brighton, England, pp. 917-919.

37. Vidhyase, P. 1978. Role of amino acids and amides in Helminthosporiose disease incidence in finger-millet. Indian Phytopathol. 30:41-46.

38. Wain, R. L., and M. S. Smith. 1976. Selectivity in relation to metabolism. Pages 279-302. In L. J. Audus, ed. Herbicides: physiology, biochemistry, ecology. Vol. 2, 2nd ed. Academic Press, New York. 564 p.

39. Weihing, J. L., S. G. Jensen, and R. I. Hamilton. 1957. Helminthosporium sativum, a destructive pathogen of bluegrass. Phytopathology 47:744-746.

40. Yoder, O. C. 1980. Toxins in pathogenesis. Ann. Rev. Phytopathol. 18:103-129.

ACKNOWLEDGEMENTS

I am deeply grateful to Dr. Clint Hodges for his guidance and friendship. He has shared his experience and knowledge and has provided more than the normal graduate education. I wish him and his family the best in the future.

I also would like to thank Dr. Lois Tiffany for her assistance as my co-major Professor. I am grateful to Dr. John Dunleavy, Dr. Jim Kelley and Dr. Cecil Stewart for their critical assistance as committee members. I appreciate the excellent typing of Sandy Aspengren.

The research presented was supported in part by grants from the Iowa State University Research Foundation, the O. J. Noer Research Foundation and the Iowa Golf Course Superintendents Association and Iowa Turfgrass Advisory Council.

I would like to thank Patti Freeman for being there when I needed her. She has helped me keep things in perspective during the preparation of this dissertation.

I dedicate my dissertation to my parents, Mr. and Mrs. William F. Madsen. I thank them for allowing me to find my interests and then supporting me in my endeavors.