Interaction of Sequential Leaf Senescence of *Poa pratensis* and Pathogenesis by *Drechslera sorokiniana* as Influenced by Postemergent Herbicides

Clinton F. Hodges

Professor of Horticulture and of Botany and Plant Pathology, Department of Horticulture, Iowa State University, Ames 50011.
Journal Paper J-9652 and Project 2308 of the Iowa Agriculture and Home Economics Experiment Station, Ames.
The research presented herein was supported in part by a research grant from the O. J. Noer Research Foundation.
Accepted for publication 10 December 1979.

ABSTRACT

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.

The influence of four chlorophenoxy (2,4-D, 2,4,5-T, 2,4,5-TP, MCPP) and one benzoic acid (dicamba) postemergent herbicides on pathogenesis by *Drechslera sorokiniana* on progressively older leaves of *Poa pratensis* was determined. Disease increased on each successively older leaf of untreated control plants and a direct relationship was established between increasing leaf senescence and pathogenesis. The soil-drench application of 2,4-D and the spray and soil-drench application of 2,4,5-T, MCPP, and dicamba increased the level of disease on leaves of all ages above that of the controls and on each older leaf of the plants in the respective treatments. Spray-applied 2,4-D had little influence on pathogenesis, and spray-applied 2,4,5-TP decreased disease. Extensive chlorosis and straw-colored blighting was associated with pathogenesis on the two oldest leaves of shoots exposed to 2,4,5-T, MCPP, and dicamba and was suggestive of premature leaf senescence. Little chlorosis and blighting occurred on the two oldest leaves of infected plants not exposed to the herbicides and no chlorosis occurred on uninoculated controls. It was hypothesized that the increase in pathogenesis by *D. sorokiniana* on progressively older leaves of plants exposed to auxinlike herbicides is the function of a host-pathogen-herbicide interaction that enhances the rate of sequential leaf senescence.

Additional key words: Bipolaris, Helminthosporium, mecoprop, silvex.

Leaf spot caused by *Dreschslera sorokiniana* (Sacc.) Subram. and Jain (*Helminthosporium sativum*) on *Poa pratensis* L. is chronic throughout the growing season (3,11) and numerous cultural practices influence disease development (4,8,11,19). Results of recent studies show that the postemergent herbicides 2,4-D, 2,4,5-T, 2,4,5-TP, MCPP, and dicamba may influence pathogenesis by *D. sorokiniana* on leaves of *P. pratensis* (10). *P. pratensis* is tolerant of chlorophenoxy and benzoic acid herbicides and absorbs them without visible effects. Plants spray- or soil-treated with 2,4,5-T, MCPP, and dicamba and inoculated with *D. sorokiniana* conidia show an increase in leaf lesion development that often includes extensive chlorosis and straw-colored blighting that rarely occurs on inoculated leaves not exposed to the herbicides and does not occur at all on uninoculated controls (10). Leaf spot development in response to 2,4-D and 2,4,5-TP varies with herbicide concentration and with the organ of the plant exposed (10). Predisposition of wheat to infection by *D. sorokiniana* (*H. sativum*) (12) and of corn to *D. heterostrophus* (*H. maydis*) (15) also occurs in response to 2,4-D. Conversely, 2,4-D reduces a barley root rot which is incited by *D. sorokiniana* (*H. sativum*) (17).

The chlorosis associated with pathogenesis by *D. sorokiniana* on leaves treated with auxinlike herbicides may be indicative of a host-pathogen-herbicide-senescence interaction (10). The chlorosis of infected leaves of treated plants resembles premature senescence. Chlorophenoxy herbicides are known to induce ethylene in plant tissue (1,2), and auxinlike herbicides can induce leaf senescence in some species, probably via ethylene (7,14). It is possible that auxinlike herbicides may interact with the pathogen to enhance the rate of leaf senescence of *P. pratensis* and thereby enhance pathogenesis.

Poa pratensis is a model species for the study of factors affecting sequential development and senescence of leaves. Each shoot maintains three to four leaves throughout its vegetative life (5). Therefore, premature through postmature (near senescent) leaves (leaves 1 to 4) are maintained in a relatively fixed number on each shoot, and pathogenesis by *D. sorokiniana* on the progressively older leaves can be easily evaluated. If pathogenesis by *D. sorokiniana* is directly related to leaf senescence, then disease should increase from the youngest to the oldest leaf on plants not exposed to herbicides. If auxinlike herbicides enhance leaf senescence and predispose the leaves to more severe attack by *D. sorokiniana,* then disease should increase on each older leaf above that on untreated plants. The research presented was initiated to examine this hypothesis.

MATERIALS AND METHODS

Poa pratensis 'Newport' was vegetatively propagated in a steamed 2:1 loam-peat soil mix (2:1, v/v) in 7.6-cm (3-inch) diameter plastic pots. Plants were grown in a glasshouse for 60 days under a 16-hr daylength supplemented by incandescent lights. Cultures of *D. sorokiniana* (Sacc.) Subram. and Jain were grown on 20 ml of 1.0% Czapek Dox broth (10 g/L) in 3.0% (w/v) Bacto-agar in sterile, plastic petri dishes (15 × 100 mm). Pathogen virulence was maintained by using conidia from 20-day-old cultures produced from hyphal-tip isolates from diseased tissue (9).

Postemergent herbicides that were evaluated for influence on pathogenesis included 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,4,5-trichlorophenoxypropionic acid (2,4,5-TP, silvex), 2(2 methyl-4-chlorophenoxy)propionic acid (MCPP, mecoprop), and 2-methoxy-3, 6-dichlorobenzoic acid (dicamba). All herbicide were utilized in the acid form without carriers and were prepared at 10^{-6} M.

Herbicides were applied to plants either in atomized sprays or as soil-drenches. The foliage of spray-treated plants received four 10-ml applications of the appropriate herbicide on alternate days and was inoculated 2 days after the last application. Soil-drench treatments were applied to plants in two applications each of 20 ml of the appropriate herbicide on alternate days, and plants were inoculated with *D. sorokiniana* 2 days after the last application. All inoculations were made on the four youngest, visible leaf blades of a single shoot of each treated plant. Suspensions of 10 conidia in 0.02 ml (500 per milliliter) of distilled water were prepared with an

0031-949X/80/07062803/$03.00/0

automatic particle counter (High Accuracy Products Corp., Montclair, CA 91763) and used for all leaf-blade inoculations. Each leaf blade was inoculated in five positions (10 conidia in 0.02 ml of distilled water at each position) 1 cm apart in a specially designed inoculation apparatus (18). Inoculated plants were incubated 6 days at 22 C under continuous low-intensity (75–85 μE) fluorescent (daylight) light and then evaluated for disease severity. Each treatment consisted of 20 shoots (one per plant) with four progressively older leaves, and each leaf-age group was independently evaluated for disease. Each treatment was replicated five times (100 plants with 100 leaves of each age group evaluated per herbicide treatment). Inoculated plants not exposed to herbicides and uninoculated control plants (blank water droplets) were incubated for each herbicide treatment.

Disease was evaluated on 10-cm lengths of each older leaf of the shoot after a 6-day-incubation period. The 10-cm lengths of each inoculated leaf were removed from the tubes in which inoculations were done (18), and total area of the leaf sample and the proportion of the leaf area with symptoms was estimated. Total leaf blade area was estimated to the nearest whole number by multiplying the 10-cm length of the leaf sample by its width (determined with an ocular micrometer) at the midpoint of its length. The area of diseased tissue on the leaf sample was estimated by multiplying the estimated length and width (longest chords) of each lesion. Lesion measurements included necrotic, chlorotic, and straw-colored blighted areas associated with lesions. Lesions with an area of less than 0.4 cm^2 were not included in the area estimation of diseased tissue. The summation of the estimated lesion areas on each leaf sample was expressed as the percentage of the leaf blade area with symptoms.

RESULTS

There were no differences in severity of leaf spotting caused by *D. sorokiniana* on the two youngest leaves of untreated control plants, but disease increased from the second to the third to the fourth leaf on all control plants (Fig. 1A to E). The application of auxin-like herbicides (except 2,4,5-TP, Fig. 1C) to the leaves or roots of *P. pratensis* preceding inoculation with *D. sorokiniana* conidia generally increased disease symptoms on leaves of all ages above that of the controls and on each successively older leaf of the shoot (Fig. 1A,B,D, and E).

Disease increased on the oldest leaf of plants spray-treated with 2,4-D, but disease was not increased on the three youngest leaves of the shoot (Fig. 1A). Disease increased on each older leaf of plants treated with 2,4-D in a soil drench, but was not influenced on the youngest leaf (Fig. 1A). Plants that received either sprays or soil drenches with 2,4,5-T, MCPP, and dicamba showed increased disease on each older leaf of the shoot (Fig. 1B, D, and E). Plants treated with foliar sprays or root drenches of 2,4,5-TP either showed no effect or decreased disease on progressively older leaves (Fig. 1C).

Disease symptoms on the youngest leaves did not differ between plants that received foliar spray or soil drenches with any of the herbicides (Fig. 1A to E). However, lesion development was greater on leaves 2 and 3 on 2,4-D soil-drenched plants than on spray-treated plants (Fig. 1A). This observation was reversed with dicamba; ie, lesion development was greater on leaves 2, 3, and 4 in response to the spray treatment than to the soil drench treatment (Fig. 1E). No differences in disease occurred on each leaf-age-group in response to spray- or soil-treatment with 2,4,5-T, 2,4,5-TP, or MCPP (Fig. 1B to D).

The lesion-types induced by *D. sorokiniana* on herbicide-treated plants were of the four types previously described (10), but the various types tended to predominate on leaves of specific ages. Small purple-brown necrotic areas without halos, or with faint halos, and somewhat larger lesions with 1–2 mm halos were most common on leaves 1 and 2. Enlarged necrotic areas, often extending to the leaf margins, with 1–4 mm halos and(or) midvein chlorotic streaks interconnecting the lesions occurred occasionally on leaf 2, but were more common on leaves 3 and 4. The most severe symptoms characterized by enlarged necrotic areas accompanied by extensive chlorosis or by straw-colored blighting of the entire leaf occurred primarily on leaves 3 and 4. The extensive chlorosis and(or) straw-colored blighting most common on the two oldest leaves occurred very rapidly; ie, these symptoms were first visible by the 4th or 5th day after incubation of inoculated plants. Few leaves showed chlorosis or blighting among inoculated plants not exposed to herbicides (controls), and no leaves showed these symptoms among uninoculated controls during the incubation period.

DISCUSSION

A direct relationship exists between the severity of *D. sorokiniana* leaf spot on *P. pratensis* and the sequential development and senescence of leaves. The relationship exists on shoots not exposed to herbicides and is enhanced by spray and soil treatments with 2,4,5-T, MCPP, and dicamba and by soil treatment with 2,4-D (Fig. 1A,B,D, and E). The decrease in disease associated with 2,4,5-TP (Fig. 1C) was previously demonstrated (10). More dilute concentrations of this herbicide, however, enhance pathogenesis, and thus the host-pathogen interaction with

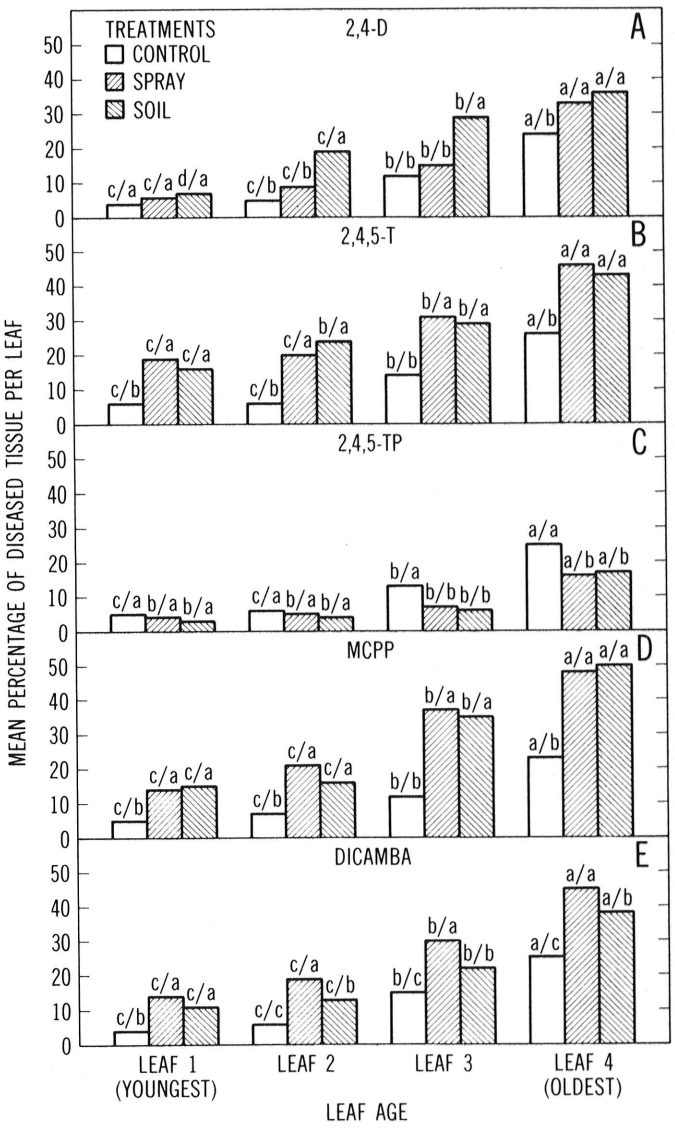

Fig. 1. The influence of auxinlike herbicides on pathogenesis by *Drechslera sorokiniana* in sequentially developing and senescing leaves of *Poa pratensis*. Mean percentage of disease on each older leaf of the shoot within treatments (letters before slash [/]) and on leaves of the same age between treatments (letters after slash [/]) followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

2,4,5-TP varies with concentration. The rapid chlorosis and straw-colored blighting that occurs by the 4th or 5th day of incubation of inoculated plants is atypical of the chlorotic halos commonly attributed to phytotoxin activity and is suggestive of ethylene-induced premature senescence (1). It is believed that the increase in pathogenesis by *D. sorokiniana* previously reported on whole shoots (10) and on the sequentially senescent leaves of plants in the pesent study supports the hypothesis that auxinlike herbicides in combination with the pathogen enhance the rate of sequential leaf senescence and, subsequently, disease development.

The increase in pathogenesis by *D. sorokiniana* on each older leaf of nonherbicide-treated *P. pratensis* exemplifies the relationship between a necrotrophic parasite and senescence-induced disease (Fig. 1A–E) (6). Disease severity increases on each older leaf and is characterized by chlorotic halos and streaking: symptoms that are reasonably attributable to the nonspecific phytotoxin helminthosporal (H-al) which is produced by *D. sorokiniana* (13,20,21). The more severe symptoms of chlorosis and blighting of entire leaves of infected, herbicide-treated plants occurs almost exclusively on the two oldest leaves and suggests that auxinlike herbicides enhance the senescence-induced disease interaction. The fact that no chlorosis occurs on leaves of uninfected, herbicide-treated plants establishes an interdependence between the auxinlike herbicides and the pathogen for the increase in disease severity on each older leaf. Auxins (including auxinlike herbicides) can promote senescence by inducing ethylene production (1,7,14), but the level of ethylene induced by auxins in grasses (2) is low (0.2–0.6 ppm) and would not be expected to effect leaf senescence. The potential presence of ethylene and H-al in the host-pathogen-herbicide interaction provides the most probable explanation for the increase in disease severity. H-al may be disruptive of membrane permeability (22) and one current view of auxin action is that it influences movement of substances across cell membranes (16). Research is in progress to examine these factors in the host-pathogen-herbicide interaction.

LITERATURE CITED

1. ABELES, F. B. 1968. Herbicide-induced ethylene production: Role of the gas in sublethal doses of 2,4-D. Weed Sci. 16:498-500.
2. ABELES, F. B. 1973. Ethylene in Plant Biology. Academic Press, New York, NY. 302 pp.
3. BEAN, G. A., and R. D. WILCOXSON. 1964. Helminthosporium leaf spot of bluegrass. Phytopathology 54:1065-1070.
4. CHEESMAN, T. H., E. C. ROBERTS, and J. 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.
5. EVANS, M. G. 1949. Kentucky bluegrass. Ohio Agric. Exp. Stn. Res. Bull. 681.
6. FARKAS, G. L. 1978. Senescence and plant disease. Pages 391–412 in: J. G. Horsfall and E. B. Cowling, eds. Plant Disease: An Advanced Treatise. Vol. 3. Academic Press, New York, NY. 487 pp.
7. HALLAWAY, M., and D. J. OSBORNE. 1969. Ethylene: A factor in defoliation induced by auxins. Science 163:1067-1068.
8. HEALEY, M. J., and M. P. BRITTON. 1968. Infection and development of *Helminthosporium sorokinianum* in *Agrostis palustris*. Phytopathology 58:273-276.
9. HODGES, C. F. 1972. Influence of culture age and temperature on germination of *Helminthosporium sorokinianum* and on pathogenicity to *Poa pratensis*. Phytopathology 62:1133-1137.
10. 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.
11. HODGES, C. F., and W. M. BLAINE. 1974. Midsummer occurrence of *Helminthosporium sorokinianum* leaf spot on selected cultivars of *Poa pratensis*. Plant Dis. Rep. 58:448-450.
12. HSIA, Y. T., and J. J. CHRISTENSEN. 1951. Effect of 2,4-D on seedling blight of wheat caused by *Helminthosporium sativum*. Phytopathology 41:1011-1020.
13. LUDWIG, R. A. 1957. Toxin production by *Helminthosporium sativum* P.K.& B. and its significance in disease development. Can. J. Bot. 35:291-303.
14. MAXIE, E. C., and J. C. CRANE. 1967. 2,4,5-trichlorophenoxyacetic acid: Effect on ethylene production by fruits and leaves of fig tree. Science 155:1548-1550.
15. OKA, I. N., and D. PIMENTEL. 1976. Herbicide (2,4-D) increases insect and pathogen pests on corn. Science 193:239-240.
16. RAY, P. M. 1974. The biochemistry of the action of indoleacetic acid on plant growth. Pages 93–122 in: V. C. Runeckles, E. Sandheimer, and D. C. Walton, eds. The Chemistry and Biochemistry of Plant Hormones. Recent Adv. Phytochem. Vol. 7.
17. RICHARDSON. L. T. 1957. Effect of insecticides and herbicides applied to soil on the development of plant disease. I. The seedling disease of barley caused by *Helminthosporium sativum* P.K.&B. Can. J. Plant Sci. 37:196-204.
18. 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.
19. 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.
20. RUDOLPH, K. 1976. Non-specific toxins. Pages 270–315 in: R. Heitefuss and P. H. Williams, eds. Physiological Plant Pathology. Encyclopedia of Plant Physiology, NS Vol. 4. Springer Verlag, Berlin.
21. SHOTWELL, O. L., and J. J. ELLIS. 1976. *Helminthosporium, Drechslera,* and *Bipolaris* toxins. Adv. Chem. Ser. 149:318-343.
22. 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.