Development of *Drechslera sorokiniana* on Sequentially Senescent Leaves of *Poa pratensis* Exposed to Postemergence Herbicide Combinations

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ABSTRACT

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Combinations of 2,4-D, 2,4,5-TP, MCPP, and dicamba at concentrations of 10^{-6} M were applied as soil drenches to *Poa pratensis* to determine their effect on development of *Drechslera sorokiniana*. Disease response to the herbicide combinations differed between the two youngest and two oldest leaves of the shoot. Disease severity on the youngest leaves decreased in response to combinations including 2,4,5-TP. Disease development was inhibited by 2,4-D + dicamba on the two youngest leaves. Disease response to the combination of 2,4-D + MCPP did not differ from that of the component herbicides. Only MCPP + dicamba showed an additive stimulatory effect on disease on the two youngest leaves. Most herbicide combinations induced an additive stimulation of leaf spot on the two oldest leaves. Only 2,4-D + 2,4,5-TP on leaf 3 and MCPP + dicamba on leaves 3 and 4 failed to stimulate disease additively.

Chlorophenoxy and benzoic acid herbicides influence pathogenesis by *Drechslera sorokiniana* (Sacc.) Subram. & Jain (= *Helminthosporium sativum*) on leaves of *Poa pratensis* (11,12). Exposure of herbicide-tolerant *P. pratensis* to foliar sprays or soil drenches of various postemergence herbicides and subsequent leaf inoculation with *D. sorokiniana* resulted in disease responses that ranged from inhibition to stimulation of leaf spot (11). A wide range of concentrations of 2,4,5-T, MCPP, and dicamba applied as foliar sprays or soil drenches stimulate development of leaf spot. 2,4-D has little effect on leaf spot when applied as a foliar spray but is stimulatory when applied as a soil drench (11). 2,4-D also predisposes wheat to infection by *D. sorokiniana* (13) and corn by *D. heterostrophus* (*H. maydis*) (20); conversely, 2,4-D reduces root rot of barley incited by *D. sorokiniana* (26). 2,4,5-TP inhibits leaf spot development at higher concentrations (10^{-4} or 10^{-6} M) but stimulates disease when diluted (11).

The host-pathogen-herbicide interaction between auxinlike herbicides and *D. sorokiniana* leaf spot also interfaces with sequential leaf senescence of *P. pratensis*. Herbicides 2,4-D, 2,4,5-T, MCPP, and dicamba substantially increase leaf spot severity on the older leaves of the shoot; only 2,4,5-TP remains inhibitory to leaf spot development on leaves of all ages (12). The increased disease severity on older leaves is characterized by extensive midvein and general chlorosis of infected leaves. Auxinlike herbicide can indirectly enhance the rate of senescence in leaves of some species, probably via ethylene (4,8,18,21,23); some plants also are known to generate ethylene in response to these herbicides (1,2). The auxinlike herbicides that stimulate leaf spot may do so by enhancing the rate of leaf senescence, which is subsequently exploited by *D. sorokiniana*.

Many postemergence herbicides applied to turfgrass are products containing combinations of two or more specific herbicides (5,16,19). Herbicide combinations may be synergistic, additive, or antagonistic on weed species (25). Mixtures of 2,4-D, MCPP, and dicamba are synergistic on some broadleaved species in turf (29), whereas mixtures of 2,4-D, MCPP, dicamba, and picloram are antagonistic on some species (14). Evidence that mixtures of postemergence herbicides may enhance or inhibit the herbicidal characteristic of the individual herbicides in the mixture is of special interest on the host-pathogen-herbicide-senescence interactions in question. It is possible that various combinations of chlorophenoxy and benzoic acid herbicides may influence the expression of leaf spot differently from that of single herbicides. The research presented was initiated to determine the effect of various combinations of postemergence herbicides on *D. sorokiniana* leaf spot on sequentially developing and senescing leaves of *P. pratensis*.

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MATERIALS AND METHODS

Postemergence herbicides evaluated for their effect on *D. sorokiniana* leaf spot on *P. pratensis* included 2,4-D, 2,4,5-TP (silvex), MCPP (mecoprop), and dicamba. Individual herbicides and all possible combinations of two herbicides were prepared at 10^{-6} M.

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

Individual herbicides and combinations of all possible pairs of each herbicide were applied to *P. pratensis* as soil drenches. Soil drenches were applied to plants in two applications of 20 ml of each individual herbicide and each combination of two herbicides on alternate days. Control plants not treated with herbicide received distilled water. All plants were inoculated with *D. sorokiniana* conidia 2 days after the last application of herbicide(s). All inoculations were made on the four youngest visible leaf blades of a single shoot. Suspensions of 10 conidia in 0.02 ml (500/ml) of distilled water were prepared with an automatic particle counter (Pacific Scientific, HIAC/ROYCO Instrument Division, Menlo Park, CA 94025). Each leaf blade was inoculated in five positions 1 cm apart in a specially designed inoculation apparatus (27). Inoculated plants were incubated 6 days at 22 C with a 10-hr photoperiod (85–90 μE, daylight fluorescent), then evaluated for disease severity. Each replicate of 10 herbicide treatments (2,4-D; 2,4,5-TP; MCPP; dicamba; 2,4-D + 2,4,5-TP; 2,4-D + MCPP; 2,4-D + dicamba; 2,4,5-TP + MCPP; 2,4,5-TP + dicamba; and MCPP + dicamba) and the controls not treated with herbicide consisted of five single-shoot plants, each with four progressively older leaves. Each herbicide treatment was replicated five times (25 plants with 25 leaves of each age group evaluated per herbicide treatment). Disease severity was evaluated independently on each leaf-age group.

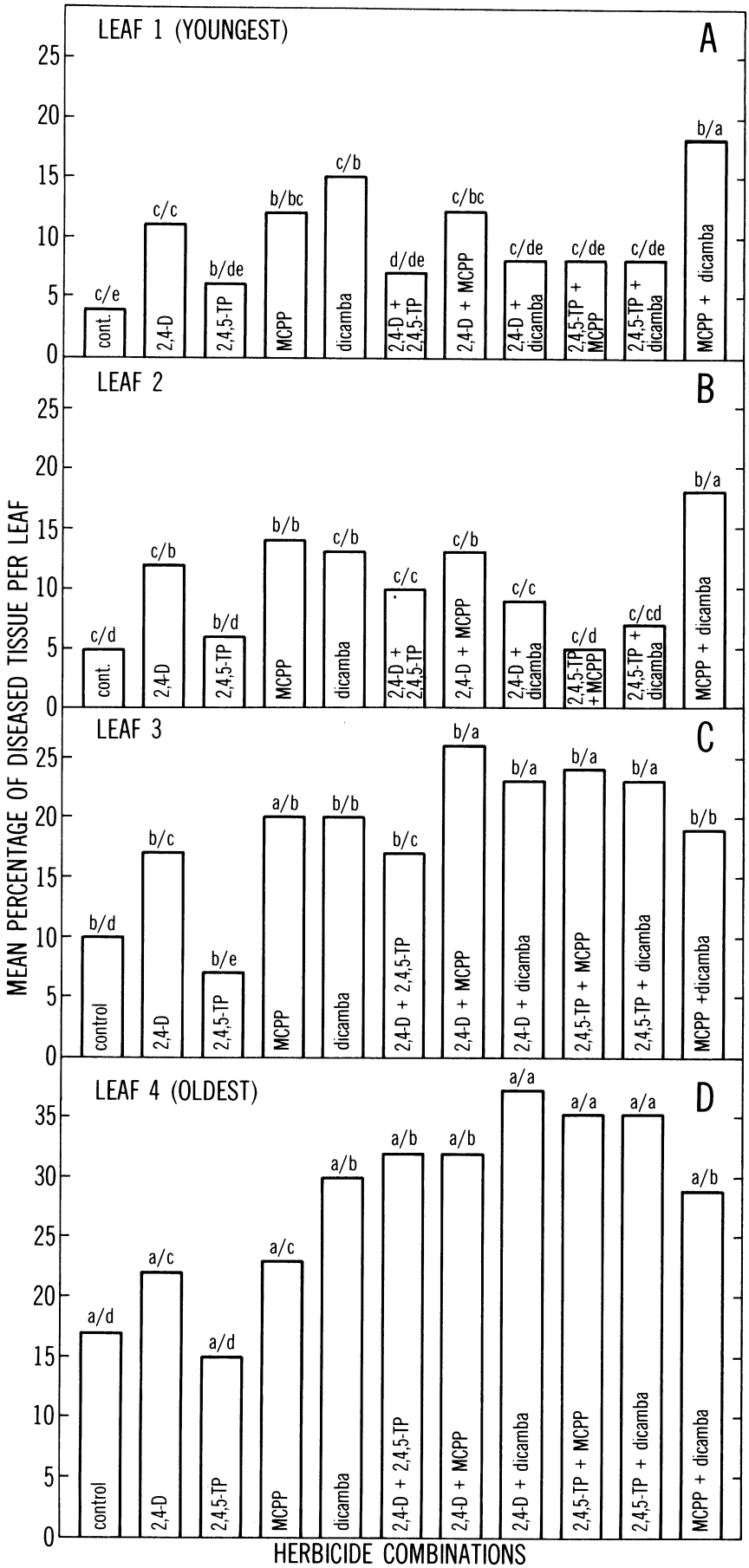

Fig. 1. Mean percentage of diseased tissue on progressively older leaves of *Poa pratensis* plants exposed to soil-drench applications of individual and combinations of postemergence herbicides and inoculated with conidia of *Drechslera sorokiniana*. Mean percentages of disease on each older leaf within treatments (a/) and on leaves of the same age between treatments (/a) followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Ten-centimeter lengths of the inoculated leaf blades of each leaf-age group were removed from the inoculation apparatus (27) at the end of the 6-day incubation period and evaluated for disease severity. Total area of the leaf sample was estimated to the nearest whole number (mm^2). The area of diseased tissue on the leaf sample was estimated by multiplying the estimated length and width (longest chords) of each lesion. Lesion measurement included necrotic, chlorotic, and straw-colored blighted areas associated with lesions. Lesions with an area of less than 0.4 mm^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

Disease severity increased from the younger to the older leaves of control plants, plants treated with individual herbicides, and plants treated with herbicide combinations. The magnitude of disease severity varied with specific treatments on each progressively older leaf; in all instances, however, the least severe disease occurred on the younger leaves and the most severe disease occurred on the oldest leaves (Fig. 1A–D). Individual herbicides 2,4-D, MCPP, and dicamba increased disease on leaves of each age group compared with the controls (Fig. 1A–D). Only 2,4,5-TP failed to change disease severity on leaves of each age group.

The herbicide combinations induced changes in disease severity that often differed from those of the individual herbicides of the combinations, and the changes were related to leaf age. Disease severity on the two youngest leaves (leaves 1 and 2) was similar in response to the herbicide combinations. The combinations of 2,4-D + 2,4,5-TP, 2,4,5-TP + MCPP, and 2,4,5-TP + dicamba produced disease severity on leaves 1 and 2 equal to that induced by 2,4,5-TP alone and less than that induced by the 2,4-D, MCPP, or dicamba components of the respective combinations (Fig. 1A, B). The combination of 2,4-D + dicamba decreased disease severity on the two youngest leaves below that associated with either herbicide alone, and the combination of 2,4-D + MCPP had no effect on disease compared with that of the component herbicides (Fig. 1A,B). Only the combination of MCPP + dicamba showed an additive effect that stimulated an increase in disease on leaves 1 and 2 greater than that associated with either herbicide alone (Fig. 1A,B).

Disease severity on the two oldest leaves of the shoot (leaves 3 and 4) generally increased in response to the herbicide combinations. The combinations of 2,4-D + MCPP, 2,4-D + dicamba, 2,4,5-TP + MCPP, and 2,4,5-

TP + dicamba showed an additive effect on the stimulation of disease on the two oldest leaves that was greater than that associated with the individual herbicides of the respective combinations (Fig. 1C, D). Disease severity in response to 2,4-D + 2,4,5-TP on leaf 3 was greater than that associated with 2,4,5-TP but did not differ from the disease level associated with 2,4-D; on the oldest leaf (leaf 4), this combination had an additive effect on increasing disease above that of 2,4-D or 2,4,5-TP alone (Fig. 1C,D). The combination of MCPP + dicamba stimulated the same level of disease on leaf 3 as that associated with the individual herbicides of the combination; on leaf 4, this combination stimulated disease more than MCPP alone but disease did not differ from that associated with dicamba (Fig. 1C,D).

DISCUSSION

Chlorophenoxy and benzoic acid herbicides can stimulate development of *D. sorokiniana* leaf spot on *P. pratensis* (11,12). The leaf spot induced by *D. sorokiniana* is characteristic of a senescence-induced disease (7) in that disease severity increases from the younger to the older leaves of the shoot (12). When the postemergence-herbicide-tolerant *P. pratensis* is exposed to chlorophenoxy or benzoic acid herbicides and inoculated with *D. sorokiniana*, disease severity generally increases on all leaves, but the increase is always greatest from the youngest to the oldest leaves. Of the several ways that herbicides are hypothesized to influence disease (3,15), the induction of physiological changes in the host that enhance leaf senescence processes probably occur and these changes are exploited by the pathogen. There also may be some direct stimulation of the pathogen by the herbicides (10).

Exposure of *P. pratensis* to combinations of postemergence herbicides before inoculation with *D. sorokiniana* resulted in some unexpected deviations in disease development relative to disease response to individual herbicides. The disease response to the herbicide combinations on the two youngest leaves (leaves 1 and 2) was primarily disease development equal to the less stimulatory of the herbicide combination and inhibition of disease (Fig. 1A,B). The disease response to the herbicide combinations on the two oldest leaves (leaves 3 and 4) was primarily additive stimulation and disease development equal to the more stimulatory herbicide of the combination (Fig. 1C,D).

The response of leaf spot development on leaves of *P. pratensis* plants exposed to chlorophenoxy and benzoic acid herbicides seems closely linked to sequential development and senescence of the leaves. Auxin-analog herbicides may slow senescence in some species by sustaining or stimulating RNA and protein synthesis and respiration in young leaves (24,28). In older leaves, auxin analogs may slow the loss of protein but do not stimulate RNA or protein synthesis (22,24,28). Oxygen utilization may be temporarily increased by auxins in middle-aged leaves, but O_2 uptake is not affected in older leaves (24). The slowing of leaf senescence induced by auxins (2,4-D, 2,4,5-T) seems to be localized in some species and establishes a metabolic sink for carbon and nitrogen compounds in the treated area (4,23). This type of auxin-delayed senescence may enhance the rate of senescence of surrounding tissue and stimulate ethylene production (1).

The known auxin-leaf senescence interactions provide the basis for a tentative hypothesis for leaf spot development on younger and older leaves of *P. pratensis* exposed to auxin-analog herbicides. The two youngest visible leaves of a *P. pratensis* shoot are components of young phytomers (6) and characteristically possess active intercalary meristems (youngest leaf) and enlarging cells (two youngest leaves). The mature leaves (leaves 3 and 4) of perennial grasses characteristically export assimilates to younger leaves (30). It is possible that the auxin-analog herbicides may increase the metabolic rate of younger leaves but not of older leaves, ie, respiration and RNA and protein synthesis may be increased in younger leaves and not be affected in older leaves. Such a differential response may enhance the rate of export of assimilates from older to younger leaves and thereby enhance the rate of senescence of the older leaves. The senescence processes may be further enhanced by auxin-induced ethylene. MCPP and 2,4,5-TP also decrease sucrose and total sugars in *P. pratensis* but have little effect on total free amino acids (17). Under these various circumstances, the older leaves of the shoot may be predisposed to more rapid aging and more severe leaf spot development.

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