Picloram-Induced Increase of Carbohydrate Exudation from Corn Seedlings

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Increased activity of soil microorganisms in a rhizosphere is primarily a result of exudation of certain substances from roots. This subject has been reviewed by several workers (1, 7, 8, 9, 11). Certain chemicals released from plant tissues may stimulate growth of parasitic organisms. Petersen et al. (5) reported that roots of kidney bean plants sprayed with gibberellin exuded more carbohydrate substances and were more susceptible to attack by one isolate of *Rhizoctonia solani* than were those of unsprayed plants. Richardson (6) indicated that seed exudates may favor pathogenesis by reversing the toxicity of certain fungicides such as thiram.

Goring et al. (2) reported that picloram (4-amino-3, 5,6-trichloropicolinic acid), depending upon soil type and moisture, lost 58 to 96% of its activity within 1 year and 78 to 100% within 2 years after it was incorporated into soil. Semeniuk & Tunac (10) showed that picloram applied to soil increased root rot of wheat and corn seedlings from a number of soil-borne fungal pathogens. Additional tests were therefore conducted to determine the effect of picloram on amino acid and carbohydrate exudation from corn seedlings.

To reduce seed contamination from fungi and bacteria during germination, healthy corn seeds of uniform size were treated with an aqueous 0.1% $HgCl_2$ solution for 3 min and thoroughly washed with sterile distilled water. Each seed was planted separately beneath the surface of 5-cm-deep moist, sterilized silica sand in 2.5 × 20-cm test tubes, and held in the greenhouse. The top of each tube was loosely covered with an aluminum foil to reduce contamination; the bottom was wrapped with black paper to exclude light from growing roots. On the 4th day after planting (seedlings about 1 inch long), the sand was treated with an aqueous solution of pure picloram for a desired concentration of the chemical in the medium. Thereafter, seedlings were watered daily as needed with sterile distilled water. On the 8th day, the seedlings and sand were carefully removed from each test tube, and both sand and roots were thoroughly washed five times with 20-ml aliquots of sterile distilled water. Washings from two tubes of a similar treatment were combined, made to 200 ml, and evaporated to dryness on a hot water-bath. The residue was carefully washed with 10 ml sterile distilled water; the aqueous solution was then stored frozen until analyzed.

TABLE 1. Effect of picloram concentration on the amount of total carbohydrate and reducing sugar exuded from corn seedlings in silica sand

Picloram concn	Total carbohydrate	Reducing sugar
ppm	*mg/ml exudate*	
0	8.8 ± 3.7[a]	1.8 ± 0.75[a]
50	13.6 ± 1.6	3.5 ± 0.65
100	12.5 ± 0.5	2.6 ± 0.15
200	18.0 ± 3.6	4.2 ± 0.30
500	37.5 ± 6.5	4.8 ± 0.12

[a] Each value is the average of three replicates, representing six seedlings. Standard error is indicated.

The concentrated exudate thus collected from each of two seedlings was quantitatively analyzed for amino acid, total carbohydrate, and reducing sugar. Amino acid was determined by the method of Yemm & Cocking (12), with glycine as the standard. Total carbohydrate was measured with Dreywood's anthrone reagent by the method of Morris (3). Reducing sugar was measured by the method of Park & Johnson (4), with glucose as the standard. Because Park & Johnson's method was sensitive, exudate preparations were diluted 1:9 for a determination of equivalent glucose per ml.

Analysis for amino acid in the concentrated exudate showed little or no ninhydrin-positive substances, hence no stimulation of amino acid exudation from applications of 50 to 500 ppm picloram. Total carbohydrate and reducing sugar, however, were increased 170 to 430% and 190 to 270%, respectively (Table 1). The increase was positively correlated with picloram concentration.

To determine the site of exudation, a quantity of surface-sterilized seed was divided into two parts. One part was planted directly into sand, the other was first germinated in a moist chamber for 48 hr for the radicles to reach approximately 2 cm in length; these were then transplanted into each test tube in such a way that the seeds were out of contact with the sand. Total carbohydrate and reducing sugar analysis (Table 2) indicated that seeds buried in the sand were the major source of these exudates, and that 500 ppm picloram stimulated exudation.

To test for a possible interaction between picloram

TABLE 2. Effect of picloram on the amount of total carbohydrate and reducing sugar exuded from corn seedlings with seeds buried and not buried

Treatment	Total carbohydrate	Reducing sugar
	mg/ml exudate[a]	
Buried	16.2 ± 4.3[b]	1.4 ± 0.24[b]
Buried + picloram[c]	26.7 ± 6.5	1.5 ± 0.25
Not buried	7.1 ± 2.8	0.9 ± 0.34
Not buried + picloram[c]	12.5 ± 3.5	1.3 ± 0.65

[a] Eighth day after planting ungerminated seed.
[b] Each value is the average of three replicates, representing six seedlings. Standard error is indicated.
[c] 500 ppm. Chemical added to each tube on the 4th day after planting ungerminated seed, on the 2nd day after planting pregerminated seed.

TABLE 3. Interaction of picloram and two pathogenic fungi on the amount of total carbohydrate and reducing sugar in corn seedlings exudates

Treatment	Total carbohydrate	Reducing sugar
	mg/ml exudate[a]	
Rhizoctonia solani	15.2 ± 2.5[b]	7.7 ± 1.1[b]
Pythium arrhenomanes	37.6 ± 5.6	21.4 ± 2.3
R. solani + picloram[c]	53.5 ± 9.4	3.5 ± 1.5
P. arrhenomanes + picloram[c]	77.0 ± 11.2	36.5 ± 1.9
Control (picloram[c])	106.2 ± 21.5	58.0 ± 2.3
Control (no picloram)	74.3 ± 10.5	26.8 ± 3.4

[a] Eighth day after seed planting.
[b] Each value is the average of three replicates, representing six seedlings. Standard error is indicated.
[c] 500 ppm.

and *Rhizoctonia solani* or *Pythium arrhenomanes* on exudation, the sand of some culture tubes was inoculated with *R. solani*, while the sand in others was inoculated with *P. arrhenomanes*. The inoculum consisted of 5-mm discs obtained from 3-day-old cultures of *R. solani* grown on water agar or of *P. arrhenomanes* grown on potato-dextrose agar. Three discs from the appropriate culture were introduced/tube. Corn seeds were planted at the time of inoculation. A picloram solution was added 3 days later to half the inoculated tubes and to half the noninoculated controls. The chemical (500 ppm) increased carbohydrate exudation from corn seedlings in the absence of the pathogens, not in their presence (Table 3), probably because the pathogens used part of the exuded carbohydrate for their own development.

The above results suggest that increased carbohydrate exudation from cereal seedlings may account for increased root damage from soil-borne root pathogens in picloram-treated soil (10).

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