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RESPONSE OF SEVERAL CUCUMBER CULTIVAR SEEDLINGS TO ETHALFLURALIN AND PENDIMETHALIN *IN VITRO*

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ABSTRACT

Seedlings of six cucumber *[Cucumis sativus* L.] cultivars were cultured from seed on agar medium containing several concentrations of ethalfluralin (N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl)benzenamine) or pendimethalin (N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine). The cultivars 'Sweet Slice' and '420 Green Pak' were the most tolerant to the herbicides, and 'Poinsett 76' and 'Ashley' were the most sensitive. The Cultivars 'Carolina' and 'Dasher' showed intermediate response to the two herbicides. The methodology of this study could be used as a valuable bioassay for crop susceptibility or tolerance to various herbicides.

INTRODUCTION

Most cucurbits are injured by many of the commonly used herbicides. Therefore, very few herbicides have been labeled for weed control in cucurbits. Two dinitroaniline herbicides, pendimethalin and ethalfluralin, control many grasses including seedling johnsongrass [*Sorghum halepense* (L.) Pers.] and goosegrass *[Eleusine indica* (L.) Gaertn.], and some common annual broadleaf weeds such as the pigweeds [*Amaranthus* spp.] when applied either preemergence or preplant incorporated. Unfortunately, cucurbit crop tolerance to these herbicides is marginal (Kennedy 1982).

In a two-year study, Cordrey et al. (1979) found that ethalfluralin and pendimethalin applied preemergence at 1.23 kg/ha reduced cucumber crop vigor and fruit yield. Talbert et al. (1979, 1980, 1981) found that pendimethalin applied preemergence at 1.1 kg/ha and ethalfluralin applied at 0.8, 1.2, and 1.7 kg/ha as preemergence treatments did not reduce the yield of cucumbers. Derr and Monaco (1981) reported no significant difference in either fresh or dry root weight between 16 cucumber cultivars treated with ethalfluralin. In field trials, Kennedy (1982) found that cucumber cultivars respond differently to ethalfluralin and pendimethalin. When ethalfluralin was applied preemergence at 1.4 kg/ha to five cultivars of pickling and fresh market cucumbers, fruit yields were 'Carolina' ≥ '420 Green Pak' ≥ 'Poinsett 76' ≥ 'Dasher' ≥ 'Ashley.' When pendimethalin was applied preplant incorporated at 0.84 kg/ha, fruit yields were '420 Green Pak' ≥ 'Carolina' ≥ 'Sweet Slice' ≥ 'Dasher' ≥ 'Poinsett 76' ≥ 'Ashley.'

Injury symptoms caused by the dinitroaniline herbicides to dicotyledonous plants are a reduction in seedling elongation or stunting of the shoot, a leathery appearance of the cotyledons, brittleness of the hypocotyl, and an intensification of green coloration. Inhibition of root growth, an increase in diameter of the root near the tip, and inhibition of secondary roots are common herbicidal symptoms (Parka and Soper 1977). Mallory and Bayer (1972) found that cotton roots treated with trifluralin (2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)benzenamine) gradually lost the root meristem. In another test, Oliver and Frans (1965) found that if this inhibitory effect of trifluralin is not great enough to cause the death of the cotton seedling, the herbicidal effect may be overcome through the production of lateral roots. Lateral roots are produced in the non-treated zone of soil after the primary root has passed through the treated soil. The greatest number of lateral roots were found in the 2.5 cm zone immediately below the treated zone. The objective of this study is to determine the response of the seedlings of six cucumber cultivars to ethalfluralin and pendimethalin by means of a root bioassay *in vitro*.

MATERIALS AND METHODS

Seeds of the cucumber cultivars '420 Green Pak,' 'Dasher,' 'Ashley,' 'Carolina,' 'Poinsett 76,' and 'Sweet Slice' were surface sterilized in a 20% v/v solution of Clorox in a 1% w/v

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aqueous solution of Alconox detergent for 10 minutes. The seeds were then rinsed three times with sterile, distilled water and placed singly in 25 x150 mm culture tubes each containing 20 ml of agar-solidified medium, and capped with a plastic closure.

The media were prepared by adjusting the pH of distilled water to 6.3 with 1 N potassium hydroxide solution, adding the appropriate quantity of herbicide, and then solidifying with 0.6% Bacto-Agar. The herbicides ethalfluralin and pendimethalin were tested singly in concentrations of 0, 0.125, 0.250, 0.375, 0.500, and 0.625 ppmw. Preliminary experiments demonstrated that both the herbicides were volatile at autoclave-sterilizing temperatures. Therefore, the media were prepared by bringing the pH adjusted water to a boil, adding and dissolving the agar, allowing each medium to cool to about 55°C, adding the appropriate quantity of herbicide, and then pouring it into the culture tubes. After the media had cooled and solidified, the seeds were placed in culture.

The cultures were maintained in a walk-in culture room. The temperature was 25±2°C. Light was furnished by white fluorescent tubes and incandescent lamps at an intensity of 2500 lux at the level of the cultures. The photoperiod was 16 hours of light and eight hours of darkness.

The surface-disinfected seeds were placed on the surface of the media in a horizontal position using flame-sterilized forceps. Two to three days later when the roots had emerged to a length of about 3 mm, the germinating seeds were reoriented to an upright (root down) position half way into the medium. This procedure ensured an ample supply of oxygen to the seeds.

A week after initial culture of the seeds, the primary root of the seedlings had grown to a length of about 2 cm. At this time, the seedlings were transferred to fresh medium without herbicides. Ten days later (17 days after initial culture), the seedlings were harvested. The length of the primary root and the number of secondary roots were recorded.

The experimental design was a randomized complete block with 12 replications, 12 chemical treatments, and six cultivars. Duncan's multiple range test was used to separate treatment means at the 5% level.

RESULTS AND DISCUSSION

Large differences were found in the growth of the primary root of the six cucumber cultivars *in vitro* (Table 1). After seven days, the length of the primary root of the six cultivars was as follows: 'Sweet Slice' ≥ '420 Green Pak' ≥ 'Carolina' ≥ 'Dasher' ≥ 'Poinsett 76' ≥ 'Ashley.' Treatment with either herbicide for seven days inhibited the growth in length of the primary root of the three most vigorous cultivars, namely 'Sweet Slice,' '420 Green Pak,' and 'Carolina.' The herbicides had no effect on root growth of 'Ashley.' The length of the primary roots of the three most vigorous cultivars was greater when treated with either herbicide than was the length of the primary roots of 'Ashley' in the non-herbicide treated controls.

Major differences among cultivars were detected after 17 days. In control cultures, the primary root lengths were 'Dasher' ≥ '420 Green Pak' ≥ 'Carolina' ≥ 'Sweet Slice' ≥ 'Poinsett 76' ≥

Table 1. Primary root length (cm) of seedlings of six cucumber cultivars after seven and seventeen days *in vitro*.

		Sweet Slice		420 Green Pak-		Carolina		Poinsett-76		Dasher		Ashley	
	ppmw	7 days	17 days	7 days	17 days	7 days	17 days	7 days	17 days	7 days	17 dys	7 days	17 days
Control	–	3.4 Aa	5.7 C-H	2.9 B	7.9 A-D	2.0 C	6.7 B-E	0.7 K-R	1.2 NO	1.2 F-K	9.9 A	0.1 S	0.6 L-O
Ethalfluralin	.125	1.1 G-K	2.4 I-O	1.9 CDE	6.3 C-G	0.8 I-N	9.7 AB	0.2 P-S	0.4 L-O	0.9 H-N	4.9 D-J	O S	0.2 NO
	.250	1.1 G-K	2.2 I-O	1.4 E-H	6.5 C-F	1.1 G-L	3.8 E-M	0.4 M-S	0.5 L-O	0.8 J-O	4.6 D-K	0 S	0.1 O
	.375	1.0 G-M	2.5 H-O	1.7 C-F	10.2 A	1.0 G-M	2.2 I-O	0.2 P-S	O.2 NO	0.8 K-O	4.6 D-K	0.1 S	0.2 N-O
	.500	1.4 E-J	2.2 I-O	1.2 F-K	5.7 C-H	0.8 K-O	3.9 E-L	0.2 P-S	0.2 NO	1.1 G-L	2.5 H-O	0 S	0.3 NO
	.625	0.8 I-N	2.2 I-O	1 0 G-M	4.8 D-J	0.8 I-N	1.7 J-O	0.3 N-S	1.4 MNO	0.5 L-S	2.3 I-O	0 S	0.2 NO
Pendimethalin	.125	0.9 H-N	2.9 G-O	1.5 C-G	8.9 ABC	1.1 F-K	9.5 AB	0.5 L-S	0.6 L-O	0.8 J-O	5.4 D-I	0 S	0.2 NO
	.250	1.0 G-M	2.4 I-O	1.3 F-K	1.9 I-O	0.8 J-N	5.2 D-I	0.5 L-S	0.4 L-O	0.8 J-N	2.8 G-O	0 S	0.2 NO
	.375	0.9 G-M	1.6 J-O	1.0 G-M	1.5 J-O	1.0 G-M	2.4 I-O	0.3 N-S	0.4 NO	0.7 K-P	3.4 F-O	0 S	0.2 NO
	.500	1.9 CD	3.5 E-N	1.5 E-G	4.4 D-L	1.0 G-M	6.8 B-E	0.2 QRS	0.4 NO	1.0 G-M	2.5 H-O	0 S	0.2 NO
	.625	1.1 G-K	2.8 H-O	1.2 F-K	3.0 F-O	0.7 K-Q	2.2 I-O	0.1 RS	0.2 NO	0.7 K-Q	1.2 L-O	0 S	0.2 NO

[a]Means of each observation date within columns and rows followed by the same letter are not significantly different at the 5% level of Duncan's multiple range test.

'Ashley' (Table 1). No differences in primary root length were found among herbicide treatments when 'Poinsett 76' and 'Ashley' were treated with either herbicide compared to their respective controls. Treatment with either herbicide reduced the growth in length of the primary roots of 'Dasher.' No differences in root length were found among treatments or among cultivars when comparing 'Ashley' and 'Poinsett 76' seedlings treated with either herbicide or their controls, or with 'Sweet Slice' treated with either herbicides.

The seedlings of 'Sweet Slice' and '420 Green Pak' were the only ones to produce secondary roots after seven days of seed germination *in vitro* (Table 2). After 17 days, the production of secondary roots in the controls was 'Sweet Slice' ≥ 'Dasher' ≥ '420 Green Pak' > 'Carolina' > 'Poinsett 76' ≥ 'Ashley.' The controls of 'Sweet Slice,' '420 Green Pak,' and 'Dasher' produced more secondary roots than their respective counterparts in the agar media containing herbicide (Table 2). The cultivar 'Carolina' produced more secondary roots in controls and an agar media containing 0.125 ppmw of either herbicide than when grown on agar media containing higher concentrations of either herbicide. The differences in length of primary root and secondary root numbers are illustrated in Figure 1 for the cultivar 'Sweet Slice' which was one of the most vigorous cultivars tested, and in Figure 2 for 'Ashley' which was the least vigorous of the cultivars tested.

The vigorous cultivars 'Sweet Slice,' '420 Green Pak,' and 'Dasher' produced larger primary roots and more secondary roots than 'Poinsett 76' and 'Ashley.' The production of secondary

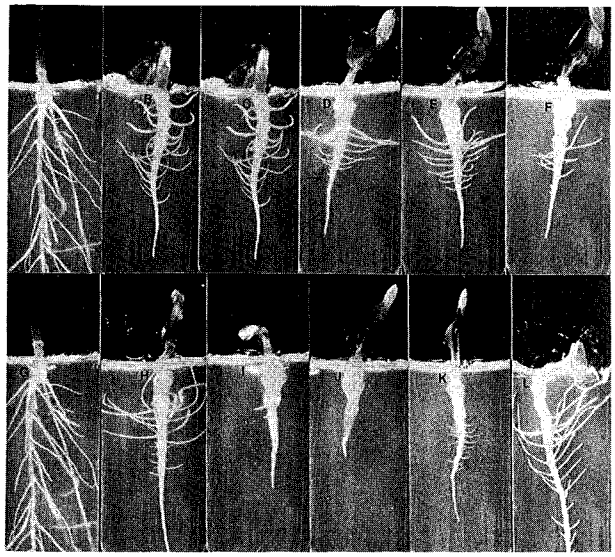

Figure 1. Effect of ethalfluralin and pendimethalin on cucumber 'Sweet Slice' seedling roots *in vitro* (A, control; B, 0.125 ppmw ethalfluralin; C, 0.250 ppmw ethalfluralin; D, 0.375 ppmw ethalfluralin; E, 0.500 ppmw ethalfluralin; F, 0.625 ppmw ethalfluralin; G, control; H, 0.125 ppmw pendimethalin; I, 0.250 ppmw pendimethalin; J., 0.375 ppmw pendimethalin; K, 0.500 ppmw pendimethalin; I, 0.625 ppmw pemdimethalin).

Table 2. Average number of secondary roots per seedling of the seedlings of six cucumber cultivars after seven and seventeen days *in vitro.*

		Sweet Slice		420 Green Pak		Carolina		Poinsett-76		Dasher		Ashley	
	ppmw	7 days	17 days	7 days	17 days	7 days	17 days	7 days	17 days	7 days	17 dys	7 days	17 days
Control	–	4 A[a]	20 A	3 A	18 AB	0 B	11 CDE	0 B	1 KLM	0 B	19 A	0 B	1 KLM
Ethalfluralin	.125	0 B	7 E-H	0 B	5 E-M	0 B	11 CDE	0 B	1 J-M	0 B	5 E-M	O B	0 M
	.250	0 B	5 E-M	0 B	6 E-M	0 B	2 H-M	0 B	1 J-M	0 B	6 E-L	0 B	0 M
	.375	0 B	9 C-G	0 B	7 E-H	0 B	1 J-M	0 B	0 M	0 B	3 G-M	0 B	0 M
	.500	0 B	5 E-M	0 B	6 E-M	0 B	2 H-M	0 B	0 M	0 B	2 H-L	0 B	0 M
	.625	0 B	6 D-J	0 B	6 D-L	0 B	2 H-M	0 B	0 M	0 B	1 I-M	0 B	0 M
Pendimethalin	.125	0 B	9 C-F	0 B	4 F-M	0 B	11 CD	0 B	1 LM	0 B	7 D-I	0 B	0 M
	.250	0 B	6 E-L	0 B	1 J-M	0 B	4 F-M	0 B	0 M	0 B	2 H-M	0 B	0 M
	.375	0 B	4 G-M	0 B	0 M	0 B	1 F-M	0 B	0 M	0 B	3 H-M	0 B	0 M
	.500	0 B	13 BC	0 B	2 H-M	0 B	6 D-K	0 B	2 H-M	0 B	0 M	0 B	0 M
	.625	0 B	10 CDE	0 B	2 H-M	0 B	2 H-M	0 B	0 M	0 B	0 M	0 B	0 M

[a]Means of each observation date within columns and rows followed by the same letter are not significantly different at the 5% level of Duncan's multiple range test.

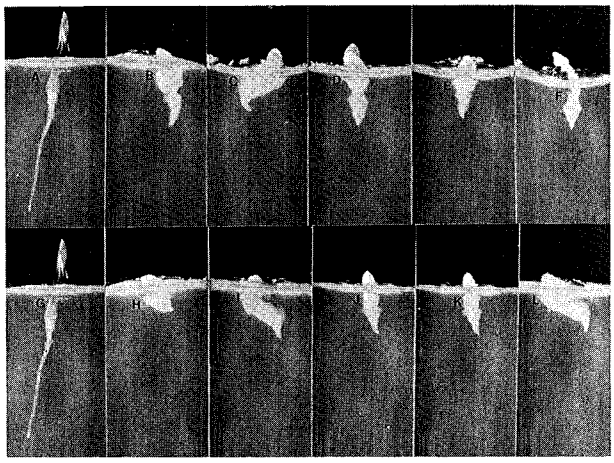

Figure 2. Effect of ethalfluralin and pendimethalin on cucumber 'Ashley' seedling roots *in vitro* (A, control; B, 0.125 ppmw ethalfluralin; C, 0.250 ppmw ethalfluralin; D, 0.375 ethalfluralin; E, 0.500 ppmw ethalfluralin; F, 0.625 ppmw ethalfluralin; G, control; H, 0.125 ppmw pendimethalin; I, 0.250 ppmw pendimethalin; J, 0.375 ppmw pendimethalin; K, 0.500 ppmw pendimethalin; L, 0.625 ppmw pendimethalin.

roots is an indication of seedling recovery from the effects of dinitroaniline herbicides. This has been demonstrated previously by Mallory and Bayer (1972) and Oliver and Frans (1965) *in vivo*, and by this study *in vitro*. Therefore, seedling recovery, as demonstrated in this study, is dependent upon cultivar and rate of herbicidal application (Table 2).

The reduced root growth of 'Poinsett 76' and 'Ashley' *in vitro* may indicate potential susceptibility of these cultivars to ethalfluralin or pendimethalin under field conditions as shown by Kennedy (1982). The results of this bioassay closely paralleled the cucumber cultivar field trials of Kennedy (1982). This bioassay may be a valuable indicator of potential cucumber cultivar susceptibility or tolerance to these two herbicides. Moreover, the methodology of this bioassay may be a valuable indicator of potential crop susceptibility or tolerance to various herbicides.

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