

Aboveground Screening for Genotypic Differences in Cucumber Root Growth in the Greenhouse and Field

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Abstract. A simple, aboveground method to study cucumber (*Cucumis sativus* L.) root growth was developed using a subsurface herbicide banding technique. Those plants with roots that grow deeper or faster reach the herbicide sooner and exhibit herbicide injury symptoms sooner. Greenhouse pot trials showed that 0.25 or 0.50 kg simazine/ha could be used to produce distinctive symptoms; time to symptom expression increased with the depth of the band from the soil surface. Root washing experiments verified that root length was associated with response time. In field trials, response time and severity of symptoms varied with herbicide concentration, depth, and distance from the seed row, thereby providing an indication of where the roots were in the soil. About 100 diverse cucumber genotypes were tested for differences in root growth rate in the greenhouse and in the field. Time to symptom expression was normally distributed among the genotypes; analysis of variance (ANOVA) indicated significant genotypic differences. This system can be used for cultural or physiological studies, or nondestructively for selection and breeding purposes. If the herbicide is placed sufficiently deep to prevent damage to the cotyledons, the plants are capable of flowering and producing fruit. Chemical name used: 6-chloro-N, N'-diethyl-1,3,5-triazine-2,4-diamine (simazine).

A well-developed root system is essential for optimal plant growth. Recognition of the importance of the root system can be seen in the many cultural practices aimed at improving the root environment (e.g., adjusting soil pH, implementing drainage systems, and the use of cultivation practices). Although roots can have a direct effect on crop growth, yield, and quality, they often are ignored in breeding efforts (Taylor, 1986; Zobel, 1986). This is the case for cucumber, especially with regard to rooting behavior in the field. The few genetic and breeding studies in the past 15 to 20 years that have examined cucumber roots, however, indicate that it may be possible to breed for improved cucumber root systems. Ghaderi and Lower (1978, 1979), using controlled environment chambers, found that the size of the root system can be highly heritable. Lebedeva (1971) and Yurina and Lebedeva (1974) reported that selecting for larger root systems can result in more aboveground growth and higher yield.

A primary function of roots is uptake of water. Short term water stress in cucumber at critical times during crop growth can result in reductions in yield and/or an increase in the percent culls (Ortega and Kretchman, 1982; O'Sullivan, 1980; Tan et al., 1983). One approach that might be helpful in reducing susceptibility to water stress would be to genetically increase the water extracting capacity of the plants by breeding for deeper or larger rooting systems (Kaspar et al., 1984; Miller, 1986). Field studies by Medina-Mora (1988) showed that cucumbers are shallow rooted; 75% to 85% of the roots were located in the top 10 to 15 cm of soil. Similarly, in greenhouse studies with ground beds, Randall and Locascio (1988) found that cuc-

umber root density was \approx 50% less at depths of 20 and 30 cm than at 10 cm.

The first step in breeding for rooting characteristics is to determine if there is genetic variability for rooting behavior among cucumber accessions. A major barrier to breeding for rooting characteristics is that work with roots, especially in the field, can be very labor intensive, difficult, and expensive (Taylor, 1986). Rhizotrons are expensive and have limited space; digging up plants is destructive and very labor intensive. To circumvent these problems we sought to develop a simple, inexpensive, aboveground screening system for cucumber roots based on the herbicide banding technique of Robertson et al. (1985). The principle of this method is to band herbicide into the root zone at a specific depth and/or lateral distance from the seed row before planting. Those plants whose roots grow faster should reach the herbicide sooner, thereby showing herbicide damage symptoms sooner. It then should be possible to monitor root growth of a large number of genotypes by simple visual observation of the shoots. In this work we demonstrate that this method can be used to monitor cucumber root growth in the greenhouse and field, and can also be used to test for differences in root growth rate among a diverse collection of genotypes. Using this technique we found significant differences in time to symptom expression among cucumber genotypes.

Materials and Methods

Cucumber genotypes. The 96 cucumber plant introductions (PIs) chosen for this study were selected to be representative of the variability present within the cucumber germplasm based on the isozyme data of Knerr et al. (1989). A list of the specific PIs tested and their response rates in the greenhouse and field is available on request. Seed was initially provided by the Plant Introduction Station at Ames, Iowa. Several plants of each genotype were self-pollinated in the greenhouse to produce sufficient seed for greenhouse and field trials. Due to space limitations, not all genotypes were multiplied at the same time. The commercial pickling cucumber cultivar 'Flurry' (Asgrow Seed

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Abbreviations: DAE, days after emergence; PI, plant introduction.

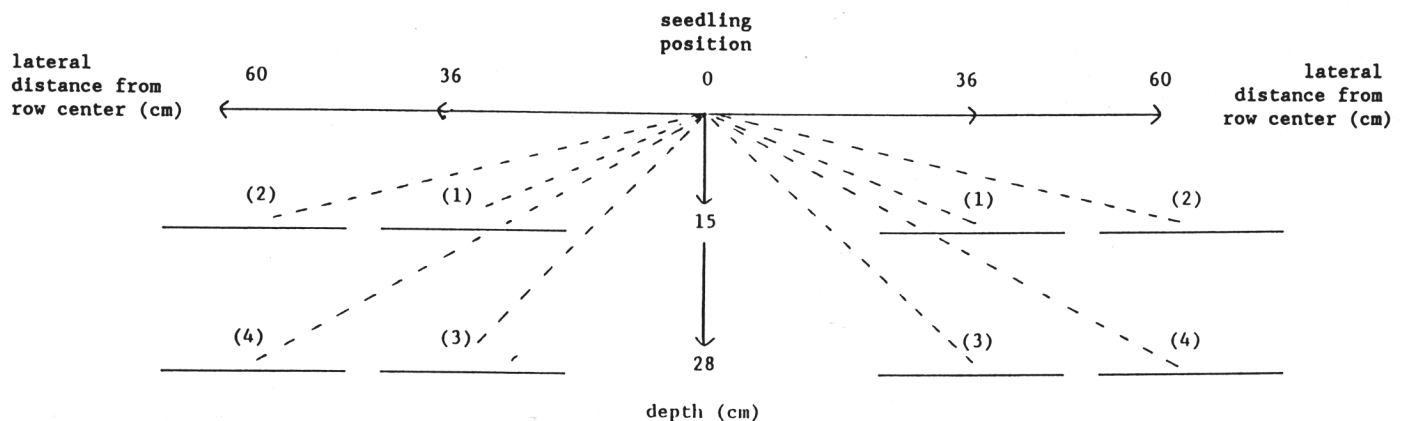
Co. Kalamazoo, Mich.) was used for all preliminary greenhouse and field herbicide banding experiments and as the control in herbicide screening experiments with the PIs.

Greenhouse studies. Plastic pots (1 or 1.8 liter) were filled with Spinks sandy loam soil (mesic Psammentic Hapludalfs) to the desired height for placement of the herbicide band (7.5, 15, or 23 cm from the top). For the genotype screen the herbicide band was 23 cm from the top. The partially filled pots were placed on a conveyor belt and passed under a sprayer calibrated to deliver herbicide (Simazine, Ciba-Geigy Corp., Greensboro, N.C.) at a rate equivalent to 0.25, 0.5, or 1 kg a.i. per ha. Simazine was selected for this study because it provided distinct symptom expression in cucumber leaves at low concentrations and was resistant to leaching from the soil. While being sprayed, the interior surfaces of the pots were lined with cut plastic pots to prevent the herbicide from coating the walls of the pots. After spraying, the liner was removed, and the pots were filled to the top with soil. Three cucumber seeds were planted in each pot at a depth of 1 to 2 cm; shortly after emergence the seedlings were thinned to two plants per pot. Plant height was measured daily. Symptoms of herbicide damage were rated daily on a 0 to 4 scale: 0 = shoots healthy and dark green; 1 = pale cotyledons or leaves; 2 = white or grey ring around edge of cotyledons or leaves and/or white patches; 3 = necrotic lesions on cotyledons or leaves; 4 = plant dead. The only plants that died were those that reached the herbicide zone early enough to affect the cotyledons. Experiments were performed using a completely random design with six to 10 plants (three to five pots) per treatment. Each experiment was performed two or three times. Because some of the accessions had poor germination, only those with at least six plants were included in the ANOVA. The genotypes varied in rate of emergence, so date of symptom expression was based on days after emergence (DAE). Although this method could introduce a possible confounding factor if there were differences in the extent of root growth before emer-

gence of the cotyledons from the soil, we felt it was appropriate to account for variation in rate of germination. For samples where the roots were measured directly, the soil was washed gently from the roots using a kitchen hose sprayer. Root lengths were measured immediately; the roots were air dried at room temperature to obtain dry weight values.

Field band placement trial. To gain information about the location of the roots in the field and to determine appropriate band placements, a field trial was performed in the summer of 1989 using four band positions with respect to the plants: 1) 15 cm deep, 36 cm lateral distance from the row center; 2) 15 cm deep, 60 cm lateral distance; 3) 28 cm deep, 36 cm lateral distance; or 4) 28 cm deep, 60 cm lateral distance (Fig. 1). In each case a pair of 30-cm-wide bands was placed equidistant from the seed row before planting; there was no herbicide directly beneath the seed row. The herbicide was banded using a pair of subsurface, V-shaped sprayer blades attached to a tractor by a three-point fixed mount. The angle of the blades and weight of the sprayer provided the necessary force to bury the blades, the mount provided the mechanism to vary and maintain the lateral distance and depth. The nine treatments (four band positions, each at two herbicide rates, 0.5 or 1 kg a.i. per ha, and a control without herbicide) were arranged in a randomized complete-block design with four replicates. Treatment rows alternated with guard rows (no herbicide band); the rows were spaced 1.2 m apart. The soil type was Marlette fine sandy loam (mesic Glossoboric Hapludalfs); the cucumber cultivar was 'Flurry'. Each plot was 6 m long; plants were thinned to 10 cm within row spacing. About 25 mm of water was applied per week via rain and/or supplemental irrigation. The central 3 m of each plot was monitored daily for symptom appearance. Data were recorded as percent plants showing herbicide symptoms in each plot.

Field genotype trial. The genotype trial was performed in the summer of 1990 with 73 genotypes. Herbicide was banded uni-



Mean distance to center of band

treatment	distance (cm)
1	39
2	62
3	46
4	66

Fig. 1. Simazine herbicide band placement in the field. Four band positions were used: 1) 15 cm deep, 36 cm lateral distance from the center of the row; 2) 15 cm deep, 60 cm lateral distance; 3) 28 cm deep, 36 cm lateral distance; and 4) 28 cm deep, 60 cm lateral distance. The position marked 0 represents the location of the seed row.

formly at a lateral distance of 36 cm and a depth of 15 cm (position 1) at a rate of 1 kg·ha⁻¹ as described above. The genotypes were arranged in a randomized complete-block design with three replicates. Each plot was 3 m long with 1.5-m borders of 'Flurry' on either end. Plants were thinned to 10-cm intervals. Rows were 1.5 m apart, and experimental genotype rows alternated with rows of 'Flurry'. Irrigation and data collection were as above. Date of symptom expression was based on days after emergence. Although the mean time to 50% emergence ranged from 7.0 to 10.3 days after planting, the time to emerge was not correlated with days after emergence to symptom development ($r = 0.008$).

Results and Discussion

Establishing the herbicide banding system. The effectiveness of the herbicide banding system for detecting the position of roots in the soil was tested in the greenhouse and field using bands placed at various depths or distances from the seeds. Simazine caused distinctive symptoms on 'Flurry' seedlings at all concentrations tested in the greenhouse; symptoms did not vary with concentration (data not shown). The time to symptom expression in the greenhouse was longer for plants in pots with deeper bands; there was a 2 to 3 day delay in symptom development with a 7.5-cm increase in band depth (Fig. 2). These results indicated that the system was providing information on root position.

To further characterize the response to herbicide, above- and below-ground growth of control plants was compared to plants grown in pots with an herbicide band at 7.5 cm. The roots were long enough to reach the band within 3 days (Fig. 3A). Although the roots of plants in pots with herbicide did not increase in weight after reaching the herbicide band (Fig. 3B), they continued to increase in length for another week. At that time the length of the herbicide treated roots began to diverge from the control roots (Fig. 3A), the shoots began to show symptoms (stage 1) (Fig. 3C), and there was a difference in aboveground mass (Fig. 3D). These data suggest that root surface area for potential herbicide uptake increases for some time after first reaching the band, and that there must be sufficient uptake before the appearance of aboveground symptoms. Interestingly, for shoots as for roots, a difference in mass was evident before

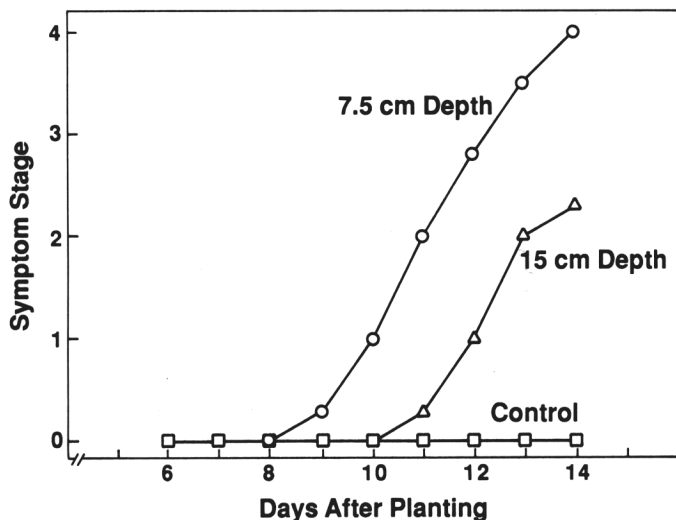


Fig. 2. Mean days after planting of 'Flurry' cucumbers to symptom development as affected by herbicide band depth in pots in the greenhouse. Each point is the mean of six to eight plants.

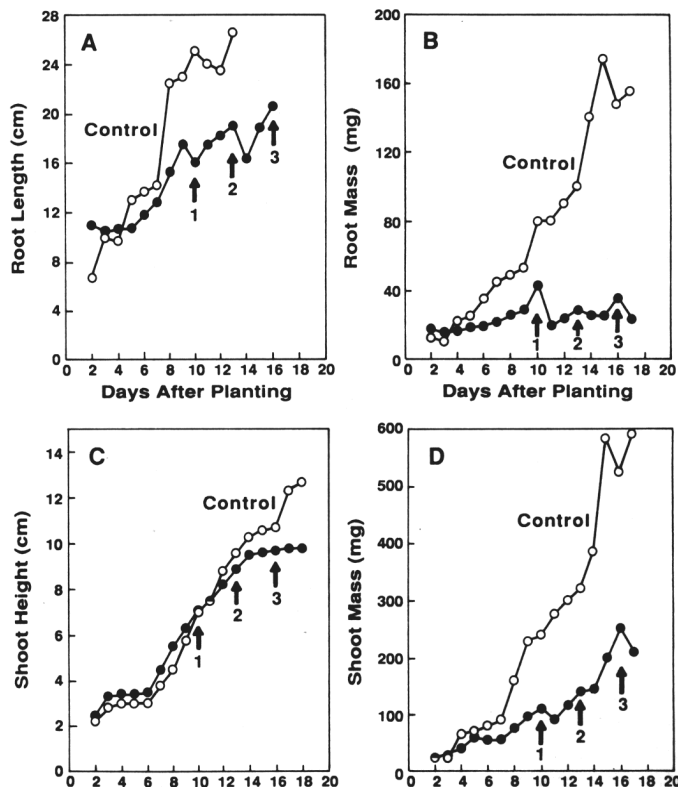


Fig. 3. Growth responses (A) root length, (B) root mass, (C) shoot height, and (D) shoot mass of greenhouse-grown control and simazine-treated cucumber plants. Simazine was banded at 7.5 cm. Arrows marked 1, 2, or 3 indicate the time at which stage 1, 2, or 3 symptoms were observed in the simazine-treated plants. Each point is the mean of six to eight replicates.

a difference in height (Fig. 3B vs. 3A, and 3D vs. 3C). Although control and treated shoots showed clear differences in mass by day 10 (stage 1) (Fig. 3D), it was almost another week until differences in height were apparent (Fig. 3C).

Translocation rate is also a likely factor influencing time to symptom development. Although trends were clearly consistent from experiment to experiment, the time to symptom expression varied among experiments (e.g., the number of days that were required for 'Flurry' plants in pots with a 7.5 cm deep herbicide band to reach stage 2 symptom expression, ranged from 9 to 15 days, depending on the experiment). The response time was faster during sunny vs. cloudy periods. This difference in response time would be predicted for a compound that must be translocated to the shoots, presumably via the transpiration stream. Similarly, hotter days may hasten symptom development; the higher temperatures also may enhance root growth rate.

The field experiment testing various banding depths and lateral distances from row center indicated root location within the soil (Fig. 4). Symptom expression for 0.5 kg simazine was most pronounced in the closer, shallower (36 cm lateral distance, 15 cm deep), treatment. There was very little symptom expression at the 60 cm lateral distance, suggesting that the majority of the roots were <45 cm laterally from the seed row (accounting for the 30-cm-band width). Randall and Locascio (1988) also observed limited lateral extension of cucumber roots. Using ground beds in the greenhouse, they observed that the majority of the roots were within a 15 cm distance from the shoot; by 27 cm the root density had decreased 2- to 3-fold. At 1 kg·ha⁻¹ simazine, symptoms occurred at both depths in the field, but with

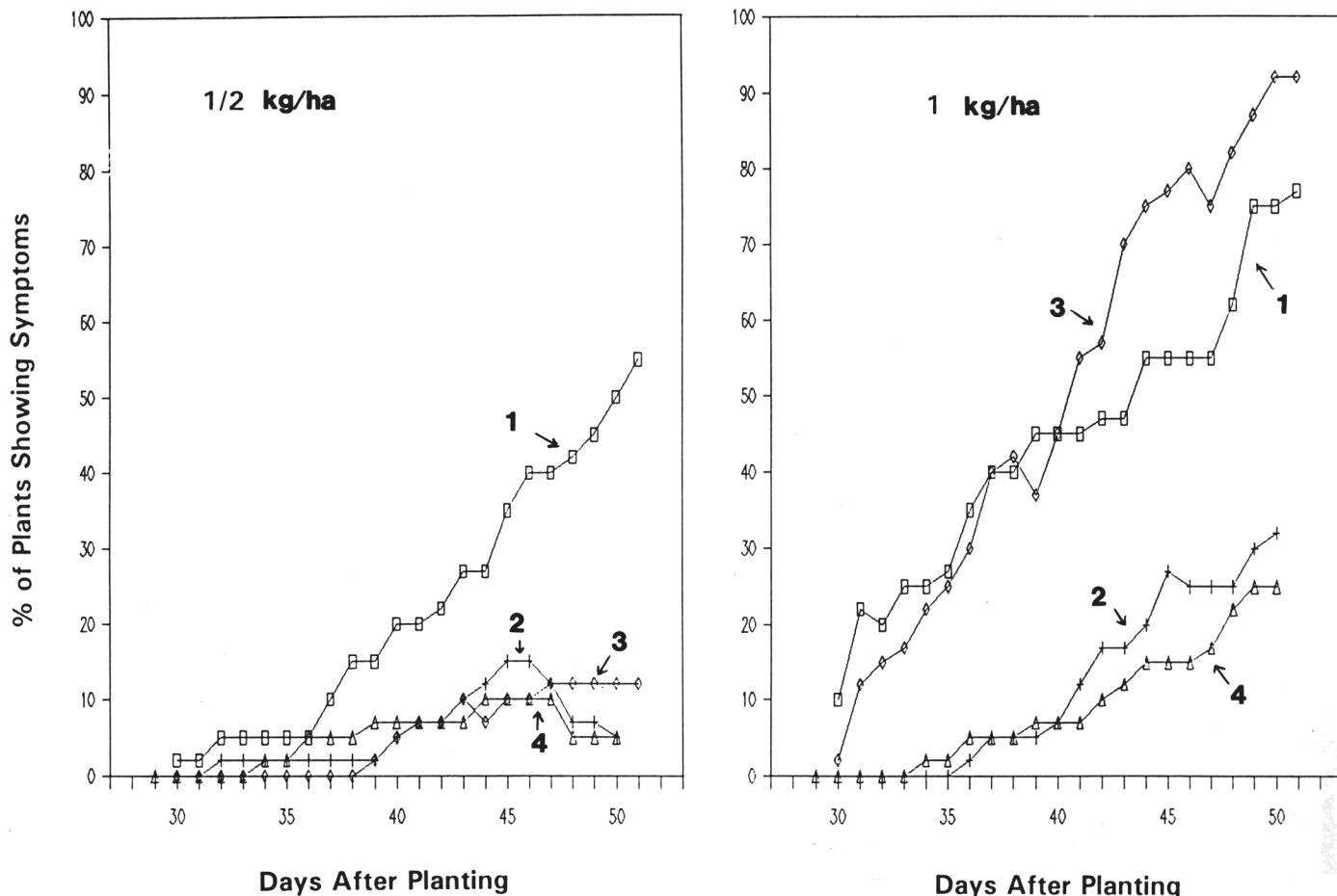


Fig. 4. Percentage of plants showing stage 2 herbicide (simazine) injury symptoms in the field in response to various herbicide placement positions and rates. Placements of 30 cm wide band: 1) 15 cm deep, 36 cm lateral distance from center of row; 2) 15 cm deep, 60 cm lateral distance; 3) 28 cm deep, 36 cm lateral distance; 4) 28 cm deep, 60 cm lateral distance. Each point is the mean of four replicates.

0.5 kg·ha⁻¹, symptoms occurred only at the shallower, 15 cm depth. This observation suggests that there were not enough roots at the location of the deeper band to take up sufficient herbicide to cause symptoms. The apparent concentration of roots within the upper 15 cm is consistent with the observations of Medina-Mora (1988) and Randall and Locascio (1988), who also reported that the majority of the roots were located in the upper 15 cm.

An additional concern was to be able to use this system non-destructively. If it is used for screening genotypes, and the most desirable genotypes are those that show symptoms first, then it may be necessary to save those individuals for further crossing and seed production. Greenhouse and field studies showed that as long as the herbicide is deep enough (at least 15 cm) to not affect the cotyledons, the plant survives in most cases to produce flowers and fruits.

Screening of diverse cucumber genotypes. In the evaluation of the diverse cucumber plant introductions in the greenhouse, time to stage 2 symptom appearance was normally distributed among the accessions (Fig. 5A). 'Flurry', the control genotype, fell in the middle of the distribution (16 DAE). ANOVA indicated that there were highly significant genotypic differences ($P = 0.01$).

The observed genotypic differences might be due to differences in sensitivity to herbicide rather than differences in root growth rate. For plants sensitive to herbicide injury, symptoms may appear sooner even though the roots have not grown as

much. To test this possibility, genotypes having either the fastest or slowest response to the herbicide (based on two greenhouse screens) were selected for growth studies in the greenhouse to examine root size directly. Plants exhibiting stage 2 symptoms were removed from their pots to measure root length and dry mass. There were highly significant differences in time (DAE) to symptom expression among the genotypes (Table 1). At the time that stage 2 symptoms were observed, however, there were not significant genotypic differences for root length or mass. Further, when orthogonal comparisons were made between the fast and slow responding groups, there was a significant difference for time to symptom expression, but not for root length or mass. This result would be expected if all roots were equivalently large at the time the symptoms appear, i.e., the difference was not due to sensitivity. Although there were significant differences in height among individual genotypes, as a group, the fast and slow responders did not differ from each other for plant height.

Field testing of the diverse genotypes showed there to be a normal distribution among PIs for time to 50% of the plants in a plot exhibiting stage 2 symptoms (Fig. 5B); the genotypic differences were highly significant (AOV; $P = 0.01$). 'Flurry' again fell in the middle of the distribution (39 DAE). There was not, however, a significant correlation ($r = 0.21$) between the greenhouse and field data. Thus, to make useful genotypic selections, screening must be done in the field. Because of the inherent variability in field conditions, this would be done best

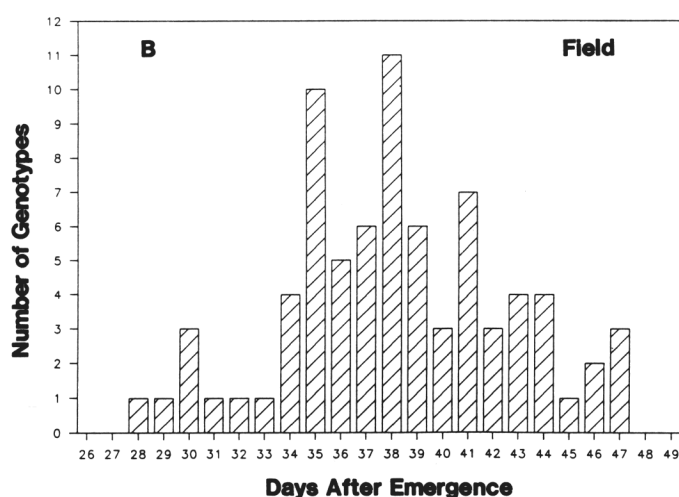
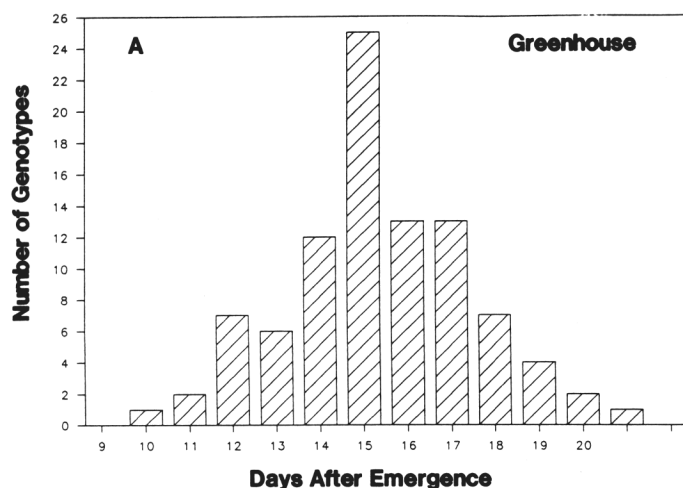


Fig. 5. DAE until herbicide (simazine) symptoms for various cucumber genotypes in the greenhouse (A) and field (B). (A) Values are DAE until stage 2 symptoms; each genotype value is the mean of five to eight plants. (B) Values are DAE until 50% of the plants showed stage 2 herbicide injury symptoms; each genotype value is the mean of 3 replicate plots.

Table 1. Analysis of variance of time to symptom appearance and root and shoot growth of cucumber genotypes selected for either fast or slow response to the herbicide.

Source	df	Mean square			
		DAE ^z	Root length ^y	Root mass ^y	Plant ht ^y
Genotypes	17	23.4**	38.6	0.055	22.8**
Fast vs. slow*	1	63.3**	17.4	<0.001	0.66
Error	150	8.35	40.6	0.033	1.98

^zDays after emergence until stage 2 symptom expression.

^yRoot length, root mass, and plant height at stage 2 symptom expression.

*Eight genotypes per group.

**Significant by F-test at $P = 0.01$.

on a family basis in a replicated design (e.g., F_3 or later) rather than on an individual basis (e.g., F_2).

The genotypes with the fastest growing root systems are of interest for further studies. The 10 genotypes with the fastest response rate in the field (time to 50% stage 2 symptom expres-

Table 2. The 10 cucumber genotypes responding fastest to simazine herbicide in the field,

Genotype	Country of origin	DAE ^z	
		Field	Greenhouse
PI 109063	Turkey	30 ± 3.4	11.7 ± 1.1 ^y
PI 175695	Turkey	29 ± 2.2	9.0 ± 0.9 ^y
PI 209069	United States (Iowa)	30 ± 2.9	16.9 ± 1.7
PI 222099	Afghanistan	30 ± 2.9	12.9 ± 0.9 ^y
PI 263048	Soviet Union	31 ± 1.7	14.7 ± 2.3
PI 267747	United States (Oklahoma)	34 ± 3.7	16.7 ± 0.9
PI 319216	Egypt	32 ± 1.5	11.5 ± 0.8 ^y
PI 376063	Israel	34 ± 2.9	11.8 ± 1.1 ^y
PI 385968	Kenya	34 ± 2.9	12.0 ± 2.1 ^y
PI 422168	Netherlands	28 ± 2.9	14.8 ± 0.5
'Flurry'	United States	39 ± 1.0	16.0 ± 1.6
Total population		$\bar{x} = 38$	$\bar{x} = 14.9$
		LSD 0.05 = 7.6 ^x	LSD 0.05 = 3.9 ^x

^zDAE to 50% of plants exhibiting stage 2 symptom expression in the field or in the greenhouse ±SE.

^yGenotypes that were among the fastest 10% in the greenhouse trial.

^xCalculated from analysis of variance.

sion was ≤ 34 days) are listed in Table 2. Seven of the 10 responded significantly faster than 'Flurry' in the field, and six were among the fastest 10% in the greenhouse. Of these, a disproportionate number originated in the Middle East (50% vs. 29% of the collection as a whole). In contrast, the slowest 10% of the responders in the field included several accessions from far eastern countries, i.e., Thailand, Hong Kong, and the Philippines (43% of the slowest were from the Far East vs. 20% of the collection as a whole). Perhaps the different geographical biases of the two groups reflect climatic differences between the two regions. Since the Middle East is noted for generally hot, dry climates, superior root growth may be among the adaptations to drought stress.

In conclusion, we have developed a simple, inexpensive, aboveground screen to monitor cucumber root growth in the greenhouse and field. Using this screen we have found that there are significant genotypic differences for time to herbicide injury symptom expression among a diverse collection of cucumber genotypes. We have identified a small number of genotypes showing either a fast or slow response to the herbicide. These should be useful for further investigations of root growth characteristics and water stress responses. In addition, by providing a simple means to assess root behavior in response to the application of a given treatment, this approach should also be of use for cultural or physiological studies in either the greenhouse or field.

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