THE PHYSIOLOGICAL BASIS AND INHERITANCE OF TOLERANCE TO CHLORAMBEN METHYL ESTER IN CUCUMBER

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ABSTRACT

THE PHYSIOLOGICAL BASIS AND INHERITANCE OF TOLERANCE TO CHLORAMBEN METHYL ESTER IN CUCUMBER

By

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The physiological basis and inheritance of differential tolerance in cucumber (Cucumis sativus L.) to methyl 3-amino-2,5-dichlorobenzoic acid (chloramben methyl ester) was investigated. Two tolerant, MSU 3207 and MSU 0612, and two susceptible, MSU 3159 and MSU 0866, lines were selected for further study following screening of more than 200 inbred lines, plant introductions and cultivars.

Intraspecific variability exhibited by these lines was related to higher concentrations of 3-amino-2,5-dichlorobenzoic acid (chloramben) in the roots of the susceptible lines, resulting from metabolic differences in both susceptible lines and rapid absorption of the herbicide by the roots of MSU 3159. Tolerance of MSU 3207 resulted primarily from low uptake and reduced translocation of the herbicide, while tolerance of MSU 0612 appeared

to be related to a peculiar ^{14}C-label distribution pattern in the leaves. Thin-layer chromatographic analysis of methanol-soluble extracts three days after ^{14}C-chloramben methyl ester treatment separated six ^{14}C-metabolites in the roots and five in the shoots. After a 4-hr treatment, ^{14}C-chloramben methyl ester was absorbed and translocated more rapidly than ^{14}C-chloramben by all four lines, although the extent of absorption among the lines was similar. Tolerance or susceptibility did not always correlate with the total concentration of radioactivity in methanol-soluble shoot and root extracts.

Progeny from crosses among the four lines were evaluated for tolerance to chloramben methyl ester in the F_1, F_2, BC_1 and BC_2 generations. Plant height, dry weight and visual appearance were evaluated following herbicide treatment. Partial dominance of genes conditioning tolerance was shown. The minimum number of effective factor pairs was variable depending on the trait evaluated; however, quantitative inheritance of tolerance was indicated. Transgressive segregation was observed in the F_2 populations of the crosses tolerant x tolerant. Heritability estimates from crosses involving tolerant MSU 0612 were consistently higher than those involving tolerant MSU 3207, also indicating that the tolerance of these two lines may be conditioned by different genes. Estimates of heritability indicated that considerable progress can be

made in selection for tolerance when the criteria used are plant dry weight, apical inhibition, or epinasty.

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By

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Guidance Committee:

Sections I and II are segments of related thesis research information condensed into formats suited and intended for publication in Weed Science (Section I) and the Journal of the American Society for Horticultural Science (Section II).

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SECTION I

THE BASIS FOR VARIABILITY IN CUCUMBER TOLERANCE TO CHLORAMBEN METHYL ESTER

ABSTRACT

The physiological basis for tolerance and susceptibility of four lines selected from inbred lines, plant introductions and cultivars of cucumber (Cucumis sativus L.) to methyl 3-amino-2,5-dichlorobenzoic acid (chloramben methyl ester) was investigated. MSU 3207 and MSU 0612 were tolerant (T), whereas MSU 3159 and MSU 0866 were susceptible (S). Intraspecific variability exhibited by these lines was related to higher concentrations of 3-amino-2,5-dichlorobenzoic acid (chloramben) in the roots of the susceptible lines, resulting from metabolic differences in both lines and rapid absorption of the herbicide by the roots of S-MSU 3159. Tolerance of T-MSU 3207 resulted primarily from low uptake and reduced translocation of the herbicide, while tolerance of T-MSU 0612 was related to a peculiar ^{14}C-label distribution pattern in the leaves. Thin-layer chromatographic analysis of methanol-soluble extracts three days after ^{14}C-chloramben methyl ester treatment separated six ^{14}C-metabolites in the roots and five in the shoots. After a 4-hr treatment, ^{14}C-chloramben methyl ester was absorbed and translocated more rapidly than ^{14}C-chloramben by all four lines, although the extent of absorption among the lines was similar. Tolerance

or susceptibility did not always correlate with the total concentration of radioactivity in methanol-soluble shoot and root extracts.

INTRODUCTION

Interspecific variability in tolerance of various crop and weed species to chloramben and its analogs has been associated with several physiological mechanisms. Chloramben is readily absorbed by roots of tolerant and susceptible species (2, 3, 7, 20, 21, 22, 23, 24). Absorption is concentration dependent (20, 21) and does not appear to be related to species sensitivity (3, 21, 22). Conversely, differential translocation is an important factor in determining species selectivity (3, 7, 9, 21, 23) and appears to be closely associated with metabolism particularly in the roots (3, 21, 24).

The metabolism of chloramben methyl ester is considered to proceed by hydrolysis to chloramben in the soil (6). The fate of chloramben in most plants is the formation of complexes or conjugates with natural plant products (5, 8, 24). These conjugates, upon either base or acid hydrolysis release free chloramben (5, 6, 8, 21, 25). Two conjugates, N-(3-carboxy-2,5-dichlorophenyl)-glucosylamine (N-glucosyl chloramben) and an unidentified chloramben conjugate (chloramben-X) have been found (5, 6, 10, 22, 25) and are considered to be detoxication products (5, 19, 20, 24).

In several species, tolerance to chloramben has been related to rate of chloramben metabolism (20, 21, 24) and composition or distribution of metabolites (5, 21, 22); however, no such relationship was observed in cucumber and squash (3). The metabolic basis for the selective action of chloramben remains unclear.

The occurrence of intraspecific variability in the response of weed and crop plants to specific herbicides has recently been reviewed (11). Weed control in cucumber with preemergence application of chloramben methyl ester has resulted in variable tolerance dependent on environmental conditions[1] and cultivars (14).

The purpose of this investigation was to determine the physiological basis for intraspecific variability in cucumber tolerance to chloramben methyl ester by studying absorption, translocation, distribution and metabolism in two tolerant and two susceptible cucumber lines.

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[1]Amchem Products Inc. 1971. Chloramben methyl ester (Vegiben 2-E) for weed control in snap beans, cucumbers and cantaloupes. Technical Service Data Sheet No. 3/71. 5p.

MATERIALS AND METHODS

Herbicide. Carboxy-labeled ^{14}C-chloramben methyl ester was prepared by reacting carboxy-labeled ^{14}C-chloramben (specific activity 1.19 mc/mmole) dissolved in a small volume of methanol with diazomethane diethyl ether solution (1, 4). This reaction yielded 100 percent ^{14}C-chloramben methyl ester as shown by thin-layer chromatography. Non-labeled herbicide was added to the radioactive form to obtain the desired concentration of 1.8×10^{-5} M in the nutrient treatment solution.

Plant material and treatment. Preliminary experiments under both greenhouse and field conditions revealed three to seven-fold differences among approximately 200 cucumber lines tested for their response to chloramben methyl ester. From these lines, two susceptible, S-MSU 3159 and S-MSU 0866, and two relatively tolerant lines, T-MSU 0612 and T-MSU 3207, were selected for this study.

Seeds of the four inbred lines were germinated in moist vermiculite. After ten days, when the first true leaf had expanded to about 0.5 cm, seedlings were transplanted to an aerated Hoagland's no. 1 nutrient solution (12). Three days after transplant, reciprocal hypocotyl

approach grafts were made in all combinations utilizing the techniques of de Stigter (18). Sealtex Latex Bandage was used to secure graft unions. Seven days later the unwanted rootstock and scion were removed. Self grafts and nongrafted plants of each line were also included in the study. Five days after excision, when two to three true leaves were evident, plants were transferred to calibrated plastic cups containing 80 ml of nutrient solution including 1.8×10^{-5} M ^{14}C-chloramben methyl ester. The cups were protected from light with aluminum foil. Plants were grown in the greenhouse under a 27 C day and 21 C night regime, with supplemental incandescent light provided during the 14-hr day. Sufficient Hoagland's solution was added daily to maintain the 80 ml volume. At the end of the 3-day treatment period, roots were rinsed three times with distilled water and blotted dry, then the plants were immediately placed on dry ice and freeze dried.

In a separate 4-hr absorption and translocation study with ^{14}C-chloramben methyl ester and ^{14}C-chloramben, 14-day-old plants were transferred at the first true leaf stage to vials containing 10 ml of treatment solution (0.5 μc radioactivity per 100 ml Hoagland's solution) and placed in a growth chamber at 30 C with a light intensity of 21,500 lux. At the end of the treatment period the seedlings were immediately placed on dry ice, freeze-dried,

mounted, radioautographed, removed from the mount and weighed. Roots and leaf blade of each plant were combusted using the Schöniger method of Wang and Willis (26), and radioactivity was determined using the scintillation solution of Lui et al. (13). Values reported for each study are the means from two separate experiments consisting of at least two replications.

Determination of absorption, translocation and metabolism. Following freeze-drying, plants were mounted and radioautographed according to the methods of Crafts and Yamaguchi (9). The mounted plants were exposed to X-ray film for two weeks. After radioautography, the second true leaf including its petiole, and the root system including the hypocotyl, of each mounted plant were removed from the blotter paper and weighed. Methanol-soluble metabolites were extracted from the leaf and root tissue according to the procedures described by Swanson et al. (25). The tissue was homogenized in warm, absolute methanol, the homogenate filtered through Whatman no. 2 filter paper and concentrated in vacuo at less than 50 C. The concentrated extracts were adsorbed on 12 by 150 mm Florisil columns, eluted with methanol and reduced to dryness in vacuo.

The extract was made to 100 µl with methanol. Fifty µl was chromatographed on 20-cm-sq glass thin-layer

plates coated with 0.25 mm of silica gel HF or GF. The chromatographs were developed ascendingly for 15 cm using a solvent system consisting of n-butanol:ethanol:ammonium hydroxide (2:1:1; v/v/v). Radioautographs of the thin-layer plates were used for detecting the precise location of the ^{14}C-components. R_f values of the five to seven ^{14}C-labeled spots obtained were compared with those of Stoller and Wax (22). Chloramben and chloramben methyl ester standards were localized with UV light, Ehrlichs reagent, and by radioautographic technique. The ^{14}C-labeled spots were scraped from the plates and radio-assayed by liquid scintillation spectrometry. The scintillation solution contained 5.0 g/L PPO plus 0.3 g/L POPOP in toluene.

The ^{14}C-metabolites were eluted from thin-layer plates and hydrolized with 1.0 N HCl for 1 hr at 70 C. The hydrolysates were then re-chromatographed. Thin-layer chromatographic plates were also sprayed with 1.0 N HCl and then heated to 70 C for hydrolysis of metabolites. Reducing sugars were detected with silver nitrate spray reagent.

Membrane study. The fatty acid composition of membranes from each of the four lines was investigated to determine whether it contributed to variation among lines tested. Membranes were isolated by differential

centrifugation using the general procedure of Raison and Lyons (17). Lipids were extracted from the membranes with chloroform and methanol (2:1, v/v). Fatty acids were extracted from the lipid fraction, methylated, and separated by gas-liquid chromatography (16).

RESULTS

Absorption and translocation. All lines readily absorbed ^{14}C-chloramben and ^{14}C-chloramben methyl ester during the 4-hr treatment period, the latter being absorbed to a greater extent (Table 1). A comparison between T-MSU 3207 and S-MSU 3159 showed a significantly lower concentration of ^{14}C activity in the root tissue of T-MSU 3207 following ^{14}C-chloramben methyl ester treatment. The other tolerant line, T-MSU 0612, absorbed as much ^{14}C-chloramben methyl ester after 4 hr as did both susceptible lines (Table 1). After 3 days, the concentration of ^{14}C-chloramben methyl ester was greater in this line than in S-MSU 0866, but less than in S-MSU 3159 (Table 2).

Concentration of ^{14}C in the leaves of the four lines did not differ significantly following 4-hr treatment with either ^{14}C-chloramben or ^{14}C-chloramben methyl ester (Table 1). Comparison of T-MSU 3207 and S-MSU 3159 following the 3-day treatment period revealed the translocation of a 3-fold higher concentration of ^{14}C to the leaf tissue of the susceptible line (Table 2). The concentration of ^{14}C in the roots of S-MSU 3159 was twice

TABLE 1.--Accumulation of ^{14}C from ^{14}C-chloramben and ^{14}C-chloramben methyl ester (CME) in the roots and leaf blade of 16-day old cucumber seedlings treated for 4 hours.

Cucumber line no.	^{14}C in roots (dpm/mg dry wt)		^{14}C in leaf (dpm/mg dry wt)	
	Chloramben	CME	Chloramben	CME
T-MSU 3207	393 a[a]	523 a	19 a	48 a
S-MSU 3159	452 a	778 b	30 a	73 a
S-MSU 0866	407 a	677 ab	28 a	79 a
T-MSU 0612	446 a	712 b	25 a	56 a

[a]Means within columns with common letters did not differ significantly at the 5% level by Duncan's Multiple Range Test.

TABLE 2.--Accumulation of ^{14}C from ^{14}C-chloramben methyl ester in the roots and second true leaves of four lines of 4-week-old cucumber plants treated for 3 days.

Cucumber line no.	^{14}C in roots	^{14}C in leaf
	(dpm/mg dry wt)	(dpm/mg dry wt)
T-MSU 3207	20,518 a[a]	542 a
S-MSU 3159	40,626 c	1,712 b
S-MSU 0866	20,360 a	714 a
T-MSU 0612	30,011 b	1,700 b

[a]Means within columns with common letters did not differ significantly at the 5% level by Duncan's Multiple Range Test.

as great as in T-MSU 3207. However, S-MSU 0866 absorbed and translocated no more ^{14}C-label to the leaf than did T-MSU 3207 (Table 2). T-MSU 0612, the other tolerant line, was intermediate between the two susceptible lines in absorption of ^{14}C, but it was equal to S-MSU 3159 in translocation of ^{14}C to the leaves (Table 2).

The role of roots and shoots in imparting tolerance or susceptibility to these four lines is shown in Table 3. Plants with T-MSU 3207 scions had significantly less ^{14}C in the leaf tissue than plants with scions of the other three lines, implying reduced translocation of ^{14}C to the leaves. Plants with S-MSU 3159 rootstocks accumulated more ^{14}C in the leaf tissue than plants with other rootstocks.

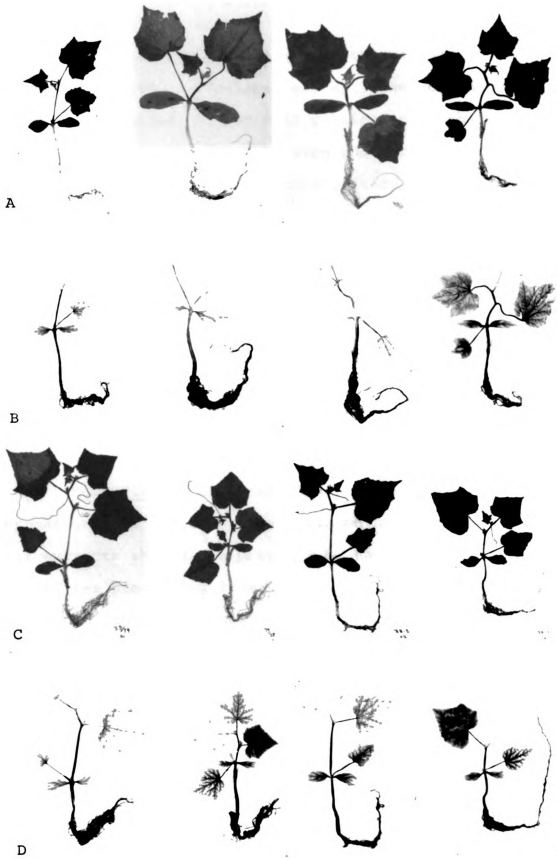
Radioautographs of plants treated with ^{14}C-chloramben methyl ester showed that movement of radioactivity was principally into the older leaves (Figure 1). The intensity of ^{14}C-labeling in S-MSU 3159 was greater than in T-MSU 3207. The grafted plant T-MSU 3207/T-MSU 3207 did not differ appreciably from the ungrafted T-MSU 3207 plants. However, when T-MSU 3207 was grafted onto S-MSU 3159 rootstocks more ^{14}C was translocated to the T-MSU 3207 shoot than when it was on its own rootstock (Figure 1-A and B and Table 3). Though tolerant, T-MSU 0612 absorbed and translocated as much ^{14}C as S-MSU 3159 (Figure 1-C and D and Table 3). T-MSU 0612 exhibited a unique

TABLE 3.--The influence of rootstock and scion as shown by reciprocal grafts, on the accumulation of ^{14}C in the second true leaf of four lines of 5-week-old cucumber plants treated for 3 days with ^{14}C-chloramben methyl ester.

Rootstock	^{14}C in leaf of scion (dpm/mg dry wt)				
	Scion				
	T-MSU 3207	S-MSU 3159	S-MSU 0866	T-MSU 0612	Mean[a]
T-MSU 3207	558	1812	1483	1518	1343 a
S-MSU 3159	1136	1824	2292	2060	1828 b
S-MSU 0866	541	1308	850	1830	1133 a
T-MSU 0612	1264	1034	1116	1732	1287 a
Mean[a]	875 a	1495 b	1436 b	1785 b	

[a]Means followed by common letters did not differ significantly at the 5% level by Duncan's Multiple Range Test.

Figure 1.--Distribution of radioactivity in grafted and ungrafted cucumber lines treated for 3 days with ^{14}C-chloramben methyl ester. Plants in Figure 1-A and B from left to right are S-MSU 3159, T-MSU 3207, T-MSU 3207/T-MSU 3207, and T-MSU 3207/S-MSU 3159. Figure 1-A: Plants; Figure 1-B: Radioautographs. Plants in Figure 1-C and D from left to right are S-MSU 3159/T-MSU 0612, T-MSU 0612/S-MSU 3159, S-MSU 3159, and T-MSU 0612. Figure 1-C: Plants; Figure 1-D: Radioautographs.


A
B
C
D

distribution of ^{14}C-label in the leaves, with an accumulation of ^{14}C-label in and adjacent to the vascular tissue. This distribution of ^{14}C-label was not observed in the other lines studied. T-MSU 0612 retained this characteristic ^{14}C distribution pattern even when grafted onto other rootstocks. Thus, control of this pattern was due to factors in the leaf rather than in the roots of T-MSU 0612. Radioautographs of S-MSU 3159/T-MSU 0612 revealed a ^{14}C-labeling intensity and distribution similar to the ungrafted S-MSU 3159 plant (Figure 1-C and D). The rootstock of T-MSU 0612 did not alter the distribution of ^{14}C-label in susceptible S-MSU 3159, as there was no accumulation of ^{14}C-label along the veins as was seen in T-MSU 0612.

Metabolism. Examination of radioautographs of chromatographic plates spotted with methanol-soluble root extracts showed the presence of the following metabolites proceeding from the origin: N-glucosyl chloramben, chloramben conjugate, nonhydrolyzable metabolite, chloramben methyl ester conjugate, free chloramben, chloramben-X (traces) and the parent compound, chloramben methyl ester (Table 4). The percentage distribution among ^{14}C-metabolites found in root tissue after ^{14}C-chloramben methyl ester treatment for three days was determined (Figure 2). T-MSU 0612 contained the least

TABLE 4.--R_f values of ^{14}C-metabolites before and after acid hydrolysis for 1 hour with 1.0 N HCl at 70 C.

Metabolites	Original R_f	Hydrolysate R_f
N-glucosyl chloramben	.14 - .17	.41
Chloramben conjugate	.21 - .24	.41
Nonhydrolyzable metabolite	.27 - .29	.22
Chloramben methyl ester conjugate	.38 - .41	.78
Chloramben standard	.41 - .45	.41
Chloramben methyl ester standard	.78 - .84	.78

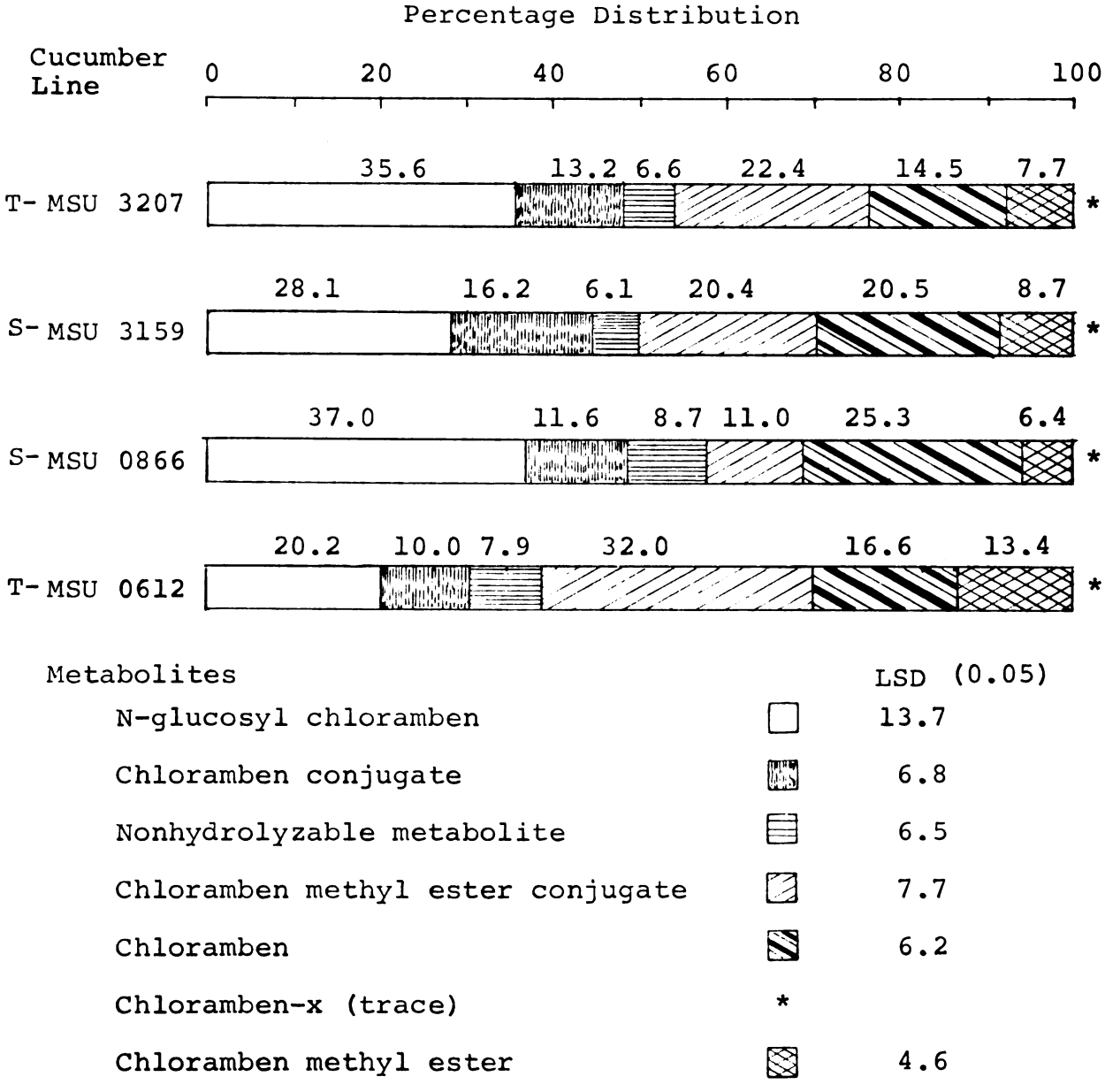


FIGURE 2.--^{14}C-metabolites in the methanol-soluble extracts of root tissue from cucumber plants treated for 3 days with ^{14}C-chloramben methyl ester. Metabolites were separated on silica gel GF TLC plates with a n-butanol:ethanol : ammonium hydroxide (2:1:1,v/v/v) solvent system.

N-glucosyl chloramben, while containing the most chloramben methyl ester conjugate and chloramben methyl ester. S-MSU 3159 and S-MSU 0866 retained more chloramben in the roots than either of the tolerant lines, possibly contributing to their susceptibility. S-MSU 0866 contained significantly less chloramben methyl ester conjugate in the roots than any of the other three lines.

The percentage distribution among ^{14}C-metabolites in methanol-soluble extracts of leaf tissue following three day treatment with ^{14}C-chloramben methyl ester was also determined (Figure 3). Of the six metabolites found in the root tissue, the nonhydrolyzable metabolite as well as chloramben methyl ester, were not detected in this tissue. Again, T-MSU 0612 contained less N-glucosyl chloramben and more chloramben and chloramben methyl ester conjugate than did the other lines. Among the other three lines there was no significant difference in the distribution of metabolites in the leaf tissue.

Following HCl hydrolysis, three of the hydrolysates (N-glucosyl chloramben, chloramben conjugate, chloramben) moved to an R_f corresponding to that of the ^{14}C-chloramben standard; two others (chloramben methyl ester, chloramben methyl ester conjugate) to an R_f corresponding to that of the chloramben methyl ester standard. One ^{14}C-metabolite proved to be nonhydrolyzable under these conditions.

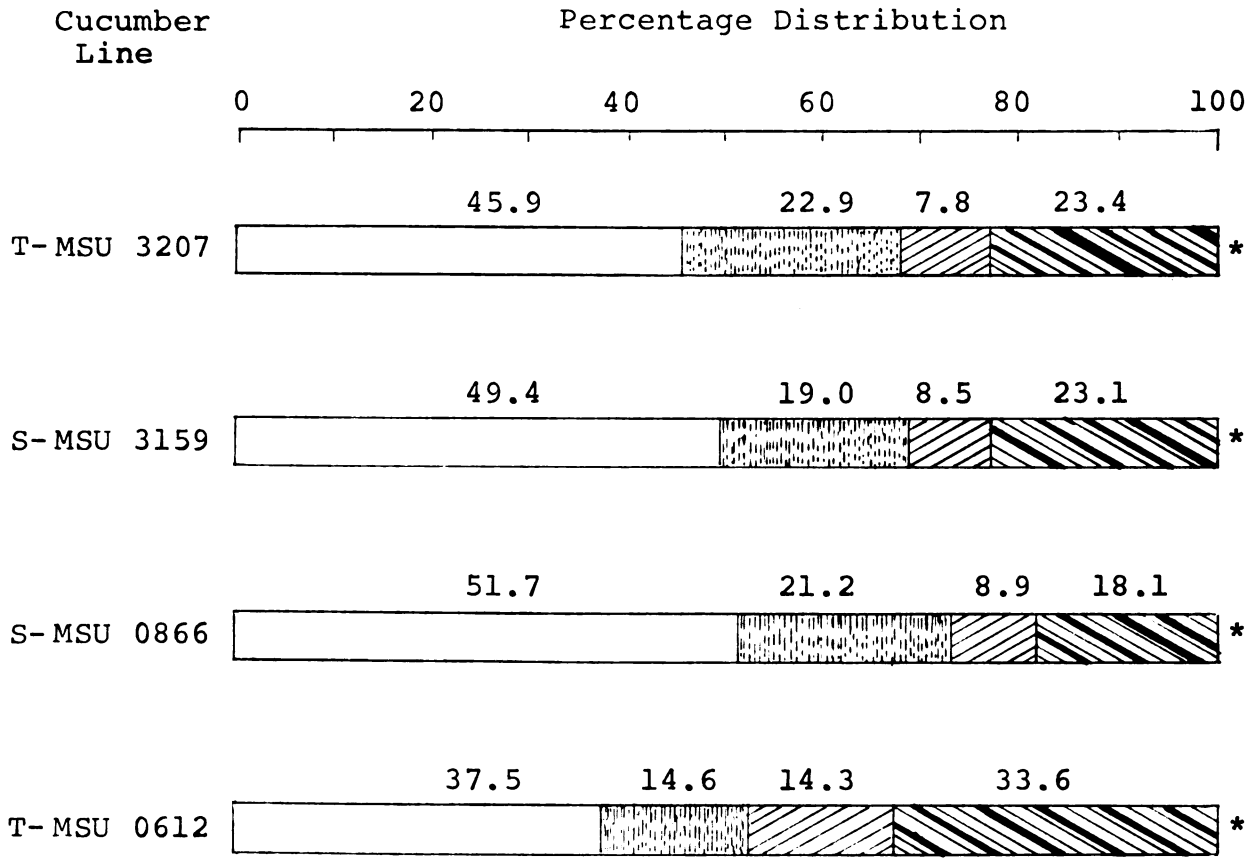


FIGURE 3.--^{14}C-metabolites in the methanol-soluble extracts of leaf tissue from cucumber plants treated for 3 days with ^{14}C-chloramben methyl ester. Metabolites were separated on silica gel GF TLC plates with a n-butanol:ethanol : ammonium hydroxide (2:1:1,v/v/v) solvent system.

Chloramben-X was present in such trace quantities that no attempt was made to hydrolyze it, and its tentative identification was based on similarity to an earlier study (22). Chloramben, chloramben-X, and chloramben methyl ester had R_f values similar to, but slightly lower than, those previously reported for these metabolites (7, 22, 24, 25).

Membrane study. The results indicated that the fatty acid fraction of cucumber membranes was composed primarily of palmitic, stearic, oleic, linoleic, and linolinic acids. There were significant differences in the fatty acid composition between roots and shoots of all lines; however, there were essentially no significant differences among root or leaf tissue of the four lines which could account for the observed differential sensitivity to chloramben methyl ester.

DISCUSSION

Cucumber lines which absorbed the most ^{14}C-chloramben methyl ester also transported the most ^{14}C to the leaves during the 3-day treatment period (Table 2). After treatment for four hours, there was greater absorption and translocation of ^{14}C-chloramben methyl ester than ^{14}C-chloramben by all four lines (Table 1). This indicated that ^{14}C-chloramben methyl ester can be absorbed directly by cucumber without first being hydrolyzed to ^{14}C-chloramben.

The tolerance of T-MSU 3207 and susceptibility of S-MSU 3159 was apparently due to inherent differences in their ability to absorb and translocate ^{14}C-chloramben methyl ester or its metabolites, resulting in higher ^{14}C accumulation in the roots and leaves of S-MSU 3159. The divergent phytotoxic reactions exhibited by S-MSU 3159 and T-MSU 3207 may be partially due to differences in metabolism, as S-MSU 3159 contained more phytotoxic chloramben and less non-phytotoxic N-glucosyl chloramben in the roots than did T-MSU 3207.

The susceptibility of S-MSU 0866 could not be attributed to absorption or translocation; however, the concentration of chloramben found in the roots of this line and S-MSU 3159 may partially explain their susceptibility. It may be that the availability of the moiety which conjugates with chloramben methyl ester or chloramben is the limiting factor in the metabolism of these two toxic compounds.

The tolerance exhibited by T-MSU 0612 cannot be attributed to low root absorption or reduced translocation to the shoot. It is also difficult to attribute the tolerance of this line solely to metabolic differences, although such differences were observed. The unique ^{14}C-labeling pattern in the leaves of T-MSU 0612 may play an important role in determining tolerance in this line. Chloramben or other metabolites may not enter the cytoplasm of this line to the same extent as in the other three lines, although the fatty acid fraction from membranes of this line did not differ in composition from that found in the other lines. The distribution of metabolites observed in T-MSU 0612 may be confounded by the mechanism responsible for the unusual labeling pattern, rendering the metabolic data an inaccurate basis on which to explain tolerance.

Three ^{14}C-chloramben methyl ester metabolites not previously reported were observed in this study and designated as chloramben conjugate (R_f .21 - .24), non-hydrolyzable metabolite (R_f .27 - .29) and chloramben methyl ester conjugate (R_f .38 - .41). The phytotoxicity and importance of these metabolites in determining selectivity was not studied.

The tolerance of these four cucumber lines to chloramben methyl ester did not always correlate with total concentration of radioactivity in the methanol-soluble shoot and root extracts. This agrees with the observations of Stoller and Wax (22) who studied six plant species with a wide range of tolerance to chloramben.

Widely divergent responses of cucumber to chloramben methyl ester have been observed (14) and tolerance is highly heritable (15). Data obtained in this study indicates that the physiological basis for tolerance of T-MSU 3207 differs from that of T-MSU 0612. Likewise, the susceptibility of S-MSU 3159 has a different physiological basis than that of S-MSU 0866. The presence of intraspecific variability in cucumber with respect to tolerance to chloramben methyl ester and of more than one physiological basis for this tolerance offers the possibility of breeding cucumber cultivars with a greater tolerance level than those presently available.

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SECTION II

INHERITANCE OF TOLERANCE TO CHLORAMBEN METHYL ESTER IN CUCUMBER

ABSTRACT

Progeny from crosses between two tolerant and two susceptible lines of cucumber (Cucumis sativus L.) were evaluated for tolerance to methyl 3-amino-2,5-dichlorobenzoic acid (chloramben methyl ester) in the F_1, F_2, BC_1 and BC_2 generations. Plant height, dry weight and visual appearance were evaluated following herbicide treatment. Partial dominance of genes controlling tolerance was noted. The minimum number of effective factor pairs was variable depending on the trait evaluated; however, quantitative inheritance of tolerance was indicated. Transgressive segregation was observed in the F_2 populations of the crosses tolerant x tolerant. Heritability estimates from crosses involving tolerant MSU 0612 were consistently higher than those involving tolerant MSU 3207, also suggesting that the tolerance of these two lines may be conditioned by different genes. Estimates of heritability indicated that considerable progress can be made in selection for tolerance when the criteria used are plant dry weight, apical inhibition, or epinasty.

INTRODUCTION

The occurrence of intraspecific variability in the response of weed and crop plants to specific herbicides has recently been reviewed (7). Methyl 3-amino-2,5-dichlorobenzoic acid (chloramben methyl ester or CME) is an effective preemergence herbicide for cucumber (Cucumis sativus L.); however, use under certain conditions has resulted in varying degrees of plant injury and occasional yield reductions (14). This variability in tolerance has been related to environmental conditions (14) and cultivars grown (12).

Although many examples of intraspecific variability within crop and weed species in their response to agricultural chemicals have been reported, relatively few investigators have studied the genetic basis for the observed variability. Resistance to insecticide damage by DDT in barley (8, 18) and trichlorfon in sorghum (15) was found to be conditioned by the recessive genes ddt and dtp respectively. Methyl parathion damage to sorghum (3) and toxaphene damage to oats (5) are determined by single dominant genes. Resistance to barban chlorosis in barley was shown to be conditioned by a single recessive gene, bc, while resistance to apical inhibition appeared to be

quantitatively inherited (9). Hayes et al. (9) reported that resistance to barban and DDT in barley is independently inherited. Most maize cultivars are resistant to injury by simazine and atrazine. However, Grogan et al. (6) isolated a susceptible inbred line and found that susceptibility was controlled by a single recessive gene. Tolerance to atrazine (4) and MCPA (17) in flax was polygenic, exhibiting relatively low heritability. Schooler et al. (16) showed that siduron tolerance in foxtail barley was controlled by three complementary factors.

Cultivar differences in growth response to CME are evidence for genetic control of this characteristic. Control of environmental conditions during and following herbicide application is often impossible; however, the development of cucumber cultivars with a relatively high degree of tolerance to CME may be possible. Knowledge of the inheritance of CME tolerance would facilitate the development of highly tolerant cultivars. The purpose of this investigation was to identify tolerant lines, and to determine the inheritance of tolerance to CME and the feasibility of incorporating this tolerance into new and existing cultivars of cucumber.

MATERIALS AND METHODS

Plant material and treatment. More than 200 inbred lines, plant introductions, and cultivars of cucumber were examined for tolerance to CME in preliminary experiments. An emulsifiable concentrate containing 0.9 kg/liter of chloramben as the methyl ester was used throughout this investigation. A treatment concentration of 3.12 ppm herbicide in Hoagland's no. 1 (10) aerated nutrient solution culture was used in the greenhouse studies, while rates of 0, 2.2, 4.5, and 6.7 kg/ha were applied in the field during 3 growing seasons.

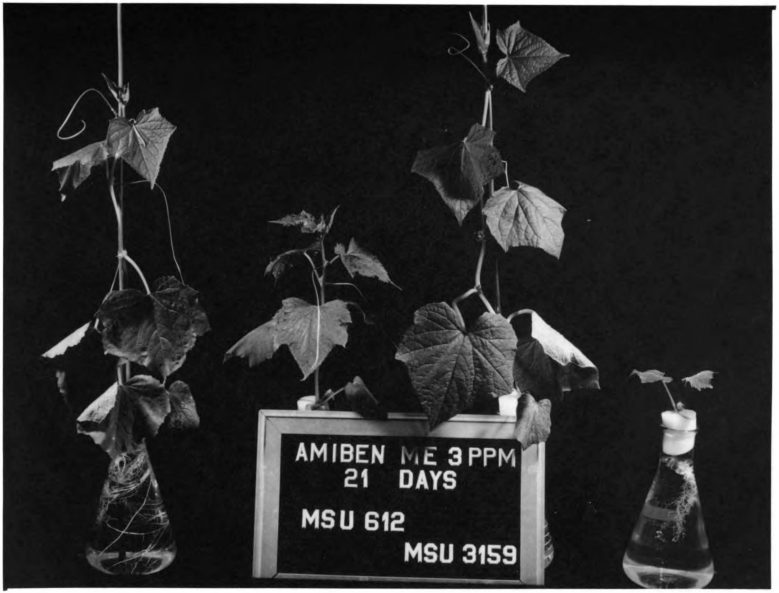
Seeds were germinated in moist vermiculite for all greenhouse experiments. After 10 days, when the first true leaf had expanded to about 0.5 cm, seedlings were transplanted to the nutrient solution culture. Herbicide was added 1 day after transplanting. Plants were grown for 3 weeks under a day and night regime of approximately 27°C and 21°C respectively, with supplemental fluorescent light provided during the 14-hr day. Fe was added to the nutrient solution every 3 days, and distilled water was added to maintain the desired level. Screening tests and all genetic studies were conducted in 6 polyethylene

trays, 74 x 117 x 11 cm, covered with stainless steel lids containing 84 holes each. Studies using carboxy-labeled ^{14}C-CME revealed that sufficient herbicide was available at the concentration used to eliminate differential competition among lines for the available herbicide. Low and high scoring lines from the greenhouse water culture test reacted similarly in the replicated field test.

Injury was assessed 3 weeks after herbicide treatment and was indexed by degree of epinasty, apical inhibition, callus formation, root inhibition and discoloration, and size (weight) reduction. There were 3 to 7-fold differences, dependent upon the trait measured, among lines tested, as related to performance of the control (untreated). Two susceptible, MSU 3159 and MSU 0866, and 2 tolerant, MSU 0612 and MSU 3207, lines were selected for use as parental material (Figure 1).

Inheritance study. Selfed single plant progeny of the 4 parental lines were increased asexually to obtain enough plants of each genotype to make a complete 4 x 4 diallel with reciprocals. For each possible cross, 10 populations were used to obtain genetic data: P_1, P_2, F_1, F_1', BC_1, BC_1', BC_2, BC_2', F_2, F_2'. To test each family, herbicide was added to 5 trays. A 6th tray without herbicide served as control. Each population was sampled 8 to 10 times per tank. The experimental design for

Figure 1.--Reactions of tolerant MSU 0612 and susceptible MSU 3159 twenty-one days after treatment with 3.12 ppm amiben methyl ester (chloramben methyl ester). Plants from left to right are control, treated, control, and treated.


AMIBEN ME 3 PPM
21 DAYS
MSU 612
MSU 3159

statistical analysis was a randomized block with multiple sampling of genotypes.

Genetic analysis. Preliminary observations of parental and segregating populations showed that the F_2 population approximated a normal distribution, indicating possible polygenic inheritance. Subsequently, quantitative inheritance analyses were used. The data obtained were subjected to analyses of variance followed by LSD tests to determine the possibility of maternal effects and of pooling reciprocal crosses. The original data were transformed to log scale (base 10). Scaling tests (11) were performed on the original and transformed data to test for conformity with the additive-dominance model. From analyses of variance, additive, dominance, and environmental variances were determined using the methods of Mather and Fisher (11). Dominance ratios were estimated using Mather's procedure (11). Narrow and broad sense heritability estimates were computed as the ratio of additive genetic variance to phenotypic variance and the ratio of additive + dominance variance to phenotypic variance, respectively. The minimum number of effective factors (k) controlling herbicide injury was determined by the methods of Mather (11) to include additive variance, Castle (2), Wright as reported by Burton (1), and Wright's (19) method modified to include BC variances.

RESULTS

Visual appearance (epinasty, rated on a 0 to 5 scale with 5 designating no epinasty, and overall plant appearance), plant height in cm, and dry weight in g were judged to accurately detect genotypic differences in response to CME. Results from Mather's (11) ABC scaling test gave insignificant values of A, B and C for all transformed data, indicating that the additive-dominance model was adequate for the analysis of variation. Some of the original data showed significant deviations from zero for A, B or C, but otherwise all analyses indicated close agreement between original and transformed data. For brevity, only results obtained from transformed data are presented. Results from each family obtained from pooled reciprocals, where such was allowable, are reported.

Visual rating. Mean values, estimated values of components of variation, degree of dominance, heritability, probable number of effective factors or blocks of factors are presented in Tables 1, 2 and 3. The F_1 means were intermediate between parental means, but were in general nearer to the tolerant parental means, indicating partial dominance of genes controlling

tolerance (Table 1). The F_2 means were lower than those of the F_1, except in the cross MSU 0612 x MSU 0866. The BC_1 and BC_2 means were located between the F_1 means and those of the respective parental means. Dominance variance was greater than additive variance in all crosses except MSU 0612 x MSU 0866 (Table 2). The values for degree of dominance indicated partial to complete dominance for tolerance. A minimum of 1 to 4 major factors appeared to be responsible for variation in tolerance, depending on the cross in question and the method used for estimation (Table 3). In the cross MSU 0612 x MSU 0866, estimates of gene number were lower than those of the other crosses.

Plant height. The F_1 values in all crosses, particularly in MSU 0612 x MSU 3159, were significantly higher than the mid-parent values and were nearer to the higher parent (Table 1). This suggests partial dominance of genes governing herbicide tolerance, as manifest by apical inhibition. The F_2 means were also higher than the mid-parent values but lower than those of the F_1, also suggesting partial dominance. The BC_1 and BC_2 means were located between those of their respective parents and the F_1. Dominance variance components were greater than the additive variance components in MSU 3207 x MSU 3159, lower in MSU 0612 x MSU 0866, and approximately

TABLE 1.--Means and standard errors of parents, F_1, F_2, BC_1, and BC_2 populations from four cucumber crosses rated for visual appearance, plant height, and dry weight 3 weeks following CME treatment.

Crosses Studied	Populations					
	P_1	P_2	F_1	F_2	BC_1	BC_2
			Visual[1]			
MSU 3207 x MSU 3159	.61±.005	.37±.012	.53±.007	.51±.013	.58±.018	.44±.015
MSU 0612 x MSU 3159	.67±.005	.03±.012	.54±.011	.46±.025	.58±.015	.22±.026
MSU 3207 x MSU 0866	.69±.005	.01±.024	.42±.009	.42±.027	.57±.022	.25±.031
MSU 0612 x MSU 0866	.63±.005	-.12±.037	.37±.012	.40±.035	.57±.018	.17±.043
			Plant height[2]			
MSU 3207 x MSU 3159	1.21±.009	.53±.026	.89±.022	.90±.036	1.07±.031	.80±.041
MSU 0612 x MSU 3159	1.34±.007	.41±.022	1.30±.009	1.03±.057	1.05±.042	.86±.052
MSU 3207 x MSU 0866	1.21±.011	.56±.025	.95±.026	.94±.036	1.09±.035	.80±.035
MSU 0612 x MSU 0866	1.05±.010	.38±.025	.73±.013	.70±.035	.98±.030	.62±.035
			Dry weight[3]			
MSU 3207 x MSU 3159	.21±.015	-.56±.022	-.06±.014	-.09±.028	.01±.048	-.25±.024
MSU 0612 x MSU 3159	-.04±.008	-.58±.017	-.08±.008	-.24±.025	-.16±.017	-.45±.026
MSU 3207 x MSU 0866	.09±.013	-.69±.022	-.23±.015	-.25±.028	-.03±.022	-.40±.033
MSU o612 x MSU 0866	-.44±.012	-1.18±.028	-.61±.016	-.58±.028	-.55±.022	-.82±.107

[1]Visual appearance (epinasty, rated on a 0 to 5 scale with 5 designating no epinasty). All data presented are transformed to log scale (base 10).

[2]Plant height in cm transformed to log scale (base 10).

[3]Dry weight in g transformed to log scale (base 10).

TABLE 2.--Estimated values of components of variation and degree of dominance for visual appearance, plant height, and dry weight of four cucumber crosses 3 weeks following treatment with CME.

Crosses Studied	Trait Evaluated		
	Visual	Height	Dry Weight
	Environmental variance (E)		
MSU 3207 x MSU 3159	0.0038	0.0206	0.0156
MSU 0612 x MSU 3159	0.0045	0.0091	0.0060
MSU 3207 x MSU 0866	0.0088	0.0186	0.0120
MSU 0612 x MSU 0866	0.0203	0.0120	0.0140
	Dominance variance (H)		
MSU 3207 x MSU 3159	0.0104	0.0127	0.0325
MSU 0612 x MSU 3159	0.0348	0.1396	0.0353
MSU 3207 x MSU 0866	0.0423	0.0520	0.0522
MSU 0612 x MSU 0866	0.0220	0.0363	0.0347
	Additive variance (D)		
MSU 3207 x MSU 3159	0.0039	0.0604	0.0331
MSU 0612 x MSU 3159	0.0283	0.1390	0.0268
MSU 3207 x MSU 0866	0.0338	0.0662	0.0278
MSU 0612 x MSU 0866	0.0708	0.0826	0.0308
	Dominance ratio		
MSU 3207 x MSU 3159	1.6397	0.9183	0.9895
MSU 0612 x MSU 3159	1.1100	1.0022	1.1482
MSU 3207 x MSU 0866	1.1183	0.8864	1.3702
MSU 0612 x MSU 0866	0.5572	0.6627	1.0623

TABLE 3.--Estimated values of heritability and probable number of effective factors for tolerance to CME of 4 cucumber crosses evaluated 3 weeks after herbicide treatment.

Crosses Studied	Trait Evaluated		
	Visual	Height	Dry Weight
	Heritability (h^2) for F_2		
MSU 3207 x MSU 3159			
broad sense	0.55	0.68	0.61
narrow sense	0.32	0.47	0.41
MSU 0612 x MSU 3159			
broad sense	0.84	0.92	0.79
narrow sense	0.52	0.61	0.48
MSU 3207 x MSU 0866			
broad sense	0.76	0.71	0.84
narrow sense	0.47	0.51	0.22
MSU 0612 x MSU 0866			
broad sense	0.66	0.81	0.63
narrow sense	0.58	0.66	0.41
	Number of Effective Factors (k)		
MSU 3207 x MSU 3159			
Castle 1921	1.2	1.5	2.5
Mather 1971	3.9	1.9	4.4
Wright 1968	3.9	1.9	4.5
Burton 1951	1.3	1.5	2.6
MSU 0612 x MSU 3159			
Castle 1921	2.4	1.0	1.4
Mather 1971	3.6	1.5	2.7
Wright 1968	3.6	1.6	2.7
Burton 1951	2.8	1.4	1.9
MSU 3207 x MSU 0866			
Castle 1921	1.7	1.4	2.5
Mather 1971	3.3	1.6	5.4
Wright 1968	3.4	1.6	5.3
Burton 1951	1.8	1.4	2.6
MSU 0612 x MSU 0866			
Castle 1921	1.2	1.0	2.1
Mather 1971	1.8	1.3	4.5
Wright 1968	1.9	1.3	4.6
Burton 1951	1.2	1.0	2.5

equal in the other crosses, as reflected by the corresponding dominance ratios (Table 2). All crosses exhibited relatively high heritability (Table 3). The minimum number of effective factor pairs involved in plant height as an expression of tolerance ranged from 1 to 2, depending on the particular cross involved and the method of calculation employed. Gene estimates for MSU 3207 x MSU 3159 were higher than those of the other crosses.

Dry weight. Partial dominance of genes controlling plant weight following herbicide treatment was evident from the F_1 mean values in all 4 crosses being higher than their corresponding mid-parent values, and closer to the mean of the tolerant parents (Table 1). Variances of the BC_2 populations were generally larger than those of the BC_1 populations. The dominance variances were higher than additive variances in all crosses, excepting MSU 3207 x MSU 3159 (Table 2). The estimate of narrow sense heritability in the cross MSU 3207 x MSU 0866 was low (Table 3). The probable number of genes controlling the expression of tolerance as measured by plant dry weight ranged from 1 to 5. In general, more genes appeared to be segregating in the expression of dry weight than in either of the other traits measured.

Transgressive combination and other observations. The F_1 plants from crosses of the 2 susceptible lines,

MSU 3159 x MSU 0866, were no more or less susceptible than either of the parents. The populations from crosses of the 2 tolerant lines, MSU 0612 x MSU 3207, were tested under conditions of environmental stress (low temperature and light intensity) and under the conditions employed throughout this investigation. Under conditions of stress the F_1 population means exceeded those of the parental means, thus exhibiting physiological homeostasis. When grown under normal conditions, the F_1 individuals did not exceed either of the tolerant parents. Approximately 4% of the F_2 population gave indication of transgressive recombination for both tolerance and susceptibility.

DISCUSSION

Several assumptions are made when the additive-dominance model (11) and the various methods (1, 2, 11, 19) of estimating the minimum number of effective factors are employed. If not all of these assumptions are met, the results obtained may be confounded. BC variances are used in estimating the number of genetic factors in the Mather (11) and Wright (19) formulas. Where BC data is available, these methods tend to be more reliable than the other 2 methods employed in this investigation (1, 2). Those estimates including BC variances generally suggested polygenic control of CME tolerance, and were consistently higher than those not including these variances.

The results illustrate one of the problems in evaluating a collection of plant material. It is difficult to determine which trait is the most reliable measure of tolerance, as each may be reflecting a different response. Plants of one line exhibited only slight apical inhibition, yet showed severe epinasty in the few leaves present. Conversely, other lines were stunted and bushy with little or no epinasty, also suggesting a complex pattern of inheritance. It must be considered that plant height and weight may reflect genes segregating for these traits

irrespective of gene segregation for CME tolerance. Also, heterosis was observed in control F_1 populations from all 4 crosses. The visual rating system was possibly the most accurate, since it is a type of discriminate function which includes primarily epinasty but also general plant appearance. The various responses observed may not have been pleiotropic *per se*.

Significant differences in tolerance were observed among some reciprocal crosses; however, it was difficult to attribute these differences conclusively to cytoplasmic effects. These differences may have been confounded by seed of low viability obtained from plants taken from cuttings.

Studies to determine the physiological basis for tolerance of cucumber to CME with these same lines showed that the tolerance of MSU 3207 and MSU 0612 resulted from 2 distinct physiological mechanisms (13). Regardless of susceptible parent used or trait studied, crosses involving MSU 0612 consistently exhibited higher narrow sense heritability than those involving MSU 3207. Results obtained in these studies would suggest that generalizations regarding the entire cultivar spectrum of a species can be misleading when based on the reactions of a single cultivar.

The results showed that both dominance and additive gene action play a role in the inheritance of tolerance to CME. The relatively high narrow sense heritabilities, particularly in crosses involving MSU 0612, indicate that considerable progress can be made by selection for CME tolerance when the criteria are plant dry weight, plant height (apical inhibition), or epinasty. These results support the idea of Weibe and Hayes (18) that plant breeding and genetics can play an important role in developing agricultural chemicals for use on crop plants.

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