A Survey of Watermelon Germplasm for Inhibitory Seed Exudates

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Abstract. Watermelon [*Citrullus lanatus* v. *lanatus* (Thunb.) Matsum & Nakai] seed exudates are inhibitory to germination and seedling growth of other plant species. A miniature bioassay experiment that measured proso millet (*Panicum miliaceum* L.) radicle growth was used to assess the inhibition caused by seed exudates of 125 genotypes of watermelon and related *Citrullus* species. Exudates of most genotypes were not inhibitory; however, exudates of 53 accessions reduced radicle growth in comparison with the control. In subsequent proso millet radicle growth experiments, genotypes were found to vary in inhibitory potential, and concentration response curves generated using filtered, pasteurized exudates were different among genotypes. Filter-sterilized seed exudates of *Citrullus* accessions also varied in the level of inhibition in a bioassay that measured their effect on sporangia formation by the watermelon pathogen, *Phytophthora capsici*. These observations suggest that constituents in *Citrullus* seed exudates affect organisms in the spermosphere and that the inhibitory potential of seed exudates varies among genotypes. Differences in concentration response curves in the millet bioassay and differences in the relative inhibition of genotypes in the millet and fungus bioassays indicate that the inhibitory constituents in seed exudates vary among genotypes.

Allelopathy has traditionally been defined as the inhibitory effect of compounds released by one plant on another plant. A broader but less accepted definition of the term includes effects of plants on microorganisms that are mediated by biological chemicals (Rice, 1984). Allelopathy in the Cucurbitaceae was first reported by Putnam and Duke (1974) who screened the U.S. PI collection of cucumber (*Cucumis sativus*) germplasms and identified accessions with varying allelopathic potentials. Lockerman and Putnam (1979, 1981) subsequently demonstrated that suppression of proso millet (*Panicum miliaceum*) and white mustard (*Brassica alba*) by cucumber was largely the result of allelopathy and that allelopathic genotypes were competitive against weeds. Yu and Matsui (1994) found that cucumber root exudates contained a number of simple and complex phenolic compounds and organic acids that were inhibitory in a lettuce (*Lactuca sativus*) seedling growth bioassay. Soil sickness observed after repeated cropping with certain cucurbit crops was attributed to autotoxicity of compounds in root

exudates and released by decomposing root tissues (Yu, 2001; Yu et al., 2000).

Germinating seeds release many compounds that affect organisms in the spermosphere. The role of seed exudates in stimulating the germination and growth of beneficial and harmful microorganisms has been extensively investigated, and this subject has been reviewed recently (Nelson, 1990, 2004). In some instances, constituents in seed exudates are inhibitory to microorganisms and protect the germinating seed against invasion by pathogens (Rose et al., 2006). Seed exudates may also be allelopathic against plants, and there are several reports of phytotoxic effects of seed exudates (Higashinakasu et al., 2004; Kushima et al., 1998; Ohno et al., 2001; Yamada et al., 1995). Kushima et al. (1998) reported that watermelon seed exudates were inhibitory to the root growth of several species in petri dish bioassays. They found that the exudates contained vanillic acid at a concentration that inhibited the growth of the test species in a bioassay. Otlewski et al. (1987) reported that watermelon seeds contained small peptides that function as protease inhibitors.

Phytophthora capsici is known to infect plants species in the families, cucurbitaceae, solanaceae, and leguminosae (Hausbeck and Lamour, 2004), and it causes an important disease in watermelon (Jester et al., 2006; Wiant, 1940). One objective of this research was to develop a miniature bioassay for inhibitory seed exudates and use it to assess the allelopathic potential of *Citrullus* germplasm. The effect of filter-sterilized seed exudates of *Citrullus* genotypes was also tested in a *Phytophthora capsici* bioassay that measured sporangia formation.

Proso millet radicle growth bioassay. The availability of seeds of most accessions was limited; thus, a bioassay that required few seeds was developed. Watermelon seeds were surface-sterilized by immersing in 70% ethanol for 2 min, thoroughly rinsed with distilled water, and air-dried at room temperature. A Whatman #1 (Whatman, Springfield, Kent, England) filter paper disk was placed in a 35-mm plastic petri dish and 0.5 mL distilled water was added. One *Citrullus* seed was added to each dish for genotypes with seed weights ≥ 7.0 g·100⁻¹ seeds, and two seeds per dish were added for genotypes with seed weights <7.0 g·100⁻¹ seeds. Ten proso millet seeds were subsequently added to each dish, and the seeds were incubated in the dark at 24 °C. After 72 h, the petri dishes were placed in a freezer to stop growth. The dishes were thawed, millet radicle lengths were measured with an electronic caliper, and average radicle length was determined for each dish.

Preliminary germplasm evaluation. Germplasm evaluated in the preliminary study included eight accessions from the U.S. Plant Introduction (USPI) collection of *Citrullus colocynthis* (L.) Schrad., nine USPI accessions from the *Citrullus lanatus* v. *citroides* (Bailey) Mansf. collection, and 108 watermelon USPI accessions, cultivars, and breeding lines. The experiment was arranged in a completely random design with three replications and was repeated three times. Data were combined, SE of the means were determined, and the F test at $P \leq 0.05$ was used to separate genotype means from the control. Linear regression analysis was used to determine if *Citrullus* seed weight (doubled for genotypes less than 7.0 g·100⁻¹ seeds) was correlated to millet radicle length.

Retesting selected genotypes. Nine genotypes that varied in the initial screening experiment (Charleston Gray, PI 432337, PI 532738, 389-5-xx-15, 406-1-xx-7, 413-5-xx-5, 416-1-xx-5-25, 416-4-xx-6, and 416-5-xx-11) were retested using the bioassay procedure described previously. The experiment was arranged in a completely random design with five replicates and repeated four times. Data were combined and subjected to analysis of variance in a factorial design in which genotype was the first factor and experiment was the second factor. Genotype means were separated using Fisher's protected least significant difference (LSD) at $P \leq 0.05$.

Concentration response for seed exudates in the millet seedling radicle growth bioassay. Eight *Citrullus* genotypes (Charleston Gray, PI 167125, PI 432337, PI 482246, PI 500354, PI 532,738, 406-1-xx-7, and 413-5-xx-7) were selected for this experiment based on activity in the previous study and seed availability. Seeds were soaked in distilled water (30 g seeds·60 mL⁻¹) on a wrist action shaker in the dark at 22 °C. After 24 h, the seeds were strained out and discarded and the volume of the exudates was adjusted to 60 mL. Exudates were filtered through Whatman #1 filter paper and then through 1.2- μ m

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Table 1. Response of proso millet radicle growth to *Citrullus* seed exudate in a petri dish bioassay.

Genotype ^z	Species ^y	Seed wt (g·100 seeds ⁻¹)	Radicle length ^x (mm)	SEM ^w	Genotype ^z	Species ^y	Seed wt (g·100 seeds ⁻¹)	Radicle length ^x (mm)	SEM ^w
Control	—	—	23.4	1.8	PI 248178	CII	11.3	20.1	7.2
PI 195297	Cc	6.1	22.3	4.4	PI 249008	CII	13.7	17.5*	5.2
PI 346082	Cc	3.3	26.6	4.4	PI 249009	CII	16.2	21.9	3.8
PI 386016	Cc	3.6	20.0	4.0	PI 306364	CII	5.4	15.4*	5.1
PI 386018	Cc	4.0	22.3	5.3	PI 306366	CII	8.3	19.4	4.4
PI 386024	Cc	4.2	22.5	2.9	PI 306782	CII	14.1	18.9	3.4
PI 386025	Cc	4.3	22.7	5.6	PI 307608	CII	9.5	24.3	8.7
PI 386026	Cc	4.4	23.9	5.1	PI 307748	CII	4.8	16.7*	5.3
PI 432337	Cc	4.2	27.0	3.4	PI 307749	CII	9.8	19.1	6.0
PI 244018	Clc	5.0	23.9	4.9	PI 307750	CII	7.3	21.7	5.9
PI 244019	Clc	4.4	21.8	4.0	PI 346787	CII	8.5	15.1	3.1
PI 255137	Clc	9.8	25.9	5.0	PI 357676	CII	8.9	27.7	4.2
PI 271779	Clc	11.4	22.6	5.1	PI 379251	CII	12.3	24.7	6.0
PI 299378	Clc	13.8	24.4	4.4	PI 379256	CII	12.0	20.7	6.1
PI 299379	Clc	15.6	16.6*	4.3	PI 381713	CII	11.8	20.1	4.2
PI 482246	Clc	20.2	23.8	5.2	PI 381741	CII	4.7	16.9*	4.0
PI 482252	Clc	12.0	21.8	6.0	PI 385964	CII	7.1	21.9	2.7
PI 500354	Clc	10.0	29.8	2.9	PI 479704	CII	10.2	32.6*	3.5
PI 532738	Clc	15.8	30.9	7.9	PI 482246	CII	8.9	26.7	7.4
Griffin 1729	CII	4.4	16.5*	4.1	PI 482256	CII	17.7	14.7*	7.6
PI 105445	CII	7.9	19.7	2.3	PI 482279	CII	12.9	22.6	4.2
PI 163204	CII	9.7	17.2*	4.0	PI 494527	CII	14.1	11.7*	4.9
PI 163574	CII	10.4	14.6*	5.5	PI 494529	CII	11.7	11.8*	3.9
PI 164146	CII	6.9	20.6	3.1	PI 494530	CII	12.8	25.0	3.8
PI 164247	CII	10.8	19.7	2.2	PI 494531	CII	14.6	19.6	7.0
PI 164543	CII	10.5	15.8*	3.7	PI 494816	CII	9.4	20.4	4.4
PI 164570	CII	9.3	15.7*	3.0	PI 500354	CII	10.0	29.8	2.9
PI 164633	CII	8.7	16.1*	4.1	PI 532810	CII	7.4	18.8	4.0
PI 164634	CII	9.1	17.7*	5.0	PI 559992	CII	14.0	19.8	4.6
PI 164636	CII	9.6	18.8	4.9	PI 599993	CII	13.4	20.8	5.7
PI 164685	CII	7.7	16.5*	3.2	PI 559995	CII	12.1	15.3*	7.4
PI 164977	CII	5.9	13.5*	4.5	PI 595203	CII	10.2	20.7	4.2
PI 165002	CII	9.4	19.0	6.4	PI 596666	CII	4.1	26.4	4.8
PI 165523	CII	11.3	20.1	7.2	205-xx-33	CII	6.5	19.0	7.9
PI 167059	CII	9.5	15.0*	6.0	210-341-31	CII	3.2	21.6	3.3
PI 167125	CII	6.4	13.5*	6.1	385-3-xx-8	CII	2.9	14.3*	5.4
PI 167126	CII	11.2	12.4*	4.6	385-3-xx-23	CII	4.0	14.9*	4.1
PI 167219	CII	10.3	15.7*	7.8	389-5-xx-15	CII	3.1	19.5	4.8
PI 169232	CII	9.9	17.9*	2.6	392-4-xx-9-28	CII	2.9	14.3*	5.4
PI 169245	CII	13.4	21.0	10.9	398-xx	CII	9.3	18.3	3.4
PI 169253	CII	6.0	17.8*	8.9	398-4-xx-4	CII	2.9	27.3	7.3
PI 169278	CII	7.5	25.9	7.0	398-4-xx-3-24	CII	3.3	13.9*	4.4
PI 169292	CII	8.8	14.0*	4.9	406-1-xx-6	CII	6.0	25.7	6.4
PI 169300	CII	10.9	21.6	3.2	406-1-xx-7	CII	5.2	14.7*	3.8
PI 171579	CII	8.5	19.8	3.0	406-1-xx-10	CII	5.9	14.0*	3.7
PI 171585	CII	11.9	21.5	4.2	413-1-xx-17	CII	5.4	14.9*	4.0
PI 172786	CII	12.8	22.6	3.0	413-5-xx-5	CII	5.2	16.1*	2.5
PI 172787	CII	8.6	15.0*	3.4	413-5-xx-17	CII	5.4	14.9*	4.0
PI 172788	CII	11.7	21.2	3.4	413-5-xx-19	CII	6.3	12.9*	4.9
PI 174106	CII	8.6	18.8	6.2	413-5-xx-32	CII	3.6	13.0*	3.6
PI 175653	CII	10.4	13.9*	4.6	413-5-xx-7-27	CII	4.3	19.5	6.4
PI 175655	CII	8.0	16.6*	3.2	413-41-xx-13	CII	5.1	15.5*	4.7
PI 175657	CII	9.7	13.9*	4.4	416-x-1-xx	CII	8.7	13.9*	3.7
PI 179908	CII	10.1	18.8	4.3	416-1-xx-5-25	CII	7.3	14.1*	4.3
PI 179910	CII	5.1	24.3	4.3	416-2-xx-14	CII	8.7	20.8	5.4
PI 177325	CII	13.5	19.9	3.0	416-2-xx-16	CII	6.5	15.4*	7.6
PI 177329	CII	10.0	20.0	4.0	416-4-xx-3	CII	8.1	23.0	5.0
PI 179237	CII	6.3	13.7*	3.6	416-4-xx-6	CII	8.2	17.6*	3.9
PI 185635	CII	8.8	18.7	4.9	416-5-xx-11	CII	5.7	19.0	6.7
PI 186975	CII	5.2	21.1	3.9	416-5-xx-20	CII	8.6	17.1	3.5
PI 189225	CII	13.8	15.7*	3.6	416-4-xx-18-29	CII	7.9	14.2*	3.0
PI 189316	CII	11.2	20.6	7.4	488-7-xx-18	CII	5.2	15.9*	3.5
PI 189317	CII	13.8	19.7	5.4	488-7-xx-22	CII	5.2	18.4	9.7
PI 189318	CII	14.3	17.5*	5.9	488-7-xx-35	CII	4.0	21.3	4.9
PI 192937	CII	9.0	12.5*	5.2	Charleston Gray	CII	7.3	15.3*	2.4

^zPI numbers are accessions from the USPI collection of *Citrullus* germplasm; other numbered genotypes are US Vegetable Laboratory experimental lines.^yCc = *Citrullus colocynthis*; Clc = *Citrullus lanatus* v. *citroides*; Cl = *Citrullus lanatus* v. *lanatus*.^xMeans followed by asterisks are different from the control based on Fisher's protected least significant difference (LSD) at $P \leq 0.05$ (LSD_{0.05} = 4.6).^wSEM = standard error of the mean.

nylon filters and pasteurized by heating to 70 °C. Exudate solutions were diluted with distilled water to form concentrations of 0%, 20%, 40%, 60%, 80%, and 100% of the initial

concentration, and 0.5-mL aliquots of each concentration were tested using the millet bioassay procedure described previously, except no watermelon seeds were added to

the dishes. The experiment was arranged in a completely random design with five replicates and the experiment was repeated. Data from the two repetitions of the experiment

were combined and subjected to analysis of variance and means were separated using Fisher's protected LSD at $P \leq 0.05$. Four genotypes (Charleston Gray, 406-1-xx-7, PI 500354, and PI 582468) were chosen to demonstrate the differences in response curves. The response curves were generated using the quadratic regression equation ($y = y_0 + ax + bx^2$).

Effect of *Citrullus* seed exudates on sporangia formation by *Phytophthora capsici*. Seven *Citrullus* genotypes included in this experiment (PI 167125, PI 432337, PI 482246, PI 500354, PI 532738, 406-1-xx-7, and Charleston Gray) were selected based on earlier observations and seed availability. PI 482246, which was not included in previous experiments, was included this experiment, because it appears to be tolerant to *P. capsici*. Seeds were soaked in distilled water (30 g seeds-60 mL⁻¹) on a wrist action shaker in the dark at 22 °C. After 72 h, the seeds were strained out and discarded, and the volume of the exudates was adjusted to 60 mL. Exudates were filtered through sterile syringe filter (Corning, Corning, NY) disks (0.20 µm) before use in bioassays. All *P. capsici* isolates used in these studies were kindly provided by A.P. Keinath, Clemson University, and R. French-Monar, University of Florida. In the first experiment, two isolates of *P. capsici* from South Carolina and two from Florida were used. Isolates were grown on parafilm wrapped V8 Juice agar plates containing the antibiotic rifampicin for 1 week using a technique described by Hausbeck and Lamour (2004). Mycelial agar plugs (6-mm diameter) of the individual isolates were placed in individual cells (57 × 108 mm) on a 10-cell Boerner (Wheaton Scientific, Millville, NJ) microscope slide. One hundred fifty microliters of each seed exudate were directly pipetted on the agar plugs in each cell. There were three replications for each isolate and exudate combination. Sterile distilled water was used as a control. The Boerner microscope slides were placed in clear plastic boxes lined with two layers of moist paper towels to prevent drying of the exudates. The plastic boxes were placed on a laboratory bench (25 ± 1 °C) for 3 d. Regular fluorescent lights in the laboratory were left on during daytime (12 h) for the 3 d. Sporangia formation was monitored everyday. After 3 d, 50 µL of sterile distilled water was added to each cell because of loss of volume of the exudates. Sporangia in each Boerner cell were dislodged from the mycelium and agar by rapidly pipetting back and forth for 10 s. One microliter of sporangial suspension from each isolate and seed exudate combination was placed on a regular glass microscope slide and total number of sporangia counted. This experiment was repeated. Based on the results of the first two experiments, we selected three accessions (Charleston Gray, PI 432337, and PI 482334) for further studies on 14 *P. capsici* isolates. Eight isolates from South Carolina and six from Florida were tested. There were two replications for each exudate and isolate

combination. This experiment was conducted as described previously. The data were analyzed using log-transformed values for the spore counts; however, this did not alter the key conclusions, and hence the actual spore counts were used in final analysis and mean number of sporangia µL⁻¹ are presented. Data were subjected to analysis of variance and means were separated by Fisher's protected LSD at $P \leq 0.05$.

Results and Discussion

The proso millet radicle growth bioassay described here was developed after preliminary experimentation to determine optimum conditions. The 70% ethanol rinse was used to reduce fungus growth associated with seeds of some genotypes, because in preliminary experiments, a dilute sodium hypochlorite bleach treatment that is often used to surface-sterilize seeds was found to reduce the inhibition by Charleston Gray seed exudates. The 70% ethanol rinse did not affect inhibition in comparison with untreated seeds. The initial germplasm assessment experiment indicated that watermelon genotypes vary in the inhibition of millet radicle growth caused by seed exudates (Table 1). Seed exudates of the *Citrullus colocynthis* accessions were not inhibitory in this bioassay, and only one accession of *Citrullus lanatus* v. *citroides* inhibited radicle growth. Exudates of most of the watermelon accessions included in this bioassay also did not inhibit proso millet radicle growth; however, radicles of seedlings grown in exudates of 53 accessions were shorter than the control. Radicles of seedlings grown in the exudates of two accessions were longer than the control. Increased growth by low concentrations of inhibitory compounds is common. Linear regression analysis indicated that the weight of the seeds added to the dishes and radicle lengths were not correlated. The frequency (61%) of inhibitory genotypes in the U.S. Vegetable Laboratory lines was higher than in the PI accessions (37%). There was considerable variation in this bioassay procedure as indicated by the relatively large standard errors for some means.

A more highly replicated experiment including selections from the preliminary germplasm evaluation indicated that detecting differences in the inhibitory potential of seed exudates of watermelon genotypes is possible. Radicle lengths of seedlings grown in seed exudates *C. colocynthis* (PI 432337) and *C. lanatus* v. *citroides* (PI 532738) accessions that were not inhibitory in the screening test were not different from the control in this study (Table 2). Radicle lengths of seedlings grown in exudates of watermelon accessions that were inhibitory in the preliminary experiment were all shorter than the control, but there were differences among the inhibitory accessions. This indicates that although a screening experiment with less replication may be useful to select genotypes with inhibitory seed exudates, highly replicated experiments are necessary to identify the most

highly inhibitory selections. Two-way factorial analysis of the radicle growth data from this experiment indicated that the effect of experiment repetition and the interaction of genotype and repetition were not significant (data not included).

The seed biomass extracted per volume water was ≈4 times higher in the procedure used to generate seed exudates for the concentration response experiments than in the whole seed bioassay. Thus, it is not surprising that seed exudates in this experiment were more inhibitory than exudates in the previous experiments (Table 3). Although PI 432337 and PI 532738 were not inhibitory in the whole seed bioassay (Tables 1 and 2), they were highly inhibitory in this experiment. The seed exudate concentration-response curves of four genotypes presented were different (Fig. 1). For instance, Charleston Gray exudate reduced radicle growth at all concentrations and maximum inhibition was 25% with the undiluted exudate; whereas PI 432337 exudate was not inhibitory at up to 60% concentration, but inhibition was 50% with undiluted exudate. The 20% concentration is approximately equivalent to the seed mass per volume water like in the whole seed bioassays (Tables 1 and 2), and at this

Table 2. Inhibition of proso millet seedling radicle growth by seed exudates of nine *Citrullus* genotypes in a petri dish bioassay.

Genotype	Species ^z	Seed wt (g-100 seeds ⁻¹)	Radicle length ^y (mm)
Control	—	—	23.4 a
PI 432337	Cc	4.2	26.0 a
PI 532738	Clc	15.8	25.8 a
Charleston Gray	ClI	7.3	17.0 cd
389-5-xx-15	ClI	3.1	17.3 c
406-1-xx-7	ClI	5.2	14.2 de
413-5-xx-5	ClI	3.6	12.9 e
416-1-xx-5-25	ClI	7.3	18.0 bc
416-4-xx-6	ClI	8.5	17.2 c
416-5-xx-11	ClI	8.1	20.2 b

^zCc = *Citrullus colocynthis*; Clc = *Citrullus lanatus* v. *citroides*; ClI = *Citrullus lanatus* v. *lanatus*.

^yMeans followed by the same letter are not different based on Fisher's protected least significant difference at $P \leq 0.05$.

Table 3. Effect of filtered, pasteurized exudates from seeds of eight *Citrullus* genotypes on proso millet radicle growth in bioassay.

Genotype	Species ^z	Radicle length ^y (mm)
Control	—	22.4 a
Charleston gray	ClI	16.7 b
406-1-xx-7	ClI	11.2 c
413-5-xx-5	ClI	15.8 b
PI-167125	ClI	12.1 c
PI-432337	Cc	11.7 c
PI-482246	ClI	10.3 c
PI-500354	Clc	8.6 c
PI-532738	Clc	11.0 c

^zCc = *Citrullus colocynthis*; Clc = *Citrullus lanatus* v. *citroides*; ClI = *Citrullus lanatus* v. *lanatus*.

^yMeans followed by the same letter are not different based on Fisher's protected least significant difference at $P \leq 0.05$.

concentration, the relative inhibition of the four lines was similar to that observed in the whole seed bioassay. The marked difference in the characteristics of the concentration-response curves indicates that exudate constituents contributing to radicle growth inhibition probably vary among genotypes.

Most of the isolates used in the *P. capsici* bioassay experiments were obtained from cucurbits or peppers. In both experiments, inhibition of sporangia formation by seed exudates varied between genotypes (Tables 4 and 5). For data averaged over four isolates, seed exudates of a *C. colocynthis* accession,

PI 432337, and a *C. lanatus* v. *citroides* accession, PI 482334, significantly inhibited sporangial formation compared with sterile, distilled water control (Table 4). The variation in the effect of seed exudates on different isolates is demonstrated by the highly significant isolate effect and the genotype-by-isolate interaction. Not all isolates were inhibited by PI 482334 and PI 432337 exudates (data not presented). In the second experiment, the seed exudates of all three genotypes inhibited sporangia formation when averaged over 14 isolates (Table 5). The isolate effect was also highly significant in this experiment, which indicates that isolates varied in response to the exudates, but the genotype $\times$ isolate interaction was not significant. No seed exudate completely inhibited sporangial formation, and at least a few sporangia were formed in all combinations. None of the seed exudates inhibited growth of the seedborne, bacterial watermelon fruit blotch pathogen, *Acidovorax avenae* subsp. *citrulli* (data not presented). We consider these results to be preliminary evidence that watermelon seed constituents may play a role in the interaction of pathogens with germinating *Citrullus* seeds. Further study is required to determine if the inhibition of sporangia formation observed in this bioassay occurs under natural conditions.

In these experiments, inhibition by watermelon seed exudates in the millet and *P. capsici* bioassays varied considerably among genotypes. The differences in the characteristics among genotypes in concentration response curves for the proso millet radicle growth bioassay (Fig. 1) and the differences among genotypes in relative inhibition in the millet and *P. capsici* bioassays (Tables 2–5) indicate that the inhibitory components of the seed exudates may vary among genotypes used in these studies. Kushima et al. (1998) reported that most of the inhibition of seedling growth was attributable to vanillic acid that was present at high concentrations in watermelon seed exudate. They did not specify the watermelon genotype that was used in their studies. It is unlikely that their genotype was included in our experiments. Preliminary chromatographic examination of phenolic constituents occurring in the exudates and extracts of seeds of 10 of the *Citrullus* genotypes included in these studies indicated that vanillic acid or its glycoside, vanillin, was not present at the high levels that they reported (Maurice E. Snook, personal communication).

It is doubtful that allelopathic potential associated with watermelon seed exudates could contribute to weed management under U.S. cultural practices, because the seeding rate is low and watermelon is often transplanted. However, if watermelon seedlings are also allelopathic, then the trait may be useful to improve competitiveness against weeds. Lockerman and Putnam (1979) found that allelopathic cucumber genotypes were more competitive than nonallelopathic genotypes in a field weed interference experiment.

Fig. 1. Concentration response curves for seed exudates from four *Citrullus* genotypes. Curves were generated using the quadratic regression equation ($y = y_0 + ax + bx^2$). R^2 s were 0.88, 0.95, 0.90, and 0.81 and P s were 0.11, 0.022, 0.048, and 0.093 for Charleston Gray, 406-1-xx-7, PI 500354, and PI 482334, respectively.

Table 4. Effect of seed exudates from nine *Citrullus* genotypes on sporangial formation by *Phytophthora capsici*.^a

Watermelon genotype	Species ^y	Number of sporangia $\cdot \mu\text{L}^{-1} \times$	
		Expt. I	Expt. II
Water control		64 a	58 a
PI 482246	Cll	67 a	60 a
PI 500354	Clc	59 a	56 ab
PI 532738	Clc	54 ab	30 cd
Charleston Gray	Cll51 ab	37 abc	
406-1-xx-7	Cll50 ab	45 abc	
PI 167125	Cll50 ab	52 abc	
PI 432337	Cc21 bc	9 de	
PI 482334	Clc 9 c	3 e	
Analysis of variance			
Expt. I			
Source	Degrees of freedom	Mean square	<i>P</i>
Rep	2	332	0.5880
Watermelon genotype	9	4158	0.0001
<i>P. capsici</i> isolate	3	31931	0.0001
Genotype $\times$ isolate	27	1697	0.0003
Expt. II			
Rep	2	194	0.7956
Watermelon genotype	9	4754	0.0001
<i>P. capsici</i> isolate	3	16462	0.0001
Genotype $\times$ isolate	27	1693	0.0101

^aData are averaged over four *P. capsici* isolates.

^yCc = *Citrullus colocynthis*; Clc = *Citrullus lanatus* v. *citroides*; Cll = *Citrullus lanatus* v. *lanatus*.

^xMeans followed by same letter within columns are not significantly different based on Fisher's protected least significant difference at $P \leq 0.05$.

Table 5. Effect of seed exudates from three *Citrullus* genotypes on sporangial formation *Phytophthora capsici*.^z

Seed exudate	Sporangia number- μl^{-1} y
Control	117 a
Charleston Gray	68 b
PI 432337	39 c
PI 482334	25 c
Analysis of variance	
Source	Degrees of freedom
Rep	1
Watermelon genotype	3
<i>P. capsici</i> isolate	13
Genotype $\times$ isolate	39
	Mean square
	<i>P</i>
	0.0001
	0.0001
	0.0001
	0.2870

^zData are averaged over 14 isolates.

^yMeans followed by same letter within columns are not significantly different based on Fisher's protected least significant difference at $P \leq 0.05$.

The biological activity of *Citrullus* seed exudates and the differences between genotypes in seed exudate activity warrant continued investigation of the trait.

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