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18 April 1974

Biological Suppression of Weeds: Evidence for Allelopathy in Accessions of Cucumber

Abstract. *Cucumber (*Cucumis sativus *L.) accessions from 41 nations were grown with two indicator species in a search for superior competitors. Of the plant introductions tested, one inhibited indicator plant growth by 87 percent and 25 inhibited growth by 50 percent or more. The toxicity of leachates from pots containing inhibitory cucumbers to indicator plants germinated in separate containers suggested allelopathy. Incorporation of an allelopathic character into a crop cultivar could provide the plant with a means of gaining a competitive advantage over certain weeds.*

Successful biological control of weeds is limited to a few instances where introduced predators have reduced populations of a target species. No biological methods have gained wide acceptance for selective use in agronomic crops (*1*). Although plant breeders have successfully incorporated both insect and disease resistance into cultivars of many crops, there has been no concerted effort to develop crops with superior competitive ability with weeds.

Differences in competitive ability have been reported among cultivars or inbred lines of *Sorghum bicolor* (L.) Moench (*2*), *Oryza sativa* L. (*3*), *Triticum aestivum* L. (*4*), and *Glycine max* (L.) Merr. (*5*) exposed to various weed species. In these instances, competitive advantage was attributed to rapid growth and an outstanding ability to compete for light, water, or nutrients.

Allelopathy, the inhibition of growth of one species by chemicals released from another, occurs widely in natural plant communities (*6, 7*) and may regulate the density and distribution of species. Certain crop plants, including those in the genera *Avena, Triticum,* and *Secale* (*8*); *Hordeum* (*9*); *Bromus* (*10*); *Brassica* (*6*); and *Nicotiana* (*11*), are known to release toxic substances from their roots. The release of allelopathic chemicals may occur by exudation from living roots or may occur upon their death and decay. The only way man has utilized allelopathy to his agronomic advantage is by planting crops such as *Hordeum vulgare* L. as interim "smoother crops" to reduce weed infestations (*9*).

We hypothesized that predecessors of many species now grown for food or fiber, when growing in their wild habitat, may have possessed allelopathic substances which allowed them to compete effectively in their native plant community. This character may have been reduced or lost as plants were bred and selected for other desirable characteristics in a weed-free environment. Furthermore, resistance to insects and diseases has been incorporated into several crops by crossing with "wild types." We chose cucumber as a test species because it has considerable genetic diversity, its tolerance to effective herbicides is less than adequate, and it may be grown at high plant populations which would favor the success of allelopathy in the field.

We obtained the collection of cucumber seed from the U.S. Department of Agriculture, North Central Introduction Station, Ames, Iowa. The collection consisted of 526 accessions of *Cucumis sativus* L. and 12 accessions of eight related *Cucumis* species representing 41 nations of origin. We also included four commercial cultivars as reference standards. In the initial, unreplicated study, we planted four seeds of each accession with ten seeds of each indicator species in 7.62-cm styrofoam pots containing 270 g of quartz sand. As indicators, we chose a monocot (*Panicum miliaceum* L.) and a dicot

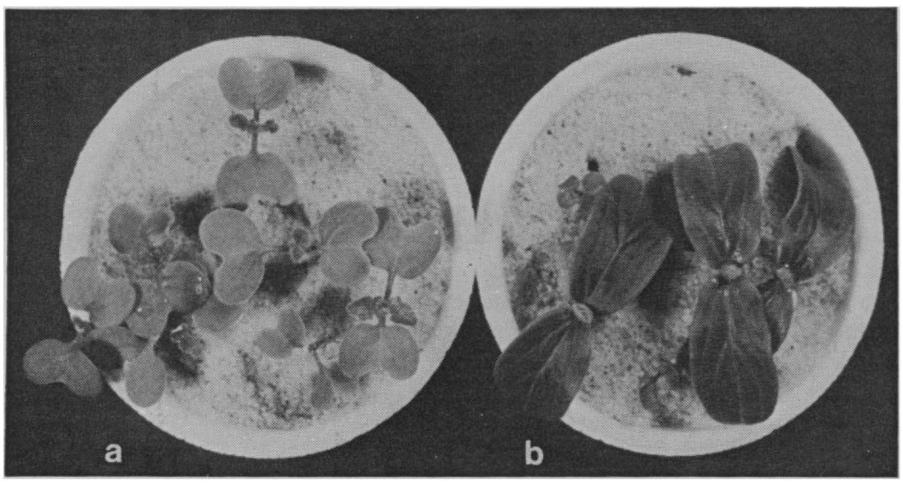

Fig. 1. Growth of *Brassica hirta* after 7 days (a) in the absence of cucumber and (b) in the presence of plant introduction 163221. Ten indicator seeds were planted in each pot.

Table 1. Growth of two indicator species after 10 days in the presence of selected plant introductions (PI) or cultivars of cucumber. Means with common letters within a column and within a test are not significantly different at $P = .05$ as determined by the least significant difference test. Weights are reported as percentages of control.

Brassica hirta		*Panicum miliaceum*	
Cucumber PI or cultivar	Fresh weight (%)	Cucumber PI or cultivar	Fresh weight (%)
	Test 1		
92806	41 a	135345	38 a
355052	53 a	227207	39 a
282450*	98 b	163216	75 b
193497	117 b	289698	80 b
	Test 2		
211728	39 a	267743	40 a
164679	47 a	169380	46 a
206053	100 b	206043	91 b
165499	101 b	165046	99 b
	Test 3		
263081	44 a	257286	38 a
257494	52 a	263081	45 a
188749	82 b	267088	75 b
183967	92 b	188807	126 c
	Test 4		
211728	41 a	172842	13 a
Wisconsin SMR-18	67 b	344444	31 a
271326	73 bc	Straight 8	60 b
Spartan Salad	93 c	164433	74 b

* *Cucumis zeyheri.*

(*Brassica hirta* Moench), which represented genera containing economically important weed species. We also grew control indicator plants in the absence of cucumbers. The plants were maintained for 10 days in controlled environment chambers which provided 15-hour days at 27°C (28,000 lu/m^2) and 9-hour nights at 21°C. Each pot received an initial watering of 80 ml of half-strength Hoagland's solution (*12*), which was supplied again on days 3, 4, 7, 8, and 9. The shoots of the plants were excised on day 10 and their numbers and fresh weights were recorded.

The initial study indicated that cucumber accessions and cultivars differ greatly in their ability to alter weed growth. The range for growth of *B. hirta* in the presence of cucumber was 4 to 125 percent of control growth with a mean of 78 percent based on 482 lines with acceptable germination. The range for *P. miliaceum* was 7 to 126 percent with a mean of 66 percent of control growth. Only 3 percent of the accessions tested inhibited growth by more than 75 percent. We expected that competition for nutrients, moisture, and light would be minimal during the first 10 days of growth, which was confirmed by the data obtained for the majority of lines. The severe toxicity exerted by some lines (Fig. 1) led us to suspect that they were releasing a substance or substances that inhibited indicator plant growth.

To confirm the results, selected toxic and nontoxic cucumber accessions from the initial study were planted with indicators as described above; three, four, or five replications were done, depending on the availability of seed. Three commercial cucumber cultivars were also included in these replicated experiments.

In general, the replicated experiments confirmed the findings of the initial study, although the mean growth reduction obtained with several replicates was not as great as that obtained with single observations. The superior accession reduced *P. miliaceum* growth by 87 percent (Table 1). In instances where severe growth inhibition occurred, there was a significant reduction in the number of indicators that emerged and in weight per plant. Accessions that exerted toxicity on one indicator were not necessarily outstanding against the other, although some were extremely effective on both. Several of the plant introductions were superior to cultivars, which indicates the potential for improvement. There was considerable variation among replicates, which might be anticipated because of the diversity maintained in these accessions by the methods of increasing seed lots (*13*). We saved the outstanding individuals from these tests so that we might later evaluate their progeny.

To substantiate the fact that allelopathy occurred and the results were not merely growth inhibition caused by other competitive factors, it was necessary to demonstrate toxicity to the indicator species grown in the absence of cucumbers. Cucumber seed were selected from two accessions that severely inhibited *P. miliaceum* growth and one accession that exerted no toxicity. Four seeds from each accession were placed in each of four pots in sterile sand, as described above. The drainage hole of each pot was fitted with a Tygon tube and stopcock to allow collection of leachate. The pots received 80 ml of nutrient solution initially, and were leached with an additional 120 ml of solution on days 4, 6, and 8. Ten-milliliter portions of the leachates collected on day 4 were applied to eight replications of *P. miliaceum* (ten seeds per lot) at the time of planting in 30 cm^3 of quartz sand in compartmented plastic trays. Fresh leachates were applied again on days 3 and 5. Control plants received nutrient solution at the same time intervals and all plants received nutrient solution on days 7 and 9. The shoots of the indicators were harvested and weighed on the tenth day after planting.

Table 2. Inhibition of emergence and growth of *Panicum miliaceum* by leachates transferred from pots containing one nontoxic and two toxic cucumber plant introductions (PI).

Cucumber PI	Inhibition (%)	
	Emergence	Fresh weight
257286	31*	38*
263081	29*	34*
109484	9	5

* Significantly different from control at $P = .01$.

Leachates from two toxic plant introductions applied to *P. miliaceum* in separate containers inhibited both the emergence of plants and their growth, while leachates from a nontoxic accession had no effect (Table 2). Examination of plants that failed to emerge indicated that the radicles had emerged from the seeds before growth terminated. Surviving plants were smaller than controls, but otherwise appeared normal. We concluded from this experiment that these particular cucumber accessions possess and release a growth inhibitor within 4 to 6 days after planting. Under the conditions of our tests, this occurred before the cotyledons were fully expanded.

We have demonstrated that within *C. sativus* there are accessions capable of severely inhibiting the growth of certain species of plants. Although these results are encouraging, it now must be demonstrated that allelopathy can contribute to the competitive ability of crops against important weed species under field conditions. If this can be proved, and if the factor is heritable, attempts could be made to incorporate the character into a commercial cultivar, as has been accomplished by plant breeding for insect and disease resistance.

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Sodium: Stimulus for Puddling Behavior by Tiger Swallowtail Butterflies, Papilio glaucus

Abstract. *Male* Papilio glaucus *butterflies are attracted to sand soaked with dilute aqueous solutions of sodium salts. A sodium ion concentration of 10^{-3} molar is sufficient to stimulate puddling behavior. Acquisition of sodium may be the main ecological function of puddling behavior.*

Adult Lepidoptera of several families are frequently seen feeding at the margins of puddles or on animal feces or carrion, sometimes in large groups (*1*, *2*). The insects extend their proboscises and imbibe fluid from the surface of the mud. On dry mud, fluid may be released from the proboscis onto the surface and then reimbibed (*2*).

Puddling behavior probably permits the butterflies to take in nutrients above those provided by larval nutrition or available from nectar; evaporation of water from the puddle and from the surrounding wet mud concentrates the desirable nutrients in the surface layer (*2*). Sugars and probably also amino acids (*3*) are available from nectar, and it has been suggested that puddling permits the insects to obtain an adequate supply of salt (*2*, *4*). Downes (*2*) has suggested that nutrients needed for probably greater flight activity by male butterflies may explain the common observation that males of most species are seen puddling much more often than are females. Here we show that puddling behavior by males of the eastern tiger swallowtail butterfly, *Papilio glaucus* L., is stimulated by sodium ions.

In June 1973, male tiger swallowtail butterflies were found puddling on mud along the northwest shore of the upper Six Mile Creek reservoir, 60 m from Burns Road and 4 km southeast of Ithaca, New York. On 7 June, at this site, ten plastic trays (44 by 30 by 6 cm) were set out in the form of a grid consisting of two rows of five trays (Fig. 1). Each tray was filled with an identical volume of children's sterilized play-sand (Campbell). Enough distilled water (1.5 liters) to saturate the sand was added to one tray in each of the two rows. Identical volumes of NaCl solutions in distilled water (1 percent, weight to volume), casein hydrolyzate (5 percent, weight to volume, "salt-free," Nutritional Biochemicals), and sucrose (5 percent, weight to volume), respectively, were added to three other trays in each row. The fifth tray in each row contained dry sand. Within each row, the positions of the trays were assigned at random with the stipulation that the two replicates of each treatment could not be adjacent to one another. A dead male tiger swallowtail butterfly was pinned on each tray to act as a decoy. From a raised bank several meters away, we watched the butterflies visiting the trays until puddling ceased. Distilled water was sprayed onto the trays to compensate for evaporation. On subsequent days the same experimental design was used to test other substances, each array of treatments being repeated for two consecutive days. The number of sampling visits (*5*) by butterflies to each tray was scored, and puddling activity was measured for each treatment (Table 1).

Although butterflies made frequent sampling visits to almost all trays, we found that extended puddling visits were restricted almost entirely to those trays containing sodium ions, regardless of which anion was also present (Table 1). The higher the sodium concentration (within the range we used), the more attractive was the sand surface as a puddling substrate. The lowest concentration at which we observed appreciable puddling was $10^{-4}M$ (experiment 8). However, since analysis of the sand at the end of experiment 8 revealed increases of as much as tenfold in sodium ion concentrations during the experiment (*6*), in spite of periodic additions of water to the trays, we can conclude only that the threshold concentration for puddling behavior lies below $10^{-3}M$.

On 15 June we watched tiger swallowtails puddling on exposed mud at an undisturbed site along Six Mile Creek

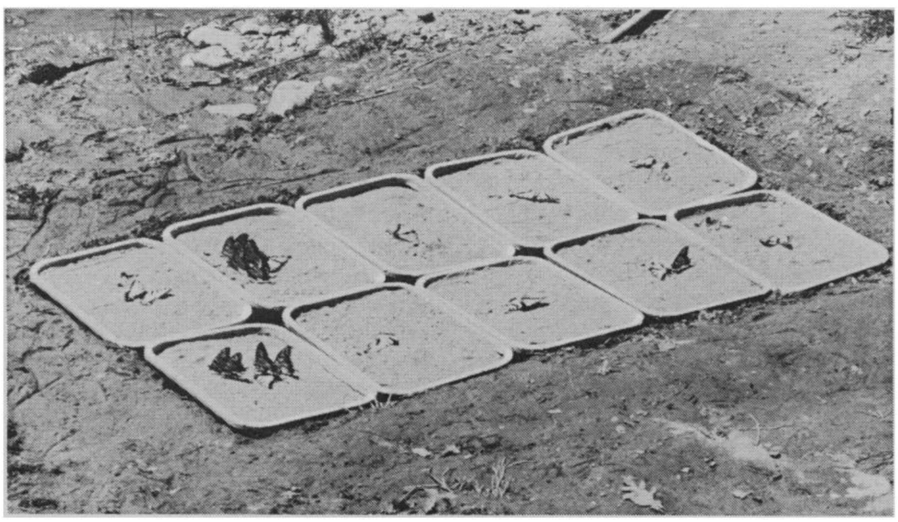

Fig. 1. Male tiger swallowtail butterflies puddling on sand trays during experiment 4 (10 June). The sand was soaked with $0.1M$ aqueous solutions of (far row, left to right) $CaCl_2$, NaCl, Na_3PO_4, $MgCl_2$, and KCl, and (near row, left to right) Na_3PO_4, $MgCl_2$, KCl, NaCl, and $CaCl_2$. A dead butterfly is pinned on each tray to serve as a decoy.