JOURNAL OF NEMATOLOGY

VOLUME 28 SEPTEMBER 1996 NUMBER 3

Journal of Nematology 28(3):259–267. 1996.

Allelopathic Interactions Involving Phenolic Acids[1]

U. Blum[2]

Abstract: A major concern regarding allelopathic interactions involving phenolic acids in no-till systems pertains to the fact that concentrations of individual phenolic acids recoverable from field soils are well below levels required for inhibition of germination and seedling growth in laboratory bioassays. Field soils contain a variety of phenolic acids as well as other toxic and nontoxic organic compounds that are available to interact with seeds and roots; whereas in laboratory bioassays, with few exceptions, single phenolic acids have been tested. Studies of mixtures of phenolic acids and other toxic (e.g., methionine) and nontoxic (e.g., glucose) organic compounds in laboratory bioassays indicate that the action of a single phenolic acid is not representative of the actions of such mixtures. Specifically, as the number of phenolic acids added to soil increased, concentrations of the individual phenolic acids required to bring about a growth inhibition declined. The addition of other organic compounds (e.g., glucose, methionine) to the soil also reduced the concentration of a phenolic acid (e.g., p-coumaric acid) required for growth inhibition. These results support the hypothesis that in the field mixtures of phenolic acids and other organic compounds can cause inhibitory effects even though the concentrations of individual compounds are well below their inhibitory levels.

Key words: allelopathy, *Cucumis sativus,* glucose, *Ipomoea hederacea,* joint action analysis, methionine, modified logistic equation, multiplicative analysis, phenolic acid, seedling growth.

Inhibition of weed germination and seedling growth by small grain residues in no-till systems may be due to: (i) the physical barriers and shading associated with the residue and reduced soil disturbance (31); (ii) the immobilization of nitrogen (7,15); and (or) (iii) allelopathic compounds (1,9,17,25–27,29). Although the relative significance of each of these factors for weed control in no-till systems is unclear, considerable emphasis has been placed on characterizing the role of allelopathic interactions in such systems over the last 10 years.

Potential allelopathic compounds identified in living and decomposing tissue of small grain-cover crops include phenolic acids (1,5,9,17), hydroxamic acids (10,20, 21,30), other organic acids (9,18,22,26,28), and volatile substances (6,8). Among these, phenolic acids have been most frequently identified as the phytotoxins.

A major concern regarding the role of phenolic acids as allelopathic agents in no-till systems pertains to the fact that concentrations of individual phenolic acids recoverable from field soils are well below levels required for inhibition of germination and growth in vitro (3–5,19,29). For example, the maximum concentration observed for p-coumaric acid, one of seven phenolic acids extracted from wheat no-till soils near Raleigh, North Carolina, was <0.03 μmol/g soil (5) (Fig. 1). Suppression of morning-glory seedling biomass by 10% and 20% required multiple applications of 0.04 and 0.13 μmol p-coumaric acid/g soil, respectively, under laboratory conditions (4). However, the action of a single compound is not necessarily representative of the action of a mixture of compounds.

Modifications of the effects of a phytotoxin (e.g., p-coumaric acid) on plant processes in the presence of other toxic or nontoxic compounds may occur in a num-

Received for publication 19 December 1995.

[1] Symposium paper presented at the Annual Meeting of the Society of Nematologists, 5–9 August 1995, Little Rock, AR.

[2] Professor of Botany, Department of Botany, North Carolina State University, Raleigh, NC 27695-7612.

The author thanks W. F. Boss, W. S. Chilton, R. C. Fites, S. R. Shafer, and T. R. Wentworth for their review of this manuscript and their constructive comments.

E-mail: udo_blum@ncsu.edu

The JOURNAL OF NEMATOLOGY for June (28:129–132) was issued 21 June 1996.

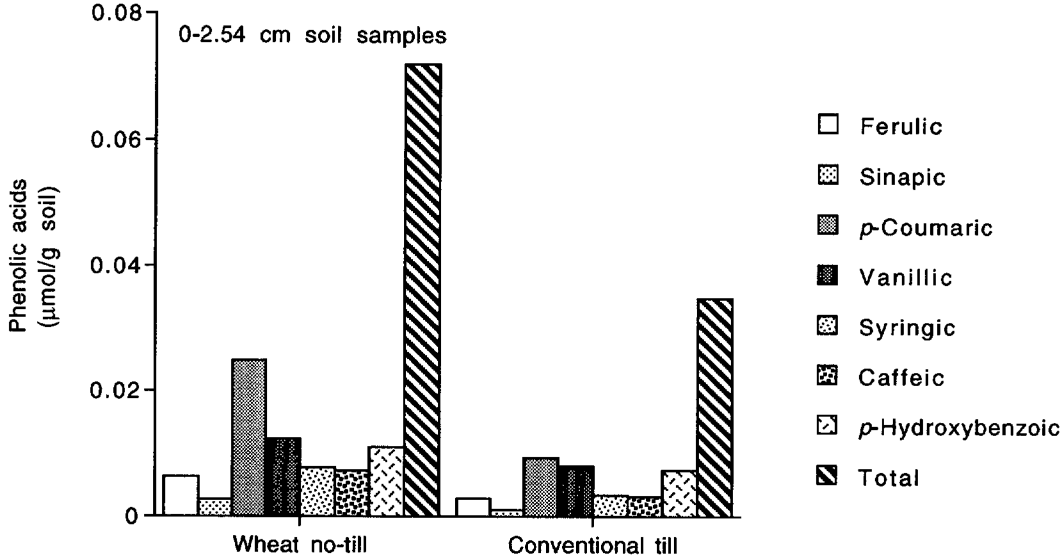


FIG. 1. Phenolic acids recovered from the top 2.54 cm of Cecil A_p soil in conventional-tilled plots without a cover crop and in wheat no-till plots. Data from Blum et al. (5).

ber of ways. If the sites or modes of action of two phytotoxins (e.g., phenolic acid mixtures) are similar, the effects on plant processes by a mixture of these phytotoxins could be antagonistic, synergistic, or additive when compared to the combined effects of the individual compounds. If their sites or modes of action are different (e.g., p-coumaric acid and methionine), the response probably will be some multiple of the individual effects. Nontoxic organic compounds (e.g., glucose) could also modify the magnitude of plant responses to a phytotoxic compound by either increasing or reducing the effectiveness or available concentration of the toxic compound. Therefore, the following hypothesis was tested in the laboratory: Mixtures of phenolic acids and other organic compounds can cause inhibitory effects even though the concentrations of individual compounds in a mixture are well below their individual inhibitory levels.

BIOASSAY SYSTEM

System: The bioassay system, its various component parts, and associated procedures have been described in detail in several publications (2–4,11,23); thus, only a short description of the system and the procedures is provided here. Seedlings were germinated in or transplanted into 155-ml plastic cups containing 150 g of a soil:sand mixture (1:2 by weight). The cucumber and morning-glory seedlings (*Cucumis sativus* L. cv. Early Green Cluster, or *Ipomoea hederacea* L. Jacquin., respectively), grown under light banks in the laboratory, were supplied with double-strength Hoagland's solution every other day (14). Water was added daily as needed. Solutions containing organic compounds (e.g., phenolic acids, methionine, glucose) of interest were added every other day and alternated with nutrient solution applications. Seedling biomass or absolute rates of leaf area expansion were determined.

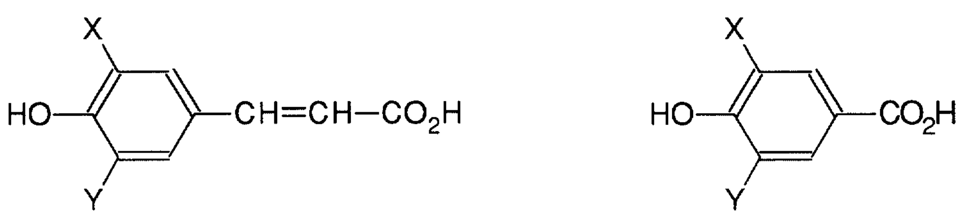
Data analysis: Similar joint action analysis (13) was used when the sites and(or) modes of action of compounds (e.g., phenolic acid mixtures) were similar and the compounds were substitutable. Multiplicative analysis (12) was used when the sites and(or) modes of action of compounds (e.g., p-coumaric acid and methionine) were different and noninteractive. A modified logistic equation (12) was used when the presence of a nontoxic compound (e.g., glucose) changed the effects of a phytotoxin (e.g., p-coumaric acid).

EFFECTS OF MIXTURES

The phenolic acids, amino acids, and carbohydrates were chosen for their differential rates of microbial utilization, different chemical characteristics, and(or) potential phytotoxicity. The selection of these compounds resulted from screening a range of concentrations of individual phenolic acids, amino acids, and carbohydrates for growth inhibition (4). Mixtures of phenolic acids and mixtures of p-coumaric acid and methionine were used to test how mixtures of toxins with similar and different sites (modes) of action, respectively, would affect growth. Mixtures of p-coumaric acid and glucose were used to test how nontoxic carbon sources might modify the behavior of a toxin.

Mixtures of phenolic acids: Cinnamic acid derivatives, such as ferulic, sinapic, p-coumaric and caffeic acids, and benzoic acid derivatives, such as vanillic, syringic, p-hydroxybenzoic, and protocatechuic acids (Fig. 2), have a broad range of phytotoxicities, but all have the same apparent mode of action. The primary effect of these phenolic acids on sensitive species appears to be a reduction of hydraulic conductivity and net nutrient uptake of roots and thus, eventually, growth (2). Cinnamic acid derivatives are generally more inhibitory than their corresponding benzoic acid derivatives. Relative toxicity to cucumber seedlings, for example, are as follows: ferulic acid and sinapic acid > p-coumaric acid, vanillic acid and syringic acid > caffeic acid, and p-hydroxybenzoic acid > protocatechuic acid (11).

Two approaches were taken to determine if the effects of individual phenolic acids in mixtures are additive, synergistic, or antagonistic when compared to the combined effects of the individual compounds. The first (3) was to apply a range of concentrations (0 to 0.5 μmol/g soil) either of ferulic or vanillic acid, or an equal-μmolar mixture (0.25 μmol/g soil each) of both compounds to a plant-soil system every other day starting when cucumber seedlings were 7 days old (Fig. 3). Leaf area of the cucumber seedlings was monitored non-destructively every other day just before phenolic acid treatment. Initially (day 9 to 11), the effects, based on similar joint action analysis, of the individual phenolic acids in the mixture for a pH 5.2 soil system were additive for absolute rates of leaf expansion (Fig. 3A). Subsequently (day 11 to day 17), however, the effects of the individual acids in the mixture became partially antagonistic (Fig. 3B). The effects of phenolic acids and phenolic acid mixtures on leaf expansion were influenced by soil acidity. For a pH 6.0 soil system the first significant inhibition of absolute rates of leaf expansion was delayed



p-Coumaric acid: X = Y = H
Caffeic acid: X = OH, Y = H
Ferulic acid: $X = OCH_3$, Y = H
Sinapic acid: $X = Y = OCH_3$

p-Hydroxybenzoic acid: X = Y = H
Protocatechuic acid: X = OH, Y = H
Vanillic acid: $X = OCH_3$, Y = H
Syringic acid: $X = Y = OCH_3$

FIG. 2. Structure of phenolic acids.

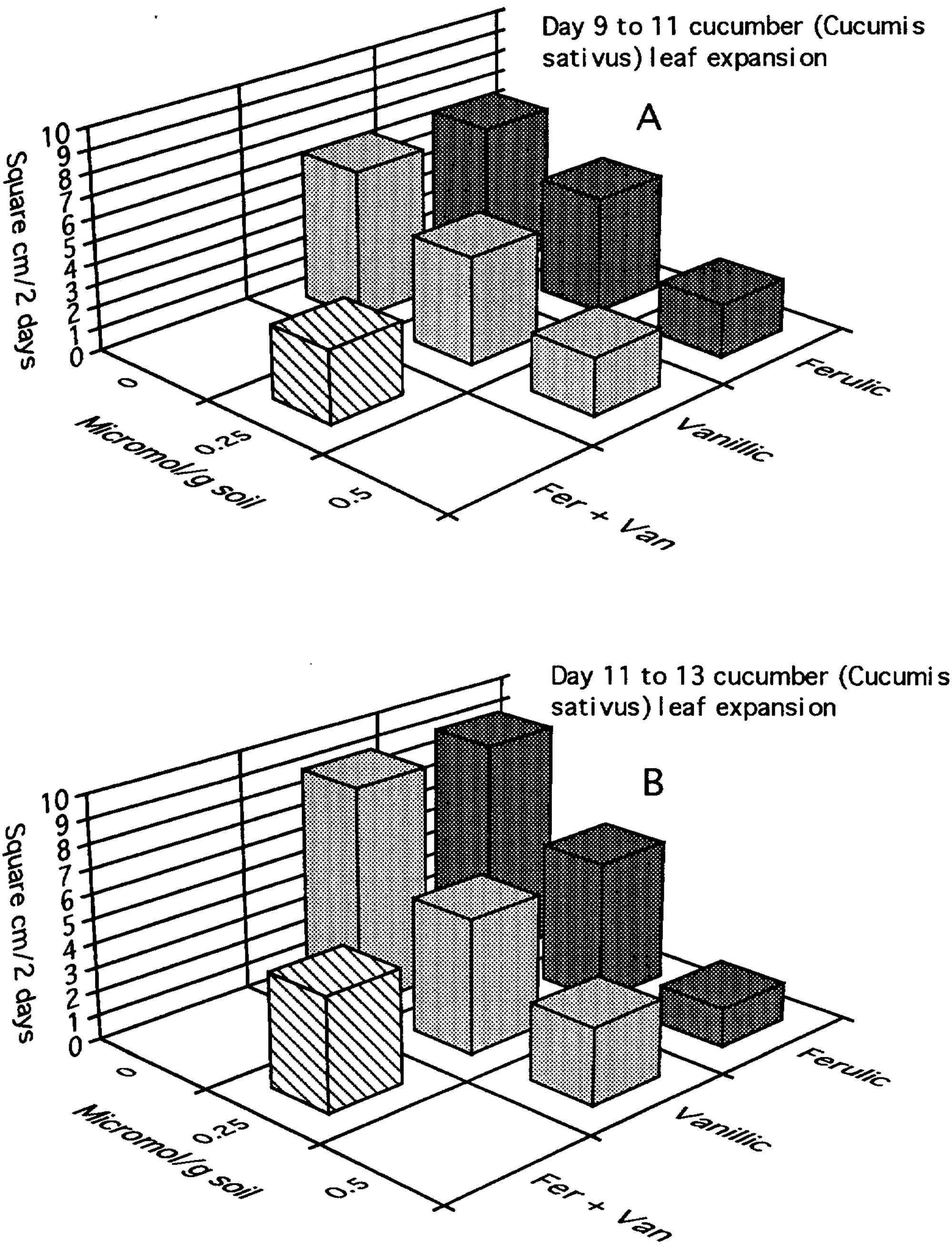


FIG. 3. Absolute rates of leaf expansion for 9- to 13-day-old cucumber seedlings growing in Portsmouth A_p soil treated with 19.6 μg/g NO_3-N and ferulic, vanillic, or an equal-molar mixture of the two phenolic acids. Nutrients and phenolic acids were supplied on alternate days. Growth rate after two, day 9 to 11 (A), and three, day 11 to 13 (B), phenolic acid treatments. Data from Blum et al. (3).

until day 11 to 13, and the effects of individual compounds in the mixture were additive (i.e., no antagonism was observed). No effects of phenolic acid on leaf expansion were observed in a pH 6.9 soil system. The pK_a of these phenolic acids is approximately 4.5. At pH 4.5, 50% of the molecules will be in an undissociated form. As the pH rises, more and more of the molecules will become charged (anionic) and as

TABLE 1. The joint action analysis of four phenolic acids on leaf area expansion of cucumber seedlings (11).

	p-Coumaric acid	Vanillic acid	p-Hydroxybenzoic acid
Ferulic acid	+	+	−
p-Coumaric acid		+	−
Vanillic acid			+

+ = additive; − = antagonistic.

such are less likely to move across cell walls and membranes of the roots (24).

The second approach (11) to studying the effects of phenolic acid mixtures was to use concentrations of individual phenolic acids and mixtures of phenolic acids that resulted in approximately the same level of inhibition for absolute rates of leaf area

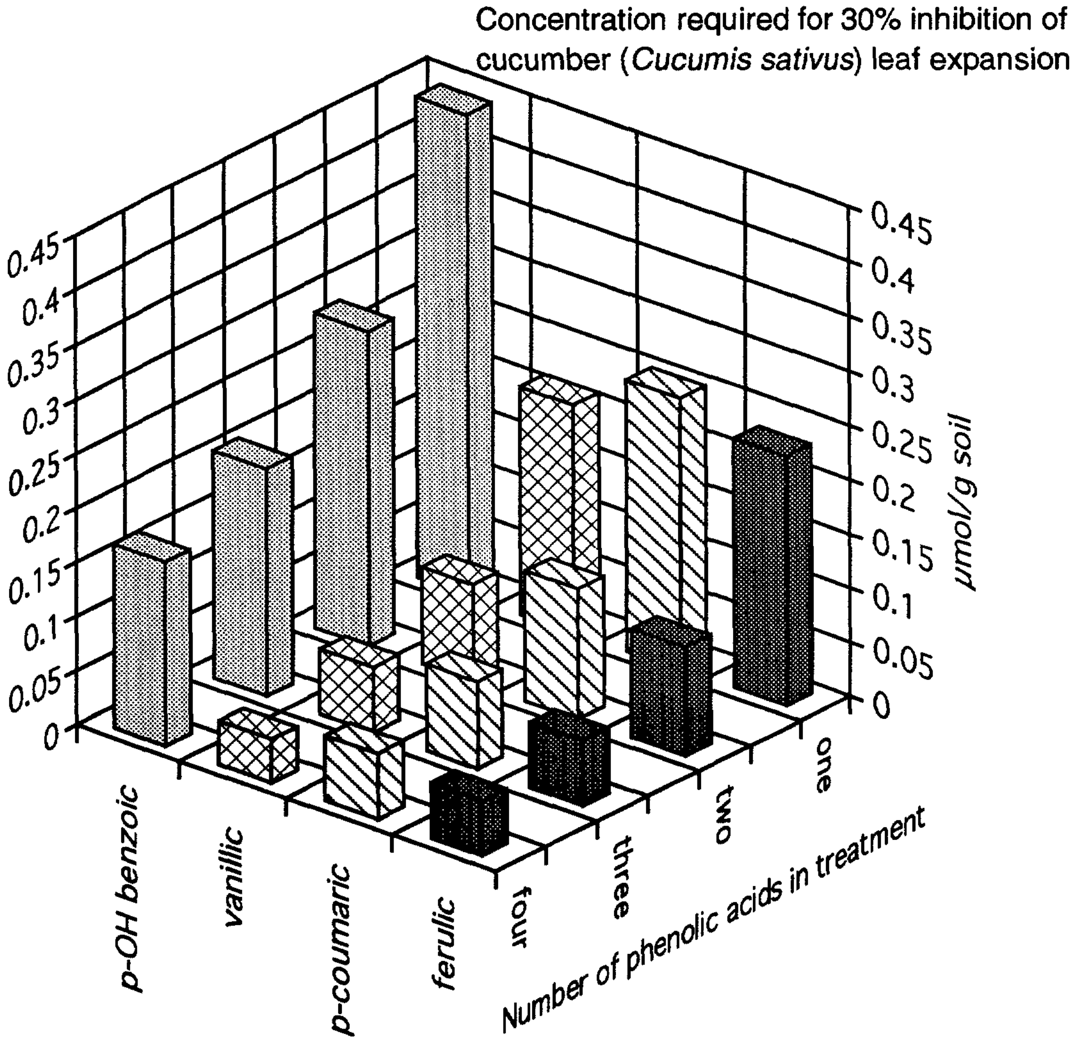


FIG. 4. Concentrations of each individual, any two-way, any three-way, or the four-way combination of phenolic acids required to reduce absolute rates of leaf expansion of cucumber seedlings by approximately 30%. Concentrations were empirically derived from the relative toxicities of phenolic acids. Seedlings were treated five times at 2-day intervals. Data from Gerig and Blum (11).

expansion. As the number of phenolic acids in a mixture was increased, the concentration of individual phenolic acids required in the mixture for a specific level of inhibition declined (Fig. 4). The effects of individual phenolic acids in mixtures of up to four phenolic acids were either additive or partially antagonistic (11) (Table 1). A 30% reduction of absolute leaf expansion, for example, required 0.23 μmol/g of ferulic acid but required only 0.05 μmol/g in the presence of 0.06, 0.17, and 0.04 μmol/g p-coumaric, p-hydroxybenzoic, and vanillic acids, respectively (Fig. 4). Thus, the joint action of phenolic acids, even though at times partially antagonistic, demonstrates that concentrations of individual phenolic acids in a mixture required for a given growth inhibition can decline as the number of phenolic acids in the mixture increases.

Methionine and p-coumaric acid: The toxic effects of methionine and p-coumaric acid on plant growth were different (4). In preliminary studies, the inhibitory effects of p-coumaric acid on water utilization and nutrient uptake of cucumber seedlings were not observed for methionine, but absolute rates of leaf area expansion were reduced by either compound. The sites or modes of action of p-coumaric acid and methionine are not known, but plant responses suggest that they are different for the two compounds. Based on this observation, multiplicative analysis (12) was used to analyze the data for morning-glory seedlings treated with p-coumaric acid and(or) methionine. A 20% biomass inhibition (Fig. 5), for example, of morning-glory seedlings required 0.13 μmol/g of soil of p-coumaric acid or 0.16 μmol/g of soil methionine, but in a mixture this level of inhibition was achieved with 0.063 μmol/g p-coumaric acid and 0.054 μmol/g methionine. Raising the NO_3-N level from 3.5 to 14 μg/g soil increased the concentration of p-coumaric acid required for a given level of inhibition but had little effect on the concentration of methionine required for inhibition (Fig. 5). Thus, the phenolic acid (e.g., p-coumaric acid) concentration required for inhibition of seedling growth can be increased in the presence of growth-stimulating substances (e.g., NO_3-N) and decreased in the presence of other growth inhibitors (e.g., methionine).

Glucose and p-coumaric acid: An experimental design similar to that described for methionine and p-coumaric acid was used to examine the interactions of p-coumaric acid and glucose (23). Glucose at concentrations of <4 μmol/g did not affect morning-glory seedling biomass. A 20% inhibition of seedling biomass required 0.42 μmol/g of p-coumaric acid in the absence of glucose and 0.33 μmol/g in the presence of 0.30 μmol/g glucose (Fig. 6). Pue et al. (23) observed that microbial utilization of p-coumaric acid in soil was reduced in the presence of various readily available carbon sources, such as glucose, phenylala-

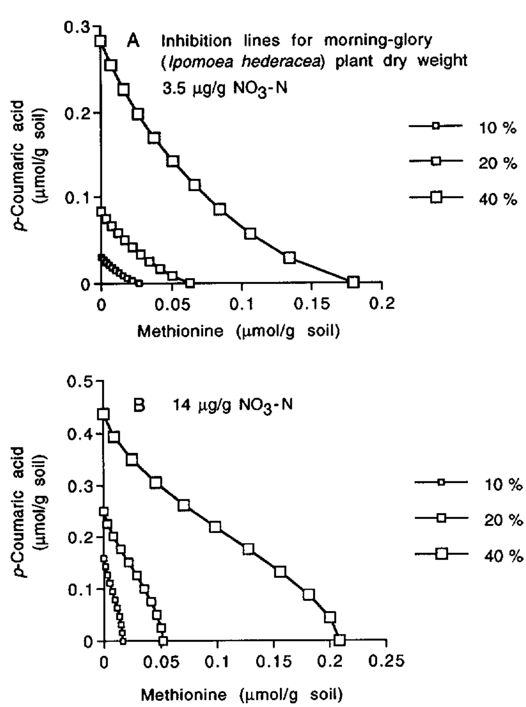


FIG. 5. Combinations of p-coumaric acid and methionine concentrations required to reduce morning-glory seedling biomass by 10%, 20%, and 40%. Seedlings were given five treatments at 2-day intervals. On alternate days seedlings were treated with either 3.5 (A) or 14 (B) μg/g NO_3-N. From Blum et al. 1993. Journal of Chemical Ecology 19:2791–2811. Used with permission.

nine, and p-hydroxybenzoic acid, but not methionine. This observation led Pue et al. (23) to suggest that greater utilization of glucose than p-coumaric acid by soil microbes increased the concentration of p-coumaric acid available for uptake by roots of morning-glory seedlings. Thus, the effectiveness of a given phenolic acid concentration in inhibiting seedling growth can be increased by differential utilization of a toxin (e.g., p-coumaric acid) and a more readily available nontoxic carbon source (e.g., glucose) by soil microbes.

Significance

All of these observations support the initial hypothesis that inhibitory effects of mixtures of phenolic acids and other organic compounds can occur when the concentrations of individual compounds in the mixture are well below their individual inhibitory levels. Inhibition of weed emergence or growth observed in wheat no-till systems is probably a result of such complex organic mixtures rather than the individual phytotoxins that are so often emphasized in the literature. Thus, dismissing the role of phenolic acids as allelopathic agents is not justified on the basis of their low specific concentrations in field soils. Finally, these observations implicate a significant role for soil pH as well as for available nitrogen and carbon sources in determining the magnitude of allelopathic effects of phenolic acids. Soil pH is important in determining the uptake of phenolic acids by roots (19,24). Available nitrogen and carbon sources, other than phenolic acids, are important in determining the rate of phenolic acid utilization as a carbon source by soil microbes and the production

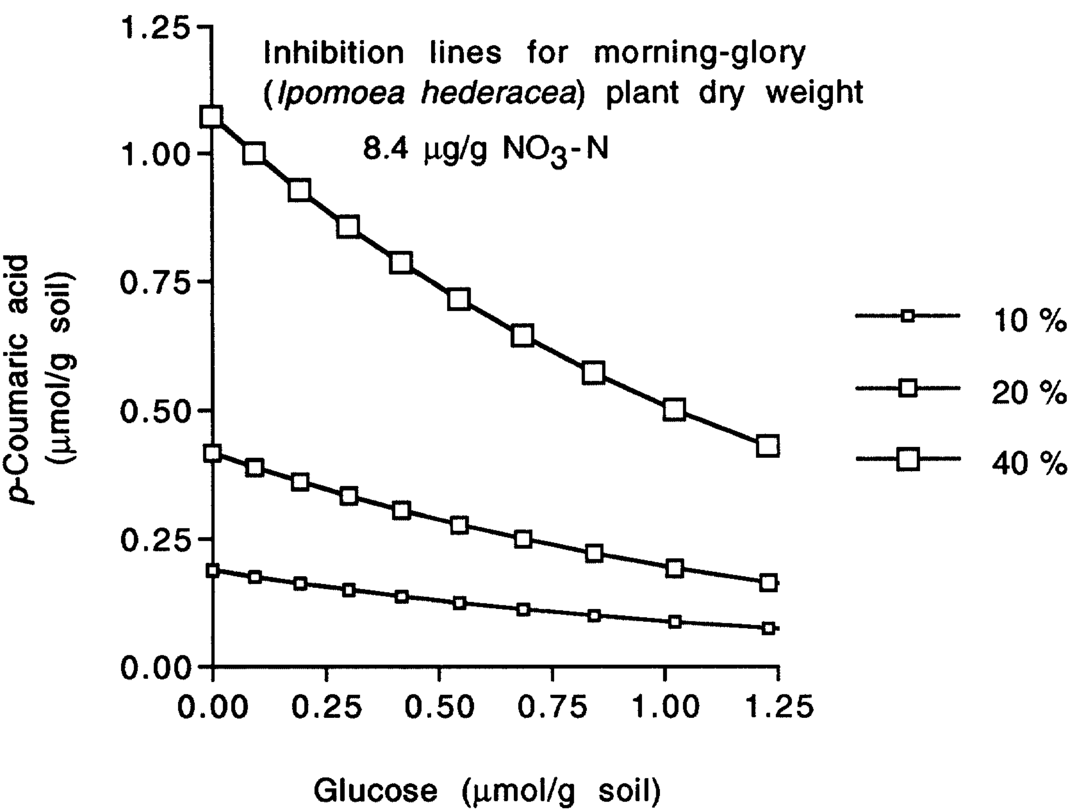


Fig. 6. Combinations of p-coumaric acid and glucose concentrations required to reduce morning-glory seedling biomass by 10%, 20%, and 40%. Seedlings were given five treatments at 2-day intervals. From Pue et al. 1995. Journal of Chemical Ecology 21:833–847. Used with permission.

of other toxins (e.g., organic acids). These observations may also explain why cover-crop residues have their highest inhibitory activities immediately after desiccation (16), the time of maximum release of water-soluble compounds, and why cover crops with similar phenolic acid content have such differing inhibitory activities in the field and in laboratory experiments.

Literature Cited

1. Barnes, J. P., A. R. Putnam, and A. Burke. 1986. Allelopathic activity of rye (*Secale cereale* L.). Pp. 271–286 *in* A. R. Putnam and C. S. Tang, eds. The science of allelopathy. New York; John Wiley.

2. Blum, U. 1995. The value of model plant-microbe-soil systems for understanding processes associated with allelopathic interactions. Pp. 127–131 *in* Inderjit, K. M. M. Dakshini, and F. A. Einhellig, eds. Allelopathy: Organisms, processes, and applications. ACS Symposium Series No. 582. Washington D.C.: American Chemical Society.

3. Blum, U., T. M. Gerig, and S. B. Weed. 1989. Effects of mixtures of phenolic acids on leaf area expansion of cucumber seedlings grown in different pH Portsmouth A_1 soil materials. Journal of Chemical Ecology 15:2413–2423.

4. Blum, U., T. M. Gerig, A. D. Worsham, and L. D. King. 1993. Modifications of allelopathic effects of p-coumaric acid on morning-glory seedling biomass by glucose, methionine, and nitrate. Journal of Chemical Ecology 19:2791–2811.

5. Blum, U., T. R. Wentworth, K. Klein, A. D. Worsham, L. D. King, T. M. Gerig, and S.-W. Lyu. 1991. Phenolic acid content of soils from wheat-no till, wheat-conventional till, and fallow-conventional till soybean cropping systems. Journal of Chemical Ecology 17:1045–1068.

6. Bradow, J. M. 1991. Relationships between chemical structure and inhibitory activity of C_6 through C_9 volatiles emitted by plant residues. Journal of Chemical Ecology 17:2193–2212.

7. Broadbent, F. E., and K. B. Tyler. 1962. Laboratory and greenhouse investigations of nitrogen mobilization. Soil Science Society of America Proceedings. 27:459–462.

8. Buttery, R. G., C.-J. Xu, and L. C. Ling. 1985. Volatile components of wheat leaves (stems): Possible insect attractants. Journal of Agricultural and Food Chemistry 33:115–117.

9. Chou, C. H., and Z. A. Patrick. 1976. Identification and phytoxic activity of compounds produced during decomposition of corn and rye residues in soil. Journal of Chemical Ecology 2:369–387.

10. Gagliardo, R. W., and W. S. Chilton. 1992. Soil transformation of 2(3H)-benzoxazolone of rye into phytotoxic 2-amino-3H-phenoxazin-3-one. Journal of Chemical Ecology 18:1683–1691.

11. Gerig, T. M., and U. Blum. 1991. Effects of mixtures of the phenolic acids on leaf area expansion of cucumber seedlings grown in Portsmouth B_1 soil material. Journal of Chemical Ecology 17:29–40.

12. Gerig, T. M., and U. Blum. 1993. Modification of an inhibitory curve to account for effects of a second compound. Journal of Chemical Ecology 19: 2783–2790.

13. Gerig, T. M., U. Blum, and K. Meier. 1989. Statistical analysis of the joint action inhibitory action of similar compounds. Journal of Chemical Ecology 15:2403–2412.

14. Hoagland, D. R., and D. J. Arnon. 1950. The water-culture method of growing plants without soil. California Agricultural Experiment Station Circular 347. Berkeley, CA.

15. Kimber, R. W. L. 1973. Phytotoxicity from plant residues III. The relative effects of toxins and nitrogen immobilization on the germination and growth of wheat. Plant and Soil 38:543–555.

16. Lehman, M. E. 1993. Effects of allelopathy on plant emergence and growth as modified by physical factors and root distribution. M.S. thesis, North Carolina State University, Raleigh, NC.

17. Liebl, R. A., and A. D. Worsham. 1983. Inhibition of morning glory (*Ipomoea lacunosa* L.) and certain other weed species by phytotoxic components of wheat (*Triticum aestivum* L.) straw. Journal of Chemical Ecology 9:1027–1043.

18. Lynch, J. M. 1977. Phytotoxicity of acetic acid produced in anaerobic decomposition of wheat straw. The Journal of Applied Bacteriology 42:81–87.

19. Lyu, S.-W., U. Blum, T. M., Gerig, and T. E. Brien. 1990. Effects of mixtures of phenolic acids on phosphorus uptake by cucumber seedlings. Journal of Chemical Ecology 16:2559–2567.

20. Nair, G. N., C. J. Whitenack, and A. R. Putnam. 1990. 2,2′-oxo-1,1′-azobenzene a microbially transformed allelochemical from 2,3-benzoxazolinone: I. Journal of Chemical Ecology 16:353–364.

21. Niemeyer, H. M., E. Pesel, S. V. Copaja, H. R. Bravo, S. Franke, and W. Francke. 1989. Changes in hydroxamic acid levels of wheat plants induced by aphid feeding. Phytochemistry 28:447–449.

22. Patrick, Z. A. 1971. Phytotoxic substances associated with the decomposition in soil of plant residues. Soil Science 111:13–18.

23. Pue, K. J., U. Blum, T. M. Gerig, and S. R. Shafer. 1995. Mechanism by which noninhibitory concentrations of glucose increase inhibitory activity of p-coumaric acid on morning-glory seedling biomass accumulation. Journal of Chemical Ecology 21: 833–847.

24. Shann, J., and U. Blum. 1987. Uptake of ferulic and p-hydroxybenzoic acid by *Cucumis sativus*. Phytochemistry 26:2959–2964.

25. Shilling, D. G., L. A. Jones, A. D. Worsham, C. E. Parker, and R. F. Wilson. 1986. Isolation and identification of some phytotoxic compounds from aqueous extracts of rye (*Secale cereale* L.). Journal of Agricultural and Food Chemistry 34:633–638.

26. Shilling, D. G., R. A. Liebl, and A. D. Worsham. 1985. Rye (*Secale cerale* L.) and wheat (*Triticum aestivum* L.) mulch: The suppression of certain weeds and the isolation and identification of phytotoxins. Pp. 243–271 in A. C. Thompson, ed. The chemistry

of allelopathy: Biochemical interactions among plants. ACS Symposium Series 268. Washington D.C.: American Chemical Society.

27. Shillling, D. G., A. D. Worsham, and D. A. Danehower. 1986. Influence of mulch, tillage, and diphenamid on weed control, yield, and quality in no-till flue-cured tobacco (*Nicotiana tabacum*). Weed Science 34:738–744.

28. Tang, C. S., and A. C. Waiss. 1978. Short-chain fatty acids as growth inhibitors in decomposing wheat straw. Journal of Chemical Ecology 4:225–232.

29. Waller, G. R., E. G. Krenzer Jr., J. K. McPherson, and S. R. McGown. 1987. Allelopathic compounds in soil from no tillage vs. conventional tillage in wheat production. Plant and Soil 98:5–15.

30. Willard, J. I., and D. Penner. 1976. Benzoxazinones: Cyclic hydroxamic acids found in plants. Residue Reviews 64:67–76.

31. Worsham, A. D. 1989. Current and potential techniques using allelopathy as an aid in weed management. Pp. 275–291 in C. H. Chou and G. R. Waller, eds. Phytochemical ecology: Allelochemicals, mycotoxins, and insect pheromones and allomones. Taipei, Taiwan: Academia Sinica Monograph Series No. 9.