The emergence of grass root chemical ecology

Stephen O. Duke*

Natural Products Utilization Research Unit, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 8048, University, MS 38677

Allelopathy is defined by most scientists as the adverse effect of one plant species on another through production of phytotoxins (allelochemicals), although more expansive definitions have been formulated. Allelopathy is but one component of plant/plant interference, the other being competition for resources such as nutrients, light, and water. Allelopathy has been a recognized phenomenon for many years (1), but prominent ecologists have argued that allelopathy is seldom a significant component of interference (e.g., ref. 2). This point of view was bolstered by the lack of scientific rigor of much of the allelopathy research that attempted to explain allelopathy through the effects of known, weakly phytotoxic, easy-to-quantify phytochemicals such as ferulic acid. More recent studies using bioassay-guided isolation and subsequent structure determination of potent, root-exuded phytotoxins built strong evidence for allelopathy, especially in grass species (reviewed in refs. 3–5, and see Table 1). The article by Bertin *et al.* (6) in this issue of PNAS adds significantly to this growing body of supportive literature.

The work provides clear evidence of a novel, root-exuded allelochemical produced by an allelopathic grass, a variety of a *Festuca rubra* subspecies. It establishes that *m*-tyrosine is a highly active allelochemical causing most, if not all, of the effects of the root exudate of this allelopathic fescue variety described in that article and an earlier one (7).

Although some nonprotein amino acids have functions in plant primary metabolism (e.g., δ-aminolevulinic acid), others are thought to be involved in protection of plants from a variety of biotic threats, particularly herbivores. The modes of action of these compounds range from direct neurotoxicity, such as produced by β-*N*-methylamino-L-alanine (8), to incorporation into proteins to produce aberrant molecules, leading to multiple physiological problems (9). Nonprotein amino acids have previously been implicated in allelopathy. For example, mimosine has been associated with allelopathy of the legume tree *Leucaena leucocephala* (10). L-DOPA, a compound structurally related to *m*-tyrosine, has been implicated in allelopathy of *Mucuna pruriens* (11). Roots of pea (*Pisum sativa*) exude β-(3-isoxazolin-5-on-2yl)-alanine, which inhibits root growth on nonlegume plant species (12), although this nonprotein amino acid is much less phytotoxic than *m*-tyrosine.

Finding that *m*-tyrosine is a potent phytotoxin leads to many interesting questions deserving further inquiry. First, how does the producing species protect itself from autotoxicity? It seems that *m*-tyrosine is broadly phytotoxic with some differences in plant species susceptibility, so what mechanism does the producing plant use to avoid the effects seen on other species? Does the plant avoid accumulation of the compound by secreting it almost as quickly as it is produced, in a manner similar to that of *Sorghum* species that produce the allelochemical sorgoleone only in root hairs that secrete it rapidly (13)? Apparently this is not the mechanism, because Bertin *et al.* (6) indicate that although the dry weight of the root exudate consists of up to 43% *m*-tyrosine, it is also a relatively abundant metabolite of the root. Is the compound sequestered into intracellular or intercellular locations where it can do little or no harm? Duke *et al.* (14) discuss this strategy for avoidance of allelochemical autotoxicity. Bertin *et al.* (7) found intracellular bodies in roots that might be associated with *m*-tyrosine sequestration. Or, is the plant resistant at a molecular target site? If the latter, this information could be helpful in determination of the mechanism of action of *m*-tyrosine on target species.

Allelopathy has been a recognized phenomenon for many years.

Bertin *et al.* (6) have circumstantial evidence that the mode of action of *m*-tyrosine is similar to that of some other nonprotein amino acids. That is, it substitutes for at least one protein amino acid (apparently phenylalanine in this case) during translation, resulting in dysfunctional proteins. Demonstration of significant loss of specific activity of phenylalanine-containing enzymes would support this hypothesis. An alternative hypothesis is that *m*-tyrosine is converted to L-DOPA, a known phytotoxin. Bertin *et al.* state that this is unlikely because L-DOPA is significantly less phytotoxic than *m*-tyrosine. However, *m*-tyrosine might be taken up more readily by plant cells than L-DOPA, leaving conversion to L-DOPA as a potentially more limiting step. Can *m*-tyrosine be converted to L-DOPA by a cell-free extract of a species susceptible to *m*-tyrosine? If so, is the process highly efficient *in vivo*? Synthetic proherbicides that are inactive at the molecular target site are much more effective when applied to intact plants than the active molecule to which they are converted *in vivo*. This is caused by superior cuticular and cellular uptake of the proherbicide. Some potent natural phytotoxins, such as hydantocidin (15), are protoxins.

How does the plant synthesize *m*-tyrosine? L-phenylalanine is a precursor of *m*-tyrosine synthesis in at least some animal systems (16). Will isotopically labeled phenylalanine fed to roots or cell-free preparations of roots of allelopathic fescue generate labeled *m*-tyrosine? If production of *m*-tyrosine is caused by one enzyme, can the gene for it be manipulated to produce fescue lines with enhanced allelopathic activity or to impart allelopathy to other species? The genetic components for root-specific production and secretion, as well as resistance, might be required for practical success. Nevertheless, this could be a simpler approach to transgenically producing weed-fighting plants than genetically engineering whole biosynthetic pathways (e.g., ref. 17).

Chemicals from target plant species have recently been found to induce both rice (18) and sorghum (19) to increase biosynthesis of their root-secreted allelochemicals, although in both cases, synthesis is constitutive. This phenomenon has not been explored with *m*-tyrosine synthesis, but water stress was found to increase its production (7).

In summary, the work of Bertin *et al.* (6) provides another convincing example of allelopathy, complete with the identi-

Author contributions: S.O.D. wrote the paper.

The author declares no conflict of interest.

See companion article on page 16964.

*E-mail: sduke@olemiss.edu.

Table 1. Highly phytotoxic root-secreted allelochemicals by grasses (5, 6)

Species	Allelochemicals	Induced by other species	Mode of action
Sorghum spp.	Sorgoleone	Yes	Photosystem II inhibition *p*-hydroxyphenylpyruvate dioxygenase inhibition
Oryza sativa	5,7,4′-trihydoxy-3′,5′-dimethoxyflavone, 2-isopropyl-5-acetoxy-cyclohexene-2-one-1, momilactone B	Yes	Unknown
F. rubra	*m*-tyrosine	?	Dysfunctional proteins? DOPA formation? Other?

fication of a highly potent allelochemical and a credible means of delivery to target plants. Practical applications of such findings are potentially significant. There are currently few economically viable alternatives to synthetic herbicides, the most heavily used of all pesticides. The most successful transgenic crops are those with transgenes imparting herbicide resistance (20), sustaining the widespread use of synthetic herbicides. The genetic information resulting from recent findings in allelopathy such as those reported by Bertin *et al.* has the potential to alter this situation.

1. Willis RJ (1985) *J History Biol* 18:71–102.
2. Harper JL (1977) *Population Biology of Plants* (Academic, London).
3. Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) *Annu Rev Plant Biol* 57:233–266.
4. Bertin C, Yang XH, Weston LA (2003) *Plant Soil* 256:67–83.
5. Duke SO, Baerson SR, Rimando AM, Pan Z, Dayan FE, Belz RG (2007) in *Novel Biotechnologies for Biocontrol Agent Enhancement and Management*, eds Vurro M, Gressel J (Springer, Dordrecht, The Netherlands), pp 75–86.
6. Bertin C, Weston LA, Huang T, Jander G, Owens T, Meinwald J, Schroeder FC (2007) *Proc Natl Acad Sci USA* 104:16964–16969.
7. Bertin C, Paul RN, Duke SO, Weston LA (2003) *J Chem Ecol* 29:1919–1937.
8. Schneider D, Wink M, Sporer F, Lounibos P (2002) *Naturwissenschaften* 89:281–294.
9. Lambein F, Kuo YH, Rozan P, Ikdegami F (2001) *R Soc Chem* 269:580–583.
10. Xuan TD, Elzaawely AA, Deba F, Fukuta M, Tawata S (2006) *Agron Sustain Dev* 26:89–97.
11. Nishihara E, Parvez MM, Araya H, Fujii Y (2004) *Plant Growth Regul* 42:181–189.
12. Schenk SU, Werner D (1991) *Phytochemistry* 30:467–470.
13. Czarnota MA, Paul RN, Weston LA, Duke SO (2003) *Int J Plant Sci* 164:861–866.
14. Duke SO, Duke MV, Paul RN, Ferreira JFS, Vaughn KC, Canel C, Tellez MR, Rimando AM, Smeda RJ (1999) in *Recent Advances in Allelopathy*, eds Macias FA, Galindo JCG, Molinillo JMG, Cutler HG (Servicio e Publicaciones, University of Cádiz, Cádiz, Spain), Vol 1, pp 211–218.
15. Fonné-Pfister R, Chemla P, Ward E, Giradet M, Kreutx KD, Hanzatko RB, Fromm HH, Schär H-P, Grütter MG, Gowan-Jacob SW (1996) *Proc Natl Acad Sci USA* 93:9431–9436.
16. Ishimitsu S, Fujimoto S, Ohara A (1980) *Chem Pharm Bull* 28:1653–1655.
17. Duke SO (2003) *Trends Biotechnol* 21:182–195.
18. Kong C, Xu X, Zhou B, Hu F, Zhang C (2004) *Phytochemistry* 65:1123–1128.
19. Dayan FE (2006) *Planta* 224:339–346.
20. Duke SO (2005) *Pest Manag Sci* 61:211–218.