p-Fluorophenylalanine Selectively Inhibits Sporulation of Two Wilt Fungi

W. L. Biehn

Assistant Plant Pathologist, The Connecticut Agricultural Experiment Station, P.O. Box 1106, New Haven 06504.

Accepted for publication 2 October 1972.

ABSTRACT

The ED_{90} of DL-*p*-fluorophenylalanine (FPA) for inhibiting sporulation of *Ceratocystis ulmi* in shake culture was about 5 μg/ml vs. an ED_{90} for inhibiting total growth in shake culture and mycelial growth in still culture of > 450 μg/ml. The ED_{90} of FPA for inhibiting sporulation and mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* was about 30 and > 200 μg/ml, respectively, after 43-hr incubation. In the presence of FPA in shake culture, growth of these fungi, especially *C. ulmi*, changed from sporulation to mycelial.

Phytopathology 63:421-423

Systemic invasion of the vascular system of plants by wilt fungi depends in part on sporulation and transport of spores in the host(4). Thus, a compound that inhibits sporulation in the infected host could reduce the amount of wilt disease.

Van Andel (11) reported that *p*-fluorophenylalanine can protect cucumber seedlings against *Cladosporium cucumerinum* Ell. & Arth. and *Colletotrichum lagenarium* (Pass.) Ell. & Halst. when applied to the roots before inoculation. We observed that DL-*p*-fluorophenylalanine (FPA) has (i) a protective action against Fusarium wilt when applied to the roots of tomato plants, and (ii) a curative action when fed to tomato cuttings after inoculation (W. L. Biehn & A. E. Dimond, *unpublished data*). Cultural studies suggested that FPA might inhibit

sporulation of *Ceratocystis ulmi* (Buis.) without noticeably affecting growth. *Ceratocystis ulmi* is known to reproduce predominantly by budding in liquid shake culture (1) and did so in our experiments. Banfield (1) observed the typical budding type of reproduction for *C. ulmi* in sap displaced from diseased elms. The buds produced by *C. ulmi* in shake culture appear to be similar to those produced by the fungus in the diseased elm (1). In the shake-culture studies reported here, *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyd. & Hans. reproduced mostly by means of microconidia. This report describes the effect of FPA on sporulation of *C. ulmi* and *F. oxysporum* f. sp. *lycopersici* in shake culture and on the growth of these fungi in both shake and still culture.

For these studies the fungi were grown on a modified Czapek-Dox casamino acids medium. The medium contained 0.83% glucose, 0.25% $NaNO_3$, 0.08% KH_2PO_4, 0.04% $MgSO_4 \cdot 7H_2O$, 0.04% KCl, 0.008% $FeSO_4$, and 10% of a casamino acids medium prepared as previously described (5). The casamino acids medium and the glucose and salts portion of the Czapek-Dox medium were each autoclaved separately; the *p*-fluorophenylalanine solution was sterilized by Millipore filtration.

Inoculum was grown by adding a small plug of mycelium from a potato-dextrose agar slant to 75 ml of casamino acids medium in a 250-ml flask. After the fungi had grown for 3 days in shake culture, 0.3 ml of a spore suspension from these cultures was transferred to a fresh flask of casamino acids medium and grown for 2 more days. The resulting spores were centrifuged and washed with sterile deionized water. A 1-ml sample of the spore suspension adjusted to 1×10^8 cells for *C. ulmi* or 1×10^6 cells for *F. oxysporum* f. sp. *lycopersici* was added to 59 ml of a modified Czapek-Dox casamino acids medium containing various amounts of FPA or Tersan LSR (maneb, 80%, plus zinc). The 250-ml Erlenmeyer flasks were incubated for 43-72 hr at 21-23 C either on a Gyrotory (New Brunswick) Shaker (146 cycles/min) or in still culture. After incubation on the shaker, the spore concentration (spores/ml) was determined with a hemacytometer. Total fungal growth (increase in dry wt) was measured by washing the contents of each flask onto a 0.45-μ Millipore-type HA filter (Millipore Corp., Bedford, Mass.) and drying the mycelium and spores overnight at 80 C.

TABLE 1. Effect of *p*-fluorophenylalanine (FPA) on sporulation and growth of *Ceratocystis ulmi* after 43-48 hr

Concentration of FPA (μg/ml)	% Inhibition of		
		Growth[b]	
	Sporulation[a]	Still culture	Shake culture
5	91	7	23
10	95	12	45
25	98	38	53
75		59	72
150		65	80
450		68	82

[a] Data are averages of nine experiments. Blank spaces indicate that no data were taken.
[b] Data are averages of at least three experiments.

In shake culture, FPA at 5 and 10 μg/ml inhibited sporulation of *C. ulmi* 91 and 95%, respectively, compared with controls; total growth was inhibited 23 and 45%, respectively (Table 1). Even at 450 μg/ml of FPA, total growth of *C. ulmi* was only inhibited 82% in shake culture and 68% in still culture. In comparison, Tersan LSR inhibited sporulation and growth of *C. ulmi* about equally. About 45 μg/ml of Tersan LSR inhibited sporulation and growth in shake culture 97%, whereas 60 μg/ml inhibited growth in still culture 93%.

FPA at 25 μg/ml inhibited sporulation and total growth of *F. oxysporum* f. sp. *lycopersici* 87 and 71%, respectively, after 43 hr in shake culture and 72 and 26%, respectively, after 72 hr (Table 2). Thus, FPA inhibits sporulation and total growth of *F. oxysporum* f. sp. *lycopersici* in shake culture more after 43 hr than after 72 hr. In still culture, approximately 200 μg/ml of FPA were required to inhibit growth 80% after 43 and 72 hr.

C. ulmi and *F. oxysporum* f. sp. *lycopersici* sporulated best in shake culture, but their vegetative growth was best in still culture. A measure of the specificity of a compound for inhibiting sporulation of these fungi was obtained by determining the ratio of the effective dose for a 90% reduction in sporulation (ED_{90}) in shake culture to the effective dose for a 90% reduction of vegetative growth (ED_{90}) in still culture, compared to the controls. Thus, sporulation of *C. ulmi* was at least 90 times more sensitive to FPA than vegetative growth based upon ED_{90} values. In contrast, sporulation of *F. oxysporum* f. sp. *lycopersici* appears to be only four to seven times more sensitive to FPA than vegetative growth.

In shake culture, FPA resulted in a very noticeable increase in mycelial growth of *C. ulmi* along with a decrease in sporulation. When von Hofsten (7) grew *Ophiostoma multiannulatum* in the presence of 5-fluorodeoxyuridine (5-FUdR) or deoxyadenosine, he observed a similar change in morphology from conidial formation to hyphae. Recently, Keen et al. (10) have found that 5-FUdR, deoxyadenosine, gossypol, semicarbazide, and phenylhydrazine inhibited sporulation by *Verticillium albo-atrum* in shake culture and changed morphology from conidial formation to hyphal growth. Ethionine and certain purine and pyrimidine analogues inhibited spore formation in *Aspergillus niger* (2, 3). Other research on compounds with antisporulant activity is reported by Horsfall & Lukens (8, 9). It is interesting to note that purine, pyrimidine, and amino acid analogues can act as antisporulants. Amino acid analogues such as FPA may act as antisporulants by interfering with protein synthesis. Protein synthesis and nucleic acid

TABLE 2. Effect of *p*-fluorophenylalanine (FPA) on sporulation and growth of *Fusarium oxysporum* f. sp. *lycopersici* in culture

Concentration of FPA (μg/ml)	% Inhibition after 43 hr[a]			% Inhibition after 72 hr[a]		
		Growth			Growth	
	Sporulation	Still culture	Shake culture	Sporulation	Still culture	Shake culture
12.5	76	26	57	30	0	1
25	87		71	72	0	26
50	99		89	89	17	48
60						
90						
100	99	73	99	99	68	83
200		79			84	
450		92			98	

[a] Blank spaces indicate that no data were taken.

synthesis are closely linked and are both known to be required for cell division of organisms. FPA may selectively inhibit sporulation of *C. ulmi* because of its lethal incorporation into enzymes required for nucleic acid synthesis. Inhibitors of nucleic acid synthesis (5-FUdR, actinomycin, and aminopterin) result in the formation of hyphal cells in *O. multiannulatum*, which generally have a low DNA content, instead of conidia, which have a relatively high DNA content (6, 7).

Since sporulation of *C. ulmi* is more sensitive to FPA than sporulation of *F. oxysporum* f. sp. *lycopersici* is, FPA might have greater chemotherapeutic activity against Dutch elm disease than against Fusarium wilt of tomato.

LITERATURE CITED

1. BANFIELD, W. M. 1941. Distribution by the sapstream of spores of three fungi that induce vascular wilt diseases of elm. J. Agr. Res. 62:637-681.
2. BEHAL, F. J. 1959. Biochemical studies of spore formation in Aspergillus niger. Arch. Biochem. Biophys. 84:151-156.
3. BEHAL, F. J., & R. E. EAKIN. 1959. Inhibition of mold development by purine and pyrimidine analogs. Arch. Biochem. Biophys. 82:439-447.
4. DIMOND, A. E. 1970. Biophysics and biochemistry of the vascular wilt syndrome. Annu. Rev. Phytopathol. 8:301-322.
5. DIMOND. A. E., G. H. PLUMB, E. M. STODDARD, & J. G. HORSFALL. 1949. An evaluation of chemotherapy and vector control by insecticides for combating Dutch elm disease. Conn. Agr. Exp. Sta. (New Haven) Bull. 531. 69 p.
6. HOFSTEN, A. VON. 1963. A study of the DNA content of different cell types of Ophiostoma multiannulatum. Physiol. Plant. 16:709-718.
7. HOFSTEN, A. VON. 1964. The effect of fluorodeoxyuridine and deoxyadenosine on growth and morphogenesis of Ophiostoma multiannulatum. Physiol. Plant. 17:177-185.
8. HORSFALL, J. G., & R. J. LUKENS. 1966. Selectivity of fungicides. Conn. Agr. Exp. Sta. (New Haven) Bull. 676. 24 p.
9. HORSFALL, J. G., & R. J. LUKENS. 1968. Aldehyde traps as antisporulants for fungi. Conn. Agr. Exp. Sta. (New Haven) Bull. 694. 27 p.
10. KEEN, N. T., M. C. WANG, MARGARET LONG, & D. C. ERWIN. 1971. Dimorphism in Verticillium albo-atrum as affected by initial spore concentration and antisporulant chemicals. Phytopathology 61:1266-1269.
11. VAN ANDEL, O. M. 1962. Fluorophenylalanine as a systemic fungicide. Nature 194:790.