Phytochemistry, Vol. 28, No. 11, pp. 2963–2969, 1989.
Printed in Great Britain.

0031–9422/89 $3.00+0.00

TOLERANCE AND METABOLISM OF FURANOCOUMARINS BY THE PHYTOPATHOGENIC FUNGUS *GIBBERELLA PULICARIS* (*FUSARIUM SAMBUCINUM*)

ANNE E. DESJARDINS,* GAYLAND F. SPENCER and RONALD D. PLATTNER

U.S. Department of Agriculture, Agricultural Research Service, Northern Regional Research Center, 1815 North University Street, Peoria, IL 61604, U.S.A.

(*Received in revised form* 3 *April* 1989)

Key Word Index—*Pastinaca sativa*; Umbellifereae; parsnip; *Gibberella pulicaris* (*Fusarium sambucinum*); phytoalexin metabolism; furanocoumarins.

Abstract—Sixty-two strains of *Gibberella pulicaris* (anamorph: *Fusarium sambucinum*) from diseased plants and from soil were tested for tolerance of the furanocoumarin xanthotoxin *in vitro*. Twenty-one (88%) of the plant-derived strains and two (5%) of the soil-derived strains were highly tolerant of xanthotoxin. Sixteen selected strains were tested further against 16 furanocoumarins or furanocoumarin precursors. All plant-derived strains tested were highly tolerant of and, in most cases, able to completely metabolize all 16 compounds. Most soil-derived strains tested were tolerant of furanocoumarin precursors but sensitive to certain furanocoumarins. Linear compounds methoxylated at C-8 appeared more toxic than both those unsubstituted and those with longer-chain ethers. Tolerance of angelicin, xanthotoxin, pimpinellin and isopimpinellin correlated in large part with their metabolism. All strains that were highly virulent on *Pastinaca sativa* root were tolerant of xanthotoxin, which is corroboration that xanthotoxin is a phytoalexin in *P. sativa*.

INTRODUCTION

Furanocoumarins are plant constituents that are characteristic of the Rutaceae and Umbelliferae but also occur widely in members of other plant families [1]. Although furanocoumarins are often present at low levels constitutively, their concentrations in plant tissues can greatly increase following fungal invasion [2–4]. Their induction by fungi and their *in vitro* fungitoxicity [3, 4] are evidence that furanocoumarins act as antimicrobial defence compounds, i.e. phytoalexins, in some plants. As plants have evolved phytoalexins that limit fungal growth in their tissues, fungi have adapted, in many cases, by evolving means to tolerate their hosts' phytoalexins. The ability of plant pathogenic fungi to metabolize, and thus detoxify, phytoalexins and the importance of detoxification for pathogenesis have been demonstrated in several systems [5]. Van Etten and coworkers, in particular, have shown that high virulence on pea of *Nectria haematococca* (anamorph: *Fusarium solani*) requires the ability to metabolize the pea phytoalexin pisatin [6].

Gibberella pulicaris (Fr.) Sacc. (anamorph: *F. sambucinum* Fuckel) is a cosmopolitan plant parasite and soil saprophyte [7]. Our collection contains more than 60 strains of this fungus which have been obtained from diseased tissues of various plants and from soil collected in widely separated geographic locations. These strains have demonstrated a wide range of natural variation for a number of traits that may be relevant to plant pathogenicity, including production of trichothecene toxins [4, 8] and metabolism of the potato phytoalexins lubumin [9] and rishitin [10]. The goals of the present study were to investigate the toxicity of furanocoumarins to field strains of *G. pulicaris*, to identify structural requirements for furanocoumarin toxicity, and to determine if furanocoumarin tolerance is related to furanocoumarin metabolism and if furanocoumarin tolerance is important for a high level of virulence on a furanocoumarin-producing plant, *Pastinaca sativa* (parsnip).

*Author to whom correspondence should be addressed.

RESULTS AND DISCUSSION

Geographic origin, habitat, source and strain number for the 62 field strains of *G. pulicaris* investigated in this study are given in Table 1. In order to determine if naturally occurring strains differ in tolerance of furanocoumarins, all 62 field strains were evaluated for tolerance of the linear furanocoumarin xanthotoxin by measurement of radial mycelial growth in duplicate plates amended at 200 and 400 μg per ml (200 μg per ml data shown in Fig. 1; 400 μg per ml data not shown). Strains with a radial growth rate more than 50% of controls were rated as highly tolerant. Neither high tolerance nor high sensitivity to furanocoumarins was restricted to strains collected from any particular geographic area; high furanocoumarin tolerance, however, did appear to be associated with adaptation of fungal strains for plant pathogenesis. Twenty-one of 24 strains isolated from diseased plant tissue were highly tolerant of xanthotoxin; their radial growth was more than 70% that of controls at both xanthotoxin concentrations tested. The only xanthotoxin sensitive strains in this group were R-110 and R-5920 from pine and KF-729 from potato. In contrast, only two of 38 strains found in soil or soil debris were highly tolerant of xanthotoxin. Although there were some differences in growth rate among field strains, these differences

Table 1. Geographic origin, strain number and source of *G. pulicaris* strains from soil and from diseased plants

Origin*		Strain numbers†	Source‡
I. Strains from soil			
Australia	NSW, pine nursery	5344, 5683, 5684, 5690, 5749, 5753	P. Nelson (L. Burgess) R. Caldwell (L. Burgess)
Australia	NSW, pasture	5867, 5928	P. Nelson
Australia	Mt. Kosciusko, mixed grasses	3032, 3084, 3245, 3248, 4170, 4187, 4263, 4268, 4272, 4273	
Great Britain		5214	
South Africa	Soil dilution	8135, 8411	P. Nelson
	Soil debris	8177, 8178, 8179, 8182, 8183, 8429, 8430, 8438, 7570	P. Nelson
United States	Alaska, tundra	A-26512, 7847, 7849, 7850, 7851	P. Nelson (M. Nelson, H. Abbas)
	Alaska, creek bank	7852, 7853	P. Nelson (M. Nelson)
	Montana	7721	P. Nelson (D. Taylor)
II. Strains from diseased plants			
Argentina	*Opuntia aurantica*	7715	P. Nelson
Australia	NSW, *Pinus*	5920	P. Nelson
Australia	*Solanum tuberosum*	2882	P. Nelson
Canada	*Zea mays*	6354	P. Nelson
Canada	New Brunswick, *S. tuberosum*	DAOM 196035, NRRL 13712, NRRL 13700, A-27940	G. Neish, A. Murphy (H. Lawrence)
Canada	P.E.I., *S. tuberosum*	DAOM 192963, DAOM 192966	G. Neish (V. Campbell)
Chile	*Dianthus*	7843	P. Nelson
Germany	*S. tuberosum*	6380	P. Nelson (Langerfeld)
Great Britain	*Polygonum siebaldii*	583	P. Nelson (W. Gordon)
Iran	*S. tuberosum*	5389, 5390	P. Nelson (C. Rabie, W. Gerlach)
Poland	*S. tuberosum*	KF 728, KF 729, KF 735	P. Gqlinski
United States	Colorado, *S. tuberosum*	NRRL 13711	Author
	Idaho, *S. tuberosum*	2633	P. Nelson
	Maine, *S. tuberosum*	NRRL 13707	S. Leach
	Minnesota, *Z. mays*	5455	P. Nelson (C. Mirocha)
	Minnesota, *Pinus*	110	R. Caldwell
	Wisconsin, *S. tuberosum*	NRRL 13500	R. Caldwell

*Information from the investigator who supplied the strain.

†Strains without an alphabetical prefix have the prefix R which was omitted to simplify the table.

‡Investigator who supplied the strain (in parenthesis is the name of the strain collector if known and if different from the supplier).

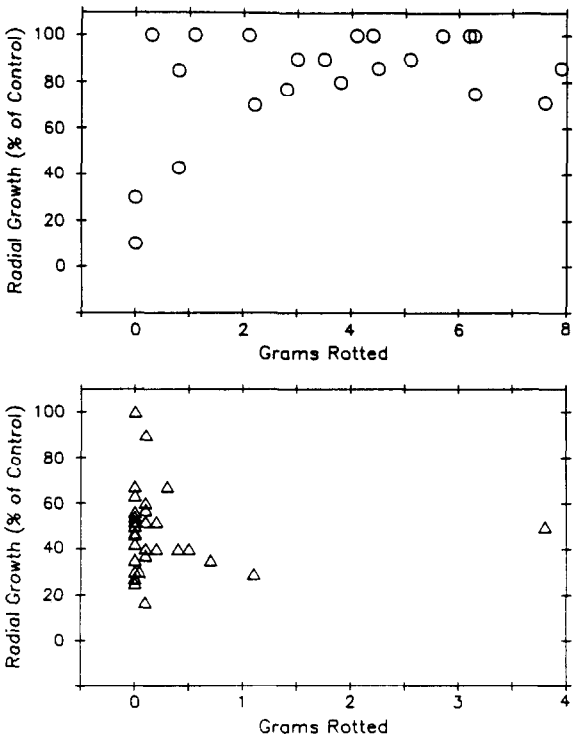
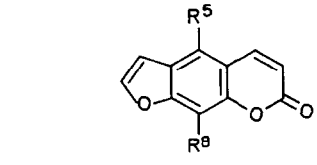


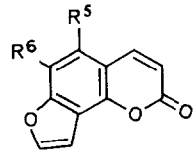
Fig. 1. The relationship between tolerance of xanthotoxin and pathogenicity on *P. sativa* root of 61 field strains of *G. pulicaris*. Each symbol represents one strain. ○, Plant-derived strains; Δ, soil-derived strains (Table 1, strain A-26512 was not tested for pathogenicity). All strains were tested simultaneously for tolerance in duplicate plates containing xanthotoxin at 200 μg per ml and were incubated for seven days. Tolerance is expressed as per cent of the radial growth of a DMSO-treated control culture. Pathogenicity is expressed as grams rotted of three root slices (total fr. *ca* 20 g) by each strain after four days incubation.

did not correlate with furanocoumarin tolerance. Adaptation to toxic plant chemicals has been well-documented in *Nectria haematococca* [6] and in other plant pathogenic fungi [5]. Such a strategy could reduce competition with other microorganisms and provide a strong selective advantage to organisms like *G. pulicaris* which are plant parasites as well as soil saprophytes.

Structural requirements for furanocoumarin toxicity to *G. pulicaris* were evaluated by measurement of radial mycelial growth of 16 strains in duplicate plates. Each plate was amended at 200 μg per ml with one of the 16 furanocoumarins or furanocoumarin precursors shown in Fig. 2. The eight plant-derived strains tested, all of which were more than 80% tolerant of xanthotoxin under these conditions, are given in Table 2. These strains were selected to represent the greatest possible biological diversity of host plant and geographical location. All eight plant-derived strains were highly tolerant (radial growth was more than 50% of that of controls) of all 16 test compounds; means and standard deviations for this group of strains against each of the test compounds are given in Table 3. The eight soil-derived strains tested, all of which were less than 50% tolerant of xanthotoxin at 200 μg per ml, are given in Table 2. Means and standard deviations for this group of strains tested against each of the 16 compounds are given in Table 3. All soil-derived strains were highly tolerant (radial growth was more than 50% of that controls) of the three furanocoumarin biosynthetic precursors tested: coumarin, umbelliferone and marmesin. Of the four angular furanocoumarins tested, angelicin was highly toxic to all eight soil-derived strains, isobergapten and pimpinellin were of intermediate toxicity to all of the soil-derived strains, and 6-isopentyloxyisobergapten was less toxic than its methoxylated analogue (Table 3). Of the nine linear furanocoumarins tested, C-8 methoxylated compounds were more toxic to soil-derived strains than either the unsubstituted compound psoralen or six substituted compounds with long-

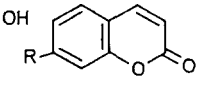


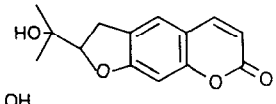
	R^5	R^8
Psoralen	H	H
Xanthotoxin	H	OMe
Isopimpinellin	OMe	OMe
Phellopterin	OPr	OMe
Bergapten	OMe	H
Isoimperatorin	OPr	H
Oxypeucedanin	OPrO	H
Oxypeucedanin hydrate	$OPrO_2$	H
Isovaleryl Oxypeucedanin hydrate	isoval-$OPrO_2$	H



	R^5	R^6
Angelicin	H	H
Isobergapten	OMe	H
Pimpinellin	OMe	OMe
6-isopentenyloxy isobergapten	OMe	OPr

Umbelliferone R = OH
Coumarin R = H





Marmesin

Pr =

PrO =

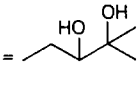
PrO_2 = 

Fig. 2. Furanocoumarins and furanocoumarin precursors investigated for fungitoxicity.

Table 2. Toxicity of xanthotoxin and angelicin to eight soil strains and eight plant strains of *Gibberella pulicaris* tested in agar culture*

Source	Strain no.	ED_{50} (μg/ml) Xanthotoxin*	Angelicin*
Soil, mixed grasses	R-4170	200–250	100–150
Soil	R-5214	150–200	50–100
Pine nursery soil	R-5684	150–200	100–150
Pasture soil	R-5867	25–50	50–100
Soil debris	R-7570	150–200	150–200
Soil	R-7721	150–200	150–200
Tundra soil	R-7851	150–200	50–100
Soil	A-26512	50–100	50–100
P. siebaldii	R-583	>400	>200
S. tuberosum	R-2882	>400	>200
S. tuberosum	R-5389	>400	>200
S. mays	R-5455	>400	>200
S. tuberosum	R-6380	>400	>200
O. aurantica	R-7715	>400	>200
Dianthus	R-7843	>400	>200
S. tuberosum	NRRL-13707	>400	>200

*The ED_{50} was calculated as described in the Experimental in a simultaneous test of all 16 strains. The highest concentrations tested were 400 and 200 μg/ml for xanthotoxin and angelicin, respectively. For soil strains the numbers given are the range of values for duplicate agar cultures.

Table 3. Furanocoumarin tolerance and metabolism of eight soil strains and eight plant strains of *Gibberella pulicaris* tested in agar culture

Compound tested	Soil strains Tolerance* (% control)	Soil strains Complete metabolism† (no. strains)	Plant strains Tolerance* (% control)	Plant strains Complete metabolism† (no. strains)
I. Furanocoumarin precursors				
Coumarin	80±20	3	90±10	6
Umbelliferone	90±20	2	100±10	7
Marmesin	80±10	0	90±10	6
II. Angular furanocoumarins				
Angelicin	10±10	0	80±10	7
Isobergapten	60±10	0	80±10	5
Pimpinellin	50±10	0	80±10	2
6-Isopentyloxy-isobergapten	80±20	1	90±10	4
III. Linear furanocoumarins				
Psoralen	70±20	2	90±10	8
Xanthotoxin	40±20	0	90±10	3
Isopimpinellin	40±30	2	80±10	8
Phellopterin	90±10	0	90±10	4
Bergapten	90±20	0	90±10	1
Isoimperatorin	90±10	7	80±10	7
Oxypeucedanin	70±20	7	90±10	4
Oxypeucedanin hydrate	90±20	3	100±10	4
Isovaleryl oxypeucedaninhydrate	80±10	0	80±10	4

*Tolerance is expressed as percent of DMSO-treated control culture radial growth rate, mean ± s.d. for eight strains, each with duplicate plates containing test compounds at 200 μg per ml and incubated for seven days.

†Number of strains from the group of eight from which no test compounds were recovered after incubation as above. Assay by TLC, detection limit was *ca* 5 μg recovered from the original 200 μg added.

er chain ethers: phellopterin, bergapten, isoimperatorin, oxypeucedanin, oxypeucedanin hydrate and isovaleryl oxypeucedaninhydrate (Table 3). The mean effective dose (ED_{50}) for 50% radial growth inhibition in agar plates for each of the 16 strains was determined for xanthotoxin and angelicin (Table 2). All plant-derived strains had an ED_{50} greater than 400 μg/ml (the highest concentration tested) for xanthotoxin and greater than 200 μg per ml (the highest concentration tested) for angelicin. The responses of the eight soil-derived strains were linear over the concentrations tested, and, for these strains, ED_{50}'s ranged from 50 to 200 μg per ml for angelicin and from 25 to 200 μg/ml for xanthotoxin (Table 2).

Whether furanocoumarin tolerance of *G. pulicaris* was due to furanocoumarin metabolism was investigated by measuring amounts of the compounds recovered from the tolerance assay plates described above. Recovery of all 16 test compounds from agar cultures of all 16 strains was monitored by thin-layer chromatography (TLC), and extracts were scored for the presence or absence (less than 5 μg recovered from 200 μg added) of each of the 16 compounds. As shown in Table 3, 13 of the 16 compounds tested were completely metabolized by at least four of the eight plant-derived strains. In contrast, few of the compounds were completely metabolized by the soil-derived strains, with the exceptions of the linear furanocoumarins, isoimperatorin and oxypeucedanin which were each completely metabolized by seven of the eight strains. Metabolism of each of the 16 test compounds by one selected plant-derived strain, R-6380, and one selected soil-derived strain, R-4170, was investigated in more detail by HPLC of the culture extracts. Furanocoumarin recoveries from R-4170 were higher than recoveries from R-6380 for 10 of the test compounds (including all compounds toxic to strain R-4170), equal (nothing recovered) for five compounds, and lower for only one compound, oxypeucedanin hydrate.

Metabolism of the four most fungitoxic furanocoumarins by the 16 selected fungal strains was quantitated by HPLC of extracts of tolerance assay plates. The relationships between tolerance and metabolism of two angular furanocoumarins, angelicin and pimpinellin, and two linear furanocoumarins, xanthotoxin and isopimpinellin, are shown in Fig. 3. For each of these four furanocoumarins, high levels of tolerance were strongly associated with low levels of furanocoumarins recovered. Correlation coefficients were −0.76, −0.89, −0.88 and −0.75 for angelicin, pimpinellin, xanthotoxin and isopimpinellin, respectively.

All bioassays in the present study were conducted in the dark in an attempt to mimic the natural conditions of fungal infection of *P. sativa* root. Very little is known, however, about light-independent effects of furanocoumarins in other systems [1, 11]. For UV light-dependent biological activities, structure–toxicity relationships of furanocoumarins have been investigated in some detail. In the Umbelliferae and Rutaceae, where furanocoumarins have been most studied, linear furanocoumarins are generally more common and more toxic than their angular analogues [1]. Among the furanocoumarins in this study, however, there were no simple patterns of taxonomic distribution and toxicity to *G. pulicaris*. Angelicin was much more fungitoxic than its more common linear analogue, psoralen; isobergapten was slightly less toxic than its linear analogue, xanthotoxin; and pimpinellin and isopimpinellin were equal in fungitoxicity. Among linear furanocoumarins, there was no relationship between frequency of occurrence in plants and fungitoxicity. For example, bergapten, which is common, and the three oxypeucedanin analogues, which are rare, were all nontoxic to all strains tested. Photosensitizing activity has been found to decrease with increasing chemical complexity of the alkyloxy substituent [13], which is similar to the results of this study.

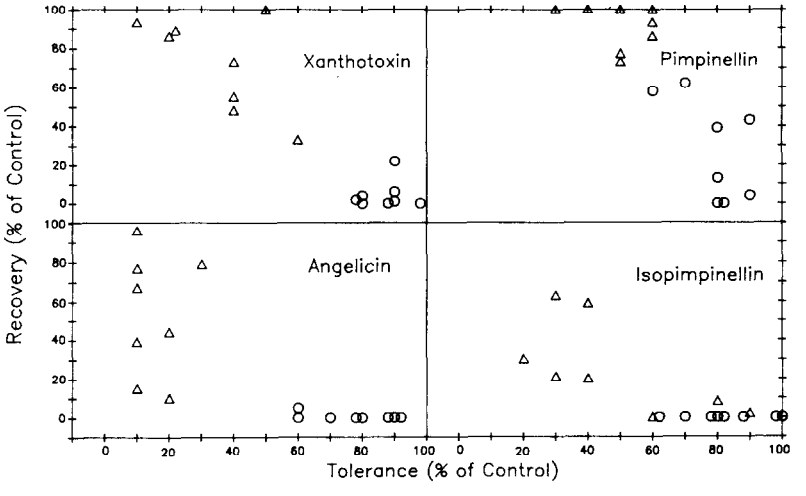


Fig. 3. The relationship between furanocoumarin tolerance and furanocoumarin metabolism of *Gibberella pulicaris* strains from diseased plants and from soil. Tolerance was assayed as in Fig. 1. Typical coefficients of variation for replicate plates were between 0 and 20%. Recoveries were determined by HPLC (one run) of extracts of one of the duplicate cultures. Each per cent recovery was normalized to the recovery from uninoculated control plates which was 160±12, 172±10, 74±9 and 200±36 (mean±range) for duplicate plates of xanthotoxin, angelicin, pimpinellin and isopimpinellin, respectively. Each symbol represents one strain. ○, Plant-derived strains; △, soil-derived strains.

The furanocoumarin precursors coumarin and umbelliferone have been shown to inhibit growth of a number of fungi [1], but were non-toxic to the 16 strains of *G. pulicaris* against which they were tested in this study. Complete metabolism of coumarin and umbelliferone was apparently not necessary for high tolerance, since only two soil-derived strains were able to metabolize all added umbelliferone but all soil-derived strains were tolerant. In contrast to furanocoumarins, which are found only in higher plants, both coumarin and umbelliferone have been found in a number of fungi and bacteria commonly found in soil [1], and might, therefore, be present in the environment of soil saprophytic strains of *G. pulicaris*. Marmesin, however, has not been reported to occur in lower plants or fungi [1], but was similar to the simpler coumarins in its low toxicity to all 16 fungal strains tested, although it was not completely metabolized by any of the eight soil strains. Marmesin was previously found to be nontoxic to 30 species of phytopathogenic fungi against which it was tested [14].

Xanthotoxin was previously shown to accumulate to high levels of parsnip root following infection with various fungi including *Fusarium sporotrichioides* [3, 4]. It was therefore of interest to determine whether xanthotoxin accumulated in parsnip root inoculated with *G. pulicaris*. After 24 hr of incubation at 25° in the dark, a dark brown lesion developed under the fungal inoculum plug in the central xylem core of the *P. sativa* root disc. Over three or more days further incubation the infected area continued to enlarge, gradually extending throughout the softer cortex tissue. Furanocoumarins were quantitated by HPLC of ethyl acetate extracts of uninfected *P. sativa* roots and of roots infected by several fungal strains. In uninoculated, peeled *P. sativa* roots, furanocoumarin levels were very low; <0.1 μmol total furanocoumarins were found per g of freshly cut roots and <0.5 μmol/g were found after six days incubation under sterile conditions. In all infected roots analysed, xanthotoxin and angelicin were always present. Concentrations of each were as high as 2 μmol/g total rotted tissue and accounted for more than 95% of furanocoumarins detected. Other furanocoumarins such as psoralen and bergapten, which previously have been found in *P. sativa* [15], were either not detected or present at much lower levels than xanthotoxin and angelicin under these experimental conditions.

The 62 strains of *G. pulicaris* investigated in this study expressed different levels of tolerance to xanthotoxin *in vitro*. In order to investigate the relationship of xanthotoxin tolerance to virulence on a xanthotoxin-producing host, all the strains were tested for the ability to rot *P. sativa* root discs. As shown in Fig. 1, xanthotoxin-tolerant strains caused the highest levels of disease on *P. sativa* root. Several xanthotoxin-tolerant strains were of low virulence on *P. sativa*, which indicates that factors other than xanthotoxin tolerance are important for disease. The eight highly xanthotoxin-tolerant plant-derived strains tested were also highly tolerant of angelicin, the other major *P. sativa* phytoalexin, and of 11 other furanocoumarins, which suggests that factors other than sensitivity to other furanocoumarins are responsible for low virulence of these xanthotoxin-tolerant strains. The soil debris-derived strain R-7570 demonstrated high (50%) tolerance of xanthotoxin and low (30%) tolerance of angelicin (tested at 200 μg per ml) and was consistently of intermediate virulence in repeated tests on *P. sativa* roots (Fig. 1).

None of the strains of *G. pulicaris* that were highly virulent on *P. sativa* root were originally isolated from diseased *P. sativa*. Similarly, strains isolated from plants that do not produce lubimin or rishitin have been found to be tolerant of lubimin and rishitin and virulent on potato tubers [9, 10]. Van Etten and coworkers [6] also found that many strains of *N. haematococca* from host plants other than pea, i.e. habitats that do not contain pisatin, were tolerant of pisatin, and that some of these strains were also virulent on pea. They speculated that these strains might have had prior contact with pea or that *N. haematococca* might be broadly adapted to a variety of plant hosts. The *P. sativa* virulence of *G. pulicaris* strains from potato, corn and other plants might be similarly explained. It should be noted that furanocoumarins, unlike pisatin, rishitin or lubimin, are quite widespread in higher plants, and have been reported to occur in the species, genera, or families of plants from which many of the furanocoumarin-tolerant strains in this study were isolated; for example, *Z. mays* and the Solanaceae [1].

It has been well-established that some insect herbivores can feed successfully and even exclusively on plants containing furanocoumarins [16], and that some of these insects can rapidly metabolize furanocoumarins to nontoxic derivatives [11, 13]. In a few instances, the mechanism for detoxification is known; oxidative cleavage of the furan ring is the major route of psoralen and xanthotoxin detoxification by caterpillars of the black swallowtail butterfly *Papilio polyxenes* [11, 13]. *O*-Demethylation of xanthotoxin to xanthotoxol is a more minor pathway in *P. polyxenes* although a major pathway in mammals [11]. Although *O*-demethylation is a phytoalexin detoxification mechanism in the genus *Fusarium* [5], and xanthotoxol is nontoxic to strains of *G. pulicaris* that are highly sensitive to xanthotoxin (data not shown), xanthotoxol has not been detected in extracts of liquid and agar cultures of strains of *G. pulicaris* that are actively metabolizing xanthotoxin. Further investigations into the chemical nature of fungal metabolites of xanthotoxin and other furanocoumarins are in progress.

EXPERIMENTAL

Chemicals and chemical analyses. Xanthotoxin was obtained from Sigma. All other compounds were purified as previously described [17], their purity was 95% or better. Furanocoumarins were added to cultures after determining their weights gravimetrically and were analysed as previously described [17]. For TLC, 20 × 20 cm silica gel 60F-254 plates (Merck) were developed to 15 cm with hexane–EtOAc (7:3).

Cultures. Most of the strains of *G. pulicaris* used in this study were identified as *F. sambucinum* and kindly supplied by P. E. Nelson (Fusarium Research Center, The Pennsylvania State University, strains with the prefix R are from this collection). Other strains were kindly supplied by G. Neish (Agriculture Canada at Ottawa); H. K. Abbas (University of Minnesota); R. Caldwell (University of Wisconsin); A. Murphy (Agriculture Canada at Fredericton); P. Golinski (Agricultural University of Poznam, Poland) and S. Leach (University of Maine). All of the strains in this study were identified as *F. sambucinum* based on standardized morphological criteria [18]. One of the soil-derived strains and 18 of the plant-derived strains have, under special

conditions, produced the perithecial stage in culture [8, 19 and M. N. Beremand and A. E. Desjardins, unpublished results]. Although many strains in this study have not yet produced the perithecial stage in culture, we have used the binomial of the teleomorph for all strains in this study, as is the recommended practice for plant pathogenic fungi [20]. All strains were re-isolated from single spores prior to this study. Cultures were routinely grown on V-8 agar medium [M-20] [21] slants or plates on an alternating 12 hr, 25° light/20° dark schedule. For long term storage, strains were maintained on V-8 agar slants at 4°, and as lyophilized conidial suspensions in the Agricultural Research Service Collection, Peoria, Illinois. For all experiments, fresh transfers of the strains were grown from stock cultures stored at 4°.

Fungitoxicity and metabolism of furanocoumarins were examined in a V-8 juice agar medium [M-20] [21]. Appropriate controls of fungal cultures without furanocoumarins and of furanocoumarins in agar media without fungal cultures were run. The growth rate of eight plant-derived strains and eight soil-derived strains was determined at xanthotoxin and angelicin concentrations of 25, 50, 100, 200 and 400 (xanthotoxin only) μg per ml. Duplicate 35 × 10 mm plastic petri dishes containing 1 ml of V-8 juice agar and 2% v/v dimethylsulphoxide with or without test compounds were inoculated with plugs (2 mm diam.) cut from the growing margin of cultures less than ten-days-old, and placed with the mycelial surface appressed to the surface of the assay medium at the edge of the plate. Plates were incubated at 25 ± 1° in the dark. The radius (from the inoculum to the growing margin) was measured daily for at least seven days or until fungal growth reached the edge of the plate. The ED_{50} was determined as the concentration at which radial growth was 50% of that of controls. The radial growth rates were approximately linear for all strains tested in the presence or absence of furanocoumarins. The 62 field strains were tested simultaneously against xanthotoxin (200 and 400 μg/ml) in duplicate plates and measured for tolerance. Per cent tolerance was calculated by dividing the radial growth rate (mm/day) on furanocoumarin-amended medium by the radial growth rate (mm/day) of controls. Sixteen selected strains were further tested simultaneously against 16 furanocoumarins and related compounds (200 μg/ml) in duplicate plates. These plates were measured for tolerance and, after seven days incubation, one of the duplicate agar cultures was extracted with EtOAc and analysed by TLC and/or HPLC. Recoveries of furanocoumarins from uninoculated control plates incubated for seven days were greater than 75% except for pimpinellin where only 40% of the compound was recovered.

Virulence assay. P. sativa roots obtained from several local suppliers (cultivars unknown) were peeled, washed in tap water, and briefly surface-sterilized with 95% EtOH. The roots were cut into 5–7 mm thick discs under aseptic conditions, and washed in several changes of sterile dist. H_2O. Each disc was transferred to a sterile plastic petri dish containing filter paper moistened with sterile dist. H_2O. The upper surface of each disc was inoculated immediately by placing an agar inoculum plug (5 mm diam.) mycelial side down in the center core of each disc. Inoculum plugs were cut from the growing margins of cultures less than ten-days-old. All fungal cultures used in each experiment were of equal age and each strain was tested on three individual discs. The petri dishes were sealed in plastic bags and incubated for four days at 25° in the dark. Root discs were weighed at the end of each experiment, then rotted tissue was removed with a spatula and the remaining tissue was re-weighed. Pathogenicity was determined from g of rotted material per three root discs (total fr. wt minus fr. wt of uninfected tissue.).

Acknowledgements—We thank Michelle Burns and Carlotta Williams for excellent technical assistance.

REFERENCES

1. Murray, R. P. H., Mendez, J. and Brown, S. A. (1982) *The Natural Coumarins*. Wiley, New York.
2. Beier, R. C. and Oertli, E. H. (1983) *Phytochemistry* **22**, 2595.
3. Johnson, C., Brannon, D. R. and Kúc, J. (1973) *Phytochemistry* **12**, 2961.
4. Desjardins, A. E., Spencer, G. F., Plattner, R. D. and Beremand, M. N. (1989) *Phytopathology* **79**, 170.
5. VanEtten, H. D., Matthews, D. E. and Smith, D. A. (1982) in *Phytoalexins* (J. A., Bailey and J. W., Mansfield, eds), p. 181. Blackie, Glasgow.
6. VanEtten, H. D., Matthews, D. E. and Mackintosh, S. F. (1987) in *Molecular Strategies for Crop Protection* (Arntzen, C. and Ryan, C. eds), p. 1. Liss, New York.
7. Booth, C. (1971) *The Genus Fusarium*. Commonwealth Mycology Institute, Kew, Surrey, England.
8. Desjardins, A. E. and Beremand, M. N. (1987) *Phytopathology* **77**, 678.
9. Desjardins, A. E., Gardner, H. W., Plattner, R. D. (1989) *Phytochemistry* **28**, 431.
10. Desjardins, A. E. and Gardner, H. W. (1989) *Mol. Plant Microbe Inter.* **2**, 26.
11. Ivie, G. W. (1987) *Rev. Latinoam. Quim.* **18**, 1.
12. Gray, A. I. and Waterman, P. G. (1978) *Phytochemistry* **17**, 845.
13. Ivie, G. W. (1987) *ASC Symp. Series* **339**, 217.
14. Shirata, A., Takahashi, K., Takasugi, M., Anetai, M. and Masamune, T. (1983) *Sanshi Shikenjo Hokoku* **28**, 781; (1984) *Chem. Abstr.* **100**, 346.
15. Ivie, G. W., Holt, D. L. and Ivey, M. C. (1981) *Science* **213**, 909.
16. Berenbaum, M. (1981) *Ecology* **62**, 1254.
17. Spencer, G. F., Tjarks, L. W. and Powell, R. G. (1987) *J. Agric. Food Chem.* **35**, 803.
18. Nelson, P. E., Toussoun, T. A. and Mavasas, W. F. O. (1983) *Fusarium Species: An Illustrated Manual for Identification*. The Pennsylvania State University Press, University Park.
19. Desjardins, A. E. and Plattner, R. D. (1989) *J. Agric. Food Chem.* (in press).
20. Yoder, O. C., Valent, B. and Chumley, F. (1986) *Phytopathology* **76**, 383.
21. Stevens, R. B. (ed.) (1974) *Mycology Guidebook*. University of Washington Press, Seattle.