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DETOXIFICATION OF THE POTATO PHYTOALEXIN LUBIMIN BY *GIBBERELLA PULICARIS*

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Key Word Index—*Solanum tuberosum*; Solanaceae; potato; *Gibberella pulicaris* (*Fusarium sambucinum*); phytoalexin metabolism; lubimin.

Abstract—The potato phytoalexin lubimin displayed a complex pattern of metabolism by strains of the potato pathogen *Gibberella pulicaris* (anamorph: *Fusarium sambucinum*). The metabolites 15-dihydrolubimin and isolubimin were common to both lubimin-sensitive and lubimin-tolerant strains. In one lubimin-tolerant strain, several unique metabolites, including the tricyclic compounds cyclodehydroisolubimin and cyclolubimin (2-dihydrocyclodehydroisolubimin) and their epoxides, accumulated at the expense of 15-dihydrolubimin and isolubimin. These tricyclic compounds were not toxic to the lubimin-sensitive strain. These results indicate that a likely pathway for lubimin detoxification in some strains of *G. pulicaris* involves cyclization of isolubimin to cyclodehydroisolubimin via an unsaturated intermediate. All highly virulent strains tested were tolerant of lubimin and were able to convert lubimin to apparently nontoxic products which is indirect evidence that lubimin detoxification contributes to virulence of *G. pulicaris* on potato tubers.

INTRODUCTION

Lubimin and other sesquiterpene phytoalexins accumulate in potato (*Solanum tuberosum* L.) tubers upon infection with a variety of microorganisms [1]. Lubimin inhibits the growth of many fungi [1, 2] and has been proposed to be part of an active defence mechanism in potatoes. It is well established that some plant pathogenic fungi are tolerant of their hosts' phytoalexins, and that they are often able to metabolize the phytoalexins to which they are tolerant [3]. Ward and Stöessl [2] found that a strain of the potato pathogen *Gibberella pulicaris* (Fries.) Sacc. [anamorph: *Fusarium sambucinum* (Fuckel)] rapidly metabolized added lubimin but that the product formed, 15-dihydrolubimin, was equally toxic. Recovery of 15-dihydrolubimin, however, represented less than 50% of the lubimin supplied, suggesting further metabolism to other, possibly less toxic, compounds. In a preliminary survey, we observed that lubimin was nontoxic to many naturally occurring strains of *G. pulicaris in vitro*, and that tolerance to lubimin correlated, in large part, with its metabolism to apparently nontoxic products. Isolation and identification of some of these lubimin metabolites has been previously described [4]. The studies reported here were carried out to determine the relationships among several structurally similar lubimin metabolites, to elucidate pathways for lubimin metabolism in selected lubimin-sensitive and lubimin-tolerant strains of *G. pulicaris*, to determine whether metabolism of lubimin leads to its detoxification, and to investigate the relationship of lubimin tolerance to virulence on potato tubers. A preliminary report of some of this research has been published [5].

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RESULTS

Lubimin tolerance

Twenty-six strains of *G. pulicaris* from a variety of habitats were each tested twice for tolerance to lubimin. All 26 strains were able to metabolize lubimin to some extent (Table 1). The 12 most lubimin-sensitive strains metabolized lubimin relatively poorly (mean of 89 ± 23 μg recovered from 200 μg added), while the 14 more lubimin-tolerant strains were able to metabolize lubimin completely (mean of 1 ± 3 μg recovered) (Table 1). Lubimin was completely stable in uninoculated control cultures for up to seven days. Loss of lubimin from all of the fungal cultures coincided with the appearance of several new compounds in extracts analysed by GC. One of these compounds was identified as 15-dihydrolubimin, which had been previously reported to be a toxic lubimin metabolite [2]. Additional compounds produced by many of the strains appeared by GC-MS analysis to be closely related to lubimin. Lubimin metabolism was investigated in greater detail with three selected strains of *G. pulicaris*. Two of these, R-7715 and R-583, were lubimin-tolerant strains and the third, R-110, was a lubimin-sensitive strain (Table 1). Although *G. pulicaris* is known to produce a variety of sesquiterpenes, including trichothecene toxins [6], lubimin and related compounds have not been found under any growth conditions in any of the 26 strains used in this study.

Metabolic products

The three selected strains of *G. pulicaris* converted lubimin and related compounds *in vitro* into a number of products. These metabolites resulted from a variety of transformations, including cyclizations and both oxida-

Table 1. Lubimin tolerance, lubimin metabolism and virulence of strains of *G. pulicaris*

Strain*	Habitat†	Lubimin tolerance‡	Phytoalexins recovered§ (μg)		Relative virulence on potato‖
			Lubimin	Lubimin metabolites	
NRRL-13503	potato	85^{abc}	0	13	98^{a}
R-6380	potato	90^{a}	0	8	78^{b}
NRRL-13500	potato	89^{a}	0	12	74^{b}
R-5389	potato	91^{a}	0	16	66^{b}
R-2882	potato	88^{ab}	0	12	49^{c}
NRRL-13504	potato	84^{abc}	0	16	36^{d}
R-5390	potato	92^{a}	0	21	32^{de}
R-7570	soil	52^{d}	10	67	31^{de}
R-5455	corn	80^{abc}	0	17	20^{ef}
R-6112	unknown	86^{abc}	0	26	9^{fg}
R-2633	potato	80^{abc}	0	19	7^{fg}
R-583	knotweed	72^{bc}	0	18	1^{g}
R-7843	carnation	74^{c}	0	39	0^{g}
R-7715	cactus	70^{c}	4	159	0^{g}
NRRL-13502	grass	20^{fgh}	96	38	5^{g}
NRRL-13501	grass	22^{efgh}	80	73	2^{g}
R-110	pine	22^{efgh}	86	76	0^{g}
R-5344	pine	14^{gh}	93	66	1^{g}
R-5753	soil	18^{fgh}	65	74	1^{g}
R-5690	soil	12^{gh}	82	57	0^{g}
R-5867	soil	37^{e}	88	42	0^{g}
R-5684	soil	32^{ef}	64	55	2^{g}
R-6354	corn	28^{efg}	87	22	0^{g}
R-5214	soil	23^{efgh}	73	21	6^{fg}
R-5185	unknown	18^{fgh}	110	49	6^{g}
R-2155	unknown	11^{h}	149	40	0^{g}

*Strains are listed approximately in order of decreasing virulence.

†Data on habitat are from the investigator who supplied the strain.

‡Tolerance is expressed as per cent of dimethysulphoxide-treated control culture radial growth rate, mean of two replicate experiments, each with duplicate plates containing lubimin 200 μg per ml and incubated for 7 days. All strains were tested simultaneously.

§Results of a single simultaneous test of all strains. Number of GC analyses per sample was two to four; initial amount of lubimin was 200 μg in a 1 ml agar culture.

‖For 25 strains, mean of six replicate tests, three of cv. Sebago and three of cv. Russett Burbank, 3 tuber slices per test. For R-7843, mean of three replicate tests on cv. Russett Burbank, 3 tuber slices per test. Virulence of each strain was normalized to strain NRRL-13503 in each experiment. Differences among means were evaluated with Duncan's multiple range test. Data on tolerance of lubimin and on virulence on tubers were analysed as standardized percentages. In both columns, means followed by the same letter are not significantly different based on 95% confidence difference intervals.

tions and reductions (Fig. 1). The three selected strains reduced the aldehyde groups of lubimin and 2-dehydrolubimin. All three strains reduced the ketone of 2-dehydrolubimin and cyclodehydroisolubimin to form the corresponding secondary alcohols. Strains R-7715 and R-110 also reduced the keto group of 11,12-epoxycyclodehydroisolubimin. The two lubimin-tolerant strains (R-7715 and R-583) also oxidized the hydroxyl group of lubimin to give 2-dehydrolubimin. In contrast, 2-dehydrolubimin was not detected at any time in cultures of the lubimin-sensitive strain (R-110) after the addition of lubimin or any lubimin metabolite. This strain was, however, able to oxidize 15-dihydrolubimin to form isolubimin. Added cyclolubimin was not oxidized to cyclodehydroisolubimin by any of these strains. The isopropenyl side chain of added cyclolubimin and cyclodehydroisolubimin was epoxidized by strains R-7715 and R-583, but no epoxides were detected after addition of any lubimin metabolite to strain R-110. Similarly, strains, R-7715 and R-583 formed tricyclic ethers from added lubimin or 2-dehydrolubimin, but these tricyclic metabolites were never detected in cultures of strain R-110 after addition of lubimin or any bicyclic metabolite.

Metabolic pathways

Metabolism of lubimin by the lubimin-sensitive strain R-110 was slow and appeared to lead to only two compounds. Only lubimin, 15-dihydrolubimin and isolubimin were detected at any time after the addition of lubimin to this strain. In liquid culture, isolubimin was a minor, transient metabolite and more than 90% of the

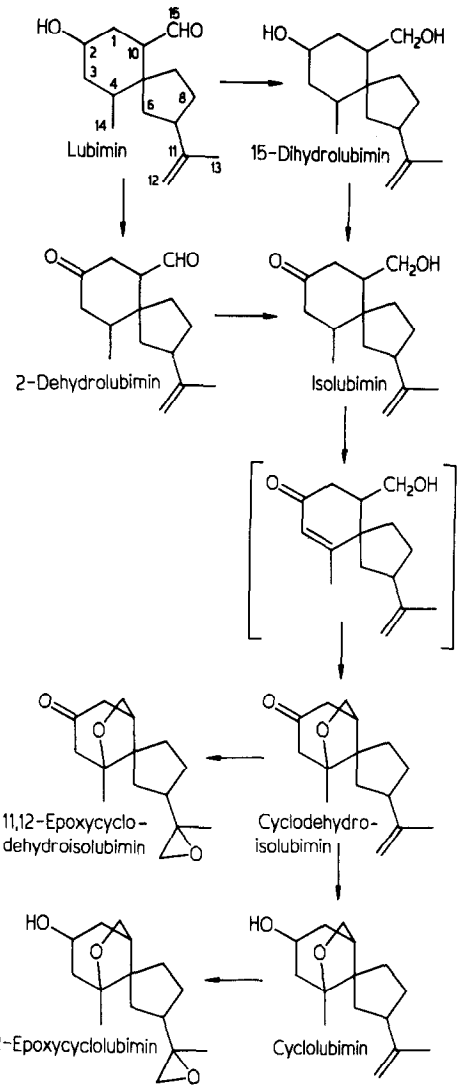

Fig. 1. Putative lubimin metabolic pathway (stereochemistry not shown) based primarily on experimental results from strain R-7715. [ ] Postulated intermediate based on specific incorporation of deuterium at C-3 of cyclodehydroisolubimin during its biotransformation from isolubimin in the presence of ^2H_2O. Arrows do not necessarily imply that these reactions are irreversible

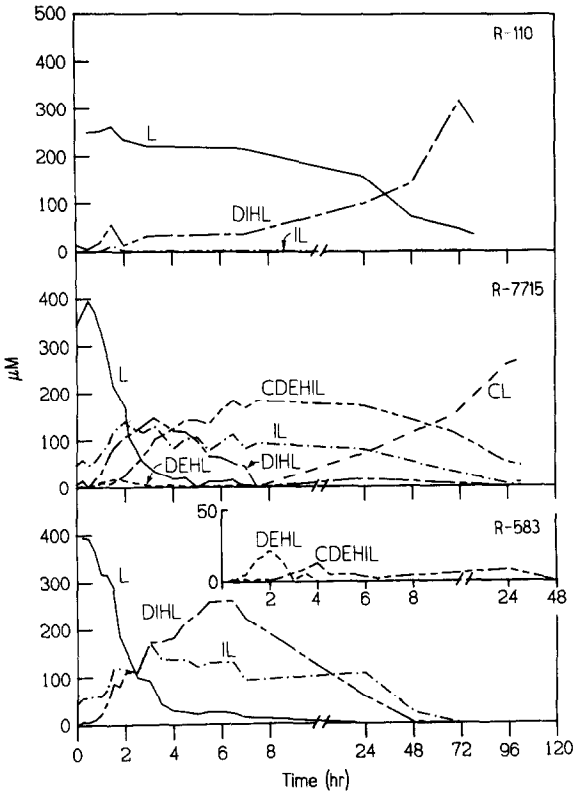

Fig. 2. Metabolism of lubimin by three strains of *G. pulicaris* in liquid shake cultures as described in Experimental. Concentrations shown (μM) are means of duplicate GC assays of trimethylsilyloxy-derivatized samples of all compounds except 2-dehydrolubimin and cyclodehydroisolubimin which were assayed without derivatization. The concentration (μM) refers to μmol of compound detected/l of culture. For strains R-7715 and R-583, 1 ml samples were taken at 15 min intervals for the first 2 hr, at 30 min intervals for the next 6 hr, and at least once a day for the following days up to 4. For strain R-110, 500 μl samples were taken at 30 min intervals for 4 hr then at least once a day for the following 4 days. Lubimin (L), 15-dihydrolubimin (DIHL), isolubimin (IL), 2-dehydrolubimin (DEHL), cyclolubimin (CL) and cyclodehydroisolubimin (CDEHIL). Several of these compounds were present in two or more isomeric forms [4] and the quantities reported for any given compound are the sum of all stereoisomers of that compound detected.

lubimin added was recovered as 15-dihydrolubimin after three and one-half days incubation (Fig. 2), but both isolubimin and 15-dihydrolubimin were major products in agar cultures after seven days incubation (Table 2). The reason for this discrepancy is not known.

Metabolism of lubimin by the two lubimin-tolerant strains was both rapid and complex. Within one hour of lubimin addition to strain R-7715, 2-dehydrolubimin, 15-dihydrolubimin and isolubimin were detected (Fig. 2). The level of 2-dehydrolubimin rapidly reached a maximum and declined. Levels of isolubimin and 15-dihydrolubimin peaked at two to four hours and then declined slowly during the next two days. Cyclodehydroisolubimin was first detected between 1.5 and 2 hr after lubimin addition, increased for 24 hr, then was slowly converted to cyclolubimin which was not metabolized significantly afterwards. After almost five days incubation, more than 75% of the lubimin added was recovered as tricyclic metabolites. After two days incubation small amounts of the epoxide of cyclodehydroisolubimin were detected by GC-MS (data not shown).

The pattern of lubimin metabolism in strain R-583 differed from that in strain R-7715 in two major respects (Fig. 2). Cyclodehydroisolubimin was a very minor metabolite in strain R-583 (≤ 13 μM), and no lubimin metabolites of any kind were detected in chloroform–methanol extracts after 48 hr incubation. Further metabolism of lubimin by this strain may involve formation of water soluble products by oxygenation or conjugation. For example, glucosides of solavetivone metabolites have been isolated from tobacco [7].

Table 2. Metabolism and fungitoxicity of lubimin and selected metabolites in strain R-110 in agar culture

Compound added	Compounds recovered*†									
	Lubimin‡	15-Dihydro lubimin	Isolubimin	2-Dehydro lubimin	Cyclodehydro isolubimin	Cyclolubimin	11,12-Epoxy cyclodehydro isolubimin	11,12-Epoxy cyclolubimin	total recovered	Radial growth‖ (% control)
Lubimin	60	72	37	—	—	—	—	—	169	28
	78	93	42	—	—	—	—	—	213	28
2-Dehydrolubimin	—§	40	134	9	—	—	—	—	183	68
		36	123	15	—	—	—	—	174	59
15-Dihydrolubimin	—	82	67	—	—	—	—	—	149	59
	—	114	94	—	—	—	—	—	208	59
Cyclodehydro	—	—	—	—	51	72	—	—	123	83
isolubimin	—	—	—	—	52	65	—	—	117	81
Cyclolubimin	—	—	—	—	—	118	—	—	118	89
	—		—	—	—	139	—	—	139	91
11,12-Epoxy cyclodehydro	—	—	—	—	—	—	134	37	171	96
isolubimin	—	—	—	—	—	—	159	44	203	96

*Duplicate 1 ml agar cultures, 200 μg/ml lubimin or metabolite added to each culture and incubated for 7 days.

†TMS derivatives, duplicate GC data on each plate.

‡Lubimin + 10-epilubimin.

§None detected.

‖Growth is expressed as per cent of dimethylsulphoxide-treated control culture radial growth rate.

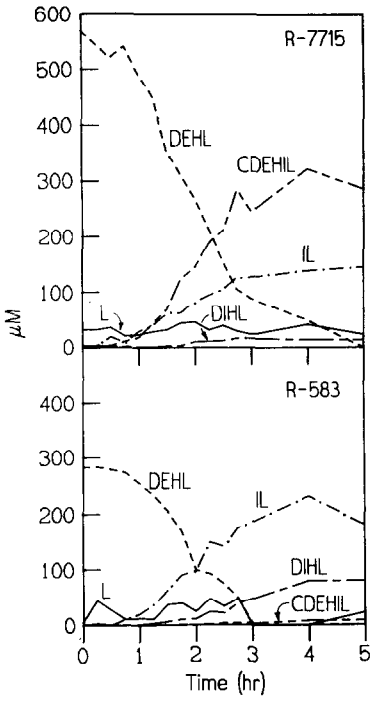

Fig. 3. Metabolism of 2-dehydrolubimin by two strains of *G. pulicaris* in liquid shake cultures. Experimental conditions, concentrations and symbols are as in Fig. 2. One ml samples were taken at 15 min intervals for the first 3 hr, then at 4 and 5 hr.

It was not evident from their structures whether 2-dehydrolubimin or 15-dihydrolubimin, or both, were precursors of isolubimin. To select among these alternate possibilities 2-dehydrolubimin was fed to strains R-7715 and R-583. Metabolism of 2-dehydrolubimin by both strains was rapid and complex (Fig. 3). Within one hour of 2-dehydrolubimin addition, lubimin, 15-dihydrolubimin and isolubimin were detected. Cyclodehydroisolubimin was a major metabolite in strain R-7715 and a minor metabolite in strain R-583, as had been observed following lubimin addition. However, since 15-dihydrolubimin appeared after 2-dehydrolubimin feeding, the relative importance of the two compounds as isolubimin precursors is still unclear.

This issue was not resolved by feeding 15-dihydrolubimin to strain R-7715 because, even after three days incubation, more than 80% of the added 15-dihydrolubimin was recovered as 15-dihydrolubimin. No isolubimin, lubimin or tricyclic metabolites were detected in the culture extracts. The lack of metabolism of exogenously supplied 15-dihydrolubimin by strain R-7715 is puzzling because this strain rapidly metabolized endogenously produced 15-dihydrolubimin.One possibility is that the enzymes responsible for 15-dihydrolubimin metabolism are not constitutively produced and are not induced by 15-dihydrolubimin. There is some evidence for inducibility of fungal metabolism of the related phytoalexin capsidiol [8].

The putative role of isolubimin in formation of cyclodehydroisolubimin was examined by isotope labelling. If the intermediate is 3,4-dehydroisolubimin, then incubation of lubimin with strain R-7715 in ^2H_2O should afford deuterium labelling at C-3 of cyclodehydroisolubimin (Fig. 1). In fact, there was *ca* 65% incorporation of one deuterium atom as determined by GC-MS, and this label was located specifically on C-3 as determined by both ^1H and ^{13}C NMR [4].

A convincing case can be presented that lubimin metabolism in strain R-7715 occurs mainly via cyclization of isolubimin. It is necessary to be cautious, however, in extrapolating these results to strain R-583 and other strains which accumulate very low levels of cyclodehydroisolubimin or cyclolubimin. The level of cyclodehydroisolubimin in these strains could be low because cyclization is not a major pathway for lubimin metabolism, or, equally, because there is a rapid conversion of cyclodehydroisolubimin to undetected product(s). Some supporting evidence for the latter hypothesis was obtained by feeding cyclodehydroisolubimin to strains R-7715 and R-583 and measuring the time course of its disappearance. Cyclodehydroisolubimin metabolism by strain R-7715 was slow; over 80% of the added compound was recovered unchanged or as cyclolubimin after three days incubation. Cyclodehydroisolubimin metabolism by strain R-583 was more rapid; only 60% was recovered after 24 hr and only 20% was recovered after three days incubation. These differential rates of cyclodehydroisolubimin metabolism, however, do not appear sufficient to account for the different levels of tricyclic compounds produced after feeding lubimin to these two strains.

Toxicity

The abilities of lubimin and five metabolites to inhibit mycelial growth of the lubimin-sensitive strain R-110 were compared (Table 2). Both 2-dehydrolubimin and 15-dihydrolubimin inhibited fungal growth. Their exact toxicity, however, could not be unambiguously determined because of interconversions to 15-dihydrolubimin and isolubimin during the bioassays. It is apparent that 15-dihydrolubimin and/or isolubimin retain some fungitoxicity since growth is inhibited to some extent when only these two compounds are present. Sufficient quantities of isolubimin were not available for direct testing. Three tricyclic intermediates tested—cyclodehydroisolubimin, 11,12-epoxycyclodehydroisolubimin and cyclolubimin—were not significantly inhibitory and were largely undegraded by strain R-110 under our bioassay conditions (Table 2).

Virulence on potato tubers

Twenty-six strains of *G. pulicaris* obtained from a wide variety of geographical areas and habitats were tested for their virulence on potato tubers (Table 1). All virulent strains produced a dry brown rot with a well-defined edge. Although variability in virulence was observed from experiment to experiment, the relative virulence of strains was consistent in all experiments. The five most virulent strains of *G. pulicaris* were very tolerant of lubimin and metabolized all lubimin added *in vitro*.

DISCUSSION

The idea that plants produce protective chemicals after exposure to microorganisms was formalized by Müller and Borger in 1940 [9] and was based on their research on potato resistance to *Phytophthora infestans*. Thirty

years later, the active compounds in potato were found to be a series of closely related sesquiterpenes, including rishitin, lubimin and solavetivone. The vetispirane lubimin was first identified by Metlitsky and coworkers [10] and the correct structure was determined by Stöessl *et al.* [11]. Several other vetispiranes have been found, usually as minor components, in fungally infected potato tubers. These include 15-dihydrolubimin, isolubimin, solavetivone and the epilubimin series [1, 12]. Cyclodehydroisolubimin was found as a minor component of potato infected with *P. infestans* [13]. In all of these cases, it was uncertain whether accumulation of these compounds was due to synthesis by the plant or to metabolism by the plant or fungus. Conversion of 15-dihydrolubimin to isolubimin, for example, has been observed in uninfected potato tissue [14]. Only one structural modification of lubimin by a fungus has been documented in pure culture; as mentioned previously, Ward and Stöessl [2] reported the conversion of lubimin to 15-dihydrolubimin by *G. pulicaris*. The data presented here conclusively demonstrate that 15-dihydrolubimin, isolubimin, 2-dehydrolubimin and the tricyclic compounds can be products of fungal metabolism. The putative lubimin metabolic pathway presented in Fig. 1 is based primarily on experimental results with strain R-7715. Lubimin, 2-dehydrolubimin, 15-dihydrolubimin, and isolubimin may be in equilibrium in some cases; for example, feeding 2-dehydrolubimin resulted in formation of lubimin, 15-dihydrolubimin, and isolubimin in both strains R-7715 and R-583 (Fig. 2). The fact that exogenous 2-dehydrolubimin can be converted to these compounds, however, does not necessarily imply that these reactions are part of the major endogenous pathway of lubimin metabolism. It is interesting to note that the pathway we have proposed for lubimin metabolism to cyclodehydroisolubimin by *G. pulicaris* (Fig. 1) is in some ways the reverse of a pathway that has been established for lubimin biosynthesis in uninfected potato slices [14–16].

The metabolism of lubimin to cyclodehydroisolubimin and related compounds represents a detoxification. Although all naturally occurring strains of *G. pulicaris* possess some ability to metabolize lubimin, only lubimin-tolerant strains apparently are able to rapidly convert lubimin to completely nontoxic products. Furthermore, only strains with a high level of lubimin detoxification *in vitro* are highly virulent on potato tubers. Lubimin metabolism, however, is apparently not sufficient to insure virulence on potato because some strains are not highly virulent, even though they can metabolize lubimin *in vitro*. This is not surprising since pathogenicity is probably a complex process depending on many genes and resistance in potato tubers to fungal pathogens can apparently involve several defence responses [17, 18]. Genetic analyses currently underway in our laboratory indicate that tolerance of rishitin is also necessary for high virulence of *G. pulicaris* on potato tubers (Desjardins and Gardner, unpublished). Although lubimin is not always a major component of the phytoalexin response in fungally infected potatoes [19], lubimin and rishitin were the major sesquiterpene phytoalexins detected by GC-MS analysis in potatoes infected with *G. pulicaris* strain R-110. Relatively high levels of tricyclic lubimin metabolites were found in potato tissue infected with strain R-7715 of *G. pulicaris*, which produces these metabolites *in vitro* (data not shown). These observations provide more direct evidence that *G. pulicaris* metabolizes lubimin during plant pathogenesis.

EXPERIMENTAL

Cultures. Strains of *G. pulicaris* used in this study were kindly supplied by P. E. Nelson from the *F. sambucinum* collection at the Fusarium Research Center, The Pennsylvania State University (strains with the prefix R are from this collection); by R. Caldwell (University of Wisconsin-Madison), G. Neish (Agriculture Canada at Ottawa) and H. K. Abbas (University of Minnesota). All strains originated from single conidium cultures. Cultures were routinely grown on V-8 agar medium [20] 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°.

Transformations of lubimin and certain metabolites by three selected strains were examined in a liquid V-8 medium. Inocula were prepared from strains grown on V-8 plates for 1–2 weeks. Conidia washed from the surface of the plates with sterile H_2O were used immediately to inoculate V-8 juice medium [5] at 10^5 conidia per ml in a 50 ml Erlenmyer flask fitted with a morton closure (Bellco Glass, Inc., Vineland, NJ). Appropriate controls of fungal cultures without lubimin and of lubimin alone in culture media were run. Cultures were incubated at 25 ± 1° in a water bath reciprocal shaker at 120 rpm. After 24 hr incubation, phytoalexins or metabolites were added as 100 × stock solns in dimethylsulphoxide to a final dimethylsulphoxide concentration of 1%. This concentration of dimethylsulphoxide had no effect on fungal growth. For lubimin experiments, 2.5 mg was added to a 25 ml liquid culture of R-7715 or R-583, and 800 μg to a 10 ml culture of R-110. For 2-dehydrolubimin experiments, 1.3 mg was added to a 10 ml culture of R-7715, and 750 μg to a 10 ml culture of R-583. Dihydrolubimin, 1 mg, was added to a 10 ml culture of R-7715, and cyclodehydroisolubimin, 750 μg, was added to a 7.5 ml culture of R-7715 or R-583. The cultures were then incubated as above for up to 4 more days, as specified in the figure legends for individual experiments, during which time 500 μl (plus 500 μl H_2O) or 1 ml homogeneous samples of whole cultures were removed at intervals, as specified in the figure legends, and immediately mixed with 3 ml $CHCl_3$–MeOH (2:1). The $CHCl_3$ layers were taken to dryness and the residues were taken up in isooctane–Me_2CO (9:1) for analysis by capillary GC [4].

For the deuterium-labelling experiment, a 20 ml culture of strain R-7715 was incubated as described above for 24 hr. The culture was then filtered and the mycelia (130 mg dry wt) were washed with H_2O and transferred to a 50 ml Erlenmyer flask containing 16 ml ^2H_2O (99.8 atom percent, Aldrich Chemical Co.) and 1.6 mg of lubimin in 320 μl dimethylsulphoxide. The flask was sealed in a plastic bag and incubated for 24 hr as described above. The culture was then mixed with 48 ml $CHCl_3$–MeOH (2:1), extracted as described above and analysed by GC-MS and NMR [4].

Toxicity of lubimin to all strains and of lubimin and selected metabolites to the lubimin-sensitive strain R-110 was examined in a V-8 juice agar medium [20]. Appropriate controls of fungal cultures without lubimin and lubimin in culture media were run. The inhibition in growth rate of a tolerant strain, R-6380, and a sensitive strain, R-110, was determined at concentrations of 50, 100, 200 and 400 μg per ml. Growth of both strains with lubimin

at 50 and 100 μg per ml was within 20% of the growth rate of controls. The tolerant strain was inhibited less than 20% and the sensitive strain was inhibited more than 70% at 200 and 400 μg per ml, thus 200 μg per ml was used for further experiments. Duplicate 35 × 10 mm plastic petri dishes containing 1 ml of V-8 juice agar and 2% dimethylsulphoxide (v/v) with or without test compounds (at 200 μg per ml), were inoculated with plugs (2 mm diam.) cut from the growing margin of cultures less than 10 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 five days. All field strains were tested against lubimin simultaneously in duplicate plates and the experiment was repeated twice. Strain R-110 was tested further against lubimin and selected metabolites in duplicate plates. Per cent tolerance was calculated by dividing radial growth on phytoalexin-amended medium by radial growth of controls. Strains with a percent tolerance greater than *ca* 50% under these conditions were rated as highly tolerant. After 7 days incubation, the agar cultures were extracted with $CHCl_3$–MeOH (2:1) as described [4] and analysed by GLC as above. Recovery of lubimin from uninoculated control plates incubated for 7 days was >95%.

Extraction and purification of lubimin and metabolites. Lubimin was prepared from potatoes by a modification [4] of a previously described method [21] and was isolated by chromatography on silica. Cyclodehydroisolubimin, 11,12-epoxycyclodehydroisolubimin, and cyclolubimin (2-dihydrocyclodehydroisolubimin) were biosynthesized from lubimin by strain R-7715 and isolated by chromatography (CC, TLC and HPLC) on silica gel [4]. 2-Dehydrolubimin and 15-dihydrolubimin were synthesized from lubimin by pyridinium chlorochromate oxidation and $NaBH_4$ reduction, respectively [4]. The purity of lubimin and all lubimin metabolites was 97% or better, and these compounds were added to cultures after determining their wt both gravimetrically and by GC peak areas compared with standards.

Virulence assay. Tubers of potato cv. Russett Burbank and Sebago were obtained from the University of Wisconsin, Lelal Starks Elite foundation seed potato farm, Rhinelander, WI. Tubers were stored at 4°, and several hr prior to use brought to room temp. Slices were prepared aseptically (0.5 to 0.8 cm thick and 3 cm in diam.) from the medullary tissue of potato tubers. Three or, occasionally, two tuber slices were placed in a 10 cm plastic Petri dish lined with filter paper moistened with 2 ml sterile H_2O. Slices were inoculated immediately by placing an inoculum plug (5 mm diam.), mycelial side down, at the top edge of each slice. Inoculum plugs were cut from the growing margins of cultures less than 10 days old. All cultures used in each experiment were of equal age. The Petri dishes were sealed in plastic bags and incubated for 4 to 5 days at 25° in the dark. Tuber slices were weighed at the beginning of each experiment. At the end, rotted tissue was removed with a spatula and the remaining tuber tissue was weighed. Virulence was estimated from percent tuber rotted; 100 − [(fr. wt of uninfected tissue after incubation/fr. wt prior to infection) × 100].

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