STUDIES ON PHYTOALEXINS

IV. THE ANTIMICROBIAL SPECTRUM OF PISATIN

By I. A. M. CRUICKSHANK*

[*Manuscript received September* 8, 1961]

Summary

Representative groups of microorganisms were assayed for sensitivity to pisatin in agar media. Detailed growth studies were carried out on a small number of filamentous fungi of special interest to the problem of disease resistance in plants. The following points were demonstrated.

(1) Pisatin is a relatively weak antibiotic with a broad biological spectrum.

(2) Mycelial growth of *Monilinia fructicola* is three times more sensitive to pisatin than is germination.

(3) Fungi pathogenic towards *Pisum sativum* are relatively insensitive to pisatin. Fungi non-pathogenic to *P. sativum* are, in general, highly sensitive.

(4) The selective toxicity of pisatin is demonstrated, not only between different genera and species, but also between varieties or strains of the same fungal species.

(5) The selective toxicity of pisatin extended to yeasts, soil bacteria (*Rhizobium* spp.), and pathogenic bacteria.

The significance of the selective toxicity is discussed in relation to the phytoalexin theory of disease resistance to fungi in plants.

I. INTRODUCTION

The isolation of pisatin from the endocarp tissues of pods of *Pisum sativum* L. following their inoculation with *Monilinia fructicola* (Wint.) Honey has been reported by Cruickshank and Perrin (1960, 1961). Its chemical structure has been described by Perrin and Bottomley (1961). A brief reference to the differential sensitivity of *M. fructicola* and *Ascochyta pisi* L. to pisatin has already appeared (Cruickshank and Perrin 1960).

The primary objective of these studies was to define in greater detail the role of pisatin as a naturally occurring defence compound in infected tissues of *Pisum sativum*. The spectrum of antibiotic activity of pisatin towards growth in culture of a representative range of plant pathogenic filamentous fungi, a small group of yeasts, and a miscellaneous group of plant, soil, and pathogenic bacteria is reported. A small number of growth comparisons of fungal groups of special interest to the study of resistance to disease in plants are also presented.

* Division of Plant Industry, C.S.I.R.O., Canberra.

II. Materials and Methods

Cultures of the microorganisms used in these tests were obtained within Australia from laboratories listed in the "British Commonwealth Collections of Micro-organisms" (Anon. 1960). Stock cultures of the filamentous fungi, yeasts, and bacteria were grown on potato dextrose agar, yeast extract agar (Whiffen 1948), and Difco nutrient agar respectively. A stock solution of pisatin was prepared by dissolving crystalline pisatin in peroxide-free ethyl ether to give a concentration of 1 mg of pisatin per millilitre of solution.

The agar media used in the antimicrobial spectrum studies were Difco prune agar for fungi, yeast extract agar for yeasts, and Difco nutrient agar for bacteria. The medium used for the detailed growth studies on selected fungi was of the following composition: glucose 30 g, Difco yeast extract 7 g, KH_2PO_4 5 g, agar 20 g, distilled water 1 l., pH 5·2. The two media chosen for fungal studies produced flat cultures with a minimum of aerial mycelium, i.e. cultures for which radial measurements were a reliable criterion of growth.

Pisatin was incorporated into agar medium as follows: known volumes of the stock solution of pisatin in ethyl ether were pipetted into dry sterile conical flasks, and the solutions evaporated to dryness over a water-bath. Ethanol (A.R. grade) was added in volumes equivalent to a final ethanol concentration in the medium of 2 or 3% to redissolve the pisatin, and assist its solution in the agar medium. The higher concentration was used for the antimicrobial spectrum studies where small total volumes were involved. The lower concentration was found adequate in the extended growth studies. Melted agar, cooled to approximately 70°C, was added under aseptic conditions to ethanol solutions of pisatin in the flasks. The medium was further cooled to 45°C before dispensing.

Inoculum consisted of agar disks (2 by 2 by 1 mm) cut from the edge of young actively growing fungal cultures on Difco prune agar, or streaks prepared from loops of actively growing yeast or bacteria from stock cultures. In the case of filamentous fungi, radial growth at the end of experiments was measured. For yeasts and bacteria, growth was scored on a 0–5 scale where 0 represented no visible growth, and 5 the growth of the organism on the control medium without pisatin. All experiments were carried out in triplicate or greater replication. In the few cases where responses between replicates were irregular, the complete tests were repeated.

III. Experimental and Results

(*a*) *Determination of Median Effective Dose and Minimum Inhibitory Concentration Values of Pisatin for Representative Groups of Filamentous Fungi, Yeasts, and Bacteria in Culture*

Sets of five watch-glasses (3·75 cm diam.) were placed in large petri dishes (15 cm diam.) and sterilized. Aliquots of melted agar (0·5 ml) containing pisatin at concentrations of 0, 25, 50, 75, and 100 μg/ml were dispensed under aseptic conditions into each set of watch-glasses. The agar in each set of watch-glasses was inoculated in the centre with the same test organism. Separate petri dish assay units were used for different test organisms.

The normal incubation periods were 96 hr for filamentous fungi and 72 hr for yeasts and bacteria. A small group of slow-growing fungi were given longer incubation periods of up to 15 days in order to obtain adequate growth of the control cultures so that reliable measurements could be made. The filamentous fungi and yeasts were incubated at 20°C. The bacteria were incubated at 26°C.

The above assay was slightly modified for the two *Ustilago* species tested. The basic agar preparative procedure was retained, but the agar surface was inoculated by applying the teliospores of the smuts to the surface as a dry dust, which was either left on the agar without further handling, or gently rubbed into the surface with the aid of a sterile platinum wire loop. The smuts were incubated at 20°C for 48 hr.

The radial growth of each fungus was plotted against pisatin concentration. The median effective dose (ED_{50}) value was read off the resulting dosage–response curves. In Table 1 are given the ED_{50} ranges for 50 fungi, i.e. the concentration ranges within which the ED_{50} values fell. In Plate 1 is shown the effect on growth of one of the two *Ustilago* species where the teliospores were dusted on the agar surface. When dusting was followed by rubbing the spores into the agar surface (not illustrated) the results at 0 and 25 μg/ml were similar to those shown in Plate 1. At 50 μg/ml germination only occurred, while at the higher pisatin concentrations there was complete inhibition of germination and growth.

The most significant result shown in Table 1 is the selective toxicity of pisatin. A comparison of the ED_{50} values of pisatin for the fungi tested shows that the organisms fell clearly into two classes, namely, those that were relatively insensitive to pisatin and those that were sensitive to pisatin. These classes corresponded to the known pea pathogens in the group and the rest of the fungi tested. In the former group of six known pathogens the ED_{50} value for five of them was greater than 100 μg/ml. For the exception (*Septoria pisi*) the ED_{50} value fell within the range 75–100 μg/ml. In the latter group of fungi, which represented many important plant pathogens of hosts other than *Pisum sativum* taken from the three main fungal classes and the Fungi Imperfecti, the individual ED_{50} values varied with the fungus. The ED_{50} values for 38 out of 44 of this group were less than 50 μg/ml, and only one of them, namely, *Fusarium graminearum* had an ED_{50} value in excess of 75 μg/ml.

A comparison of the degree of inhibition of growth of each fungus at 100 μg/ml is also given in Table 1. These results show that five out of the six pea pathogens were inhibited by less than 50% while 37 of the 44 fungi in the non-pathogenic group were inhibited by more than 90%. There is evidence (unpublished) which suggests that the rate of formation and ultimate concentration of pisatin varies with the fungus used as inoculant. When this is taken into consideration, it seems probable that the few anomalies that exist in terms of growth inhibition at 100 μg/ml may be due to the limitations of the standard assay used in this work which does not take such factors into account.

For yeasts and bacteria, growth was assessed as described above. From the data obtained, the minimum inhibitory concentration (M.I.C.) value for each organism in these two groups was determined. The results are presented in Tables 2

TABLE 1

ANTIFUNGAL SPECTRUM OF PISATIN

Assays carried out *in vitro* for 96 hr (first part of table) and for 10–15 days at 20°C (Table 1 contd.)

Microorganism	Median Effective Dose Range of Pisatin (μg/ml of agar)	Inhibition of Growth at Pisatin Concentration of 100 μg/ml (%)
Phycomycetes		
Phytophthora cactorum (Lebert & Cohn) Schroet.	25–50	>90
Pythium de baryanum Hesse.	25–50	>90
Pythium ultimum Trow.	25–50	>90
Thamnidium sp.	25–50	>90
Mucor mucedo (L.) Fres.	25–50	>90
Ascomycetes		
Glomerella cingulata (Stonem.) Spauld. & Schrenk	25–50	>90
Leptosphaeria maculans (Desm.) Ces. & de Not.	<25	>90
Monilinia fructicola (Wint.) Honey	<25	>90
**Mycosphaerella pinodes* (Berk. & Blox.) Vestergr.	>100	<50
Sclerotinia libertiana (Lib.) Fekl.	25–50	>90
Basidiomycetes		
Corticium fuciforme (Berk.) Wakef.	<25	>90
Pellicularia filamentosa (Pat.) Rogers strain 1	50–75	50–90
Pellicularia filamentosa (Pat.) Rogers strain 2	50–75	50–90
**Pellicularia filamentosa* strain 3 (ex *Pisum sativum* L.)	>100	<50
Stereum purpureum Pers.	<25	>90
Ustilago bullata Berk.	<25	>90
Ustilago avenae (Pers.) Rostr.	<25	>90
Fungi Imperfecti		
Alternaria solani (Ellis & Martin) Sorauer	25–50	>90
Aspergillus nidulans (Eidam) Wint.	25–50	50–90
**Ascochyta pisi* Lib.	>100	<50
Ascochyta pisi (Lib.) var. *fabae* Speg.	25–50	>90
**Ascochyta pinodella* L. K. Jones	>100	<50
Botrytis cinerea Pers.	25–50	>90
Botrytis allii Munn.	25–50	>90
Colletotrichum lindemuthianum (Sacc. & Magn.) Bri. & Cav.	<25	>90
Colletotrichum graminicolum (Ces.) G. W. Wils.	25–50	>90
Colletotrichum linicolum Pethybr. & Laff.	25–50	>90
Fusarium graminearum Schw.	75–100	50–90
Fusarium oxysporum var. *melonis* Snyder & Hansen	50–75	50–90
Fusarium oxysporum var. *gladioli* Snyder & Hansen	50–75	50–90
Fusarium oxysporum var. *lycopersici* (Sacc.) Snyder & Hansen	25–50	50–90
**Fusarium solani* var. *martii* Appel & Wollenw.	>100	<50
Helminthosporium cynodontis Marig.	25–50	>90
Kabatiella caulivora (Kirch.) Karak.	25–50	>90
Penicillium gladioli McCull. & Thom	25–50	>90
Penicillium digitatum (Fr.) Sacc.	25–50	>90
Phoma foveata Foister	25–50	>90
Phomopsis viticola (Sacc.) Sacc.	<25	>90
Polyspora lini Laff.	25–50	>90
Sphaerella linorum Wollenw.	25–50	>90
Thielaviopsis basicola (Berk. & Br.) Ferraris	25–50	>90
Trichoderma lignorum (Tode.) Harz.	<25	>90
Verticillium albo-atrum Reinke & Berth.	25–50	>90

TABLE 1 (*Continued*)

Microorganism	Median Effective Dose Range of Pisatin (μg/ml of agar)	Inhibition of Growth at Pisatin Concentration of 100 μg/ml (%)
Ascomycete		
Venturia inaequalis (Cooke) Wint. emend. Aderh.	<25	>90
Basidiomycetes		
Armillarea mellea (Fr.) Quel.	50–75	>90
Fomes australis (Fr.) Che	<25	>90
Fungi Imperfecti		
Fusicladium carpophilum Thun.	25–50	>90
Septoria apii Chester	25–50	>90
Septoria lycopersici Speg.	25–50	>90
**Septoria pisi* Westend.	75–100	>90

* Known pea pathogens.

and 3. Differences in M.I.C. values between yeasts were shown. The most marked differences were apparent at the pisatin concentration of 100 μg/ml. At this level, *Cryptococcus neoformans*, two species of *Saccharomyces*, and *Torulopsis utilis* showed no visible signs of growth. *Endomyces magnusii*, on the other hand, appeared completely tolerant to pisatin at this level.

From the point of view of possible biological significance the bacteria tested may be considered in three groups, viz. the plant pathogenic bacteria, the strains of *Rhizobium* spp., and the remainder. No toxicity was observed in the first group. In the second group, 6 strains of 5 species of *Rhizobium* represented isolates from 5 leguminous hosts including 2 from *P. sativum*. It may be significant to note that the two pea strains were less sensitive to pisatin than the others. The pea strains also differed from each other, SU302 being the less sensitive (Table 3) of the two. At the 100 μg/ml level, however, all six strains were almost fully inhibited. This result was confirmed for the pea strain (SU302) and two others, using a defined liquid medium (Bergersen 1961), and standard bacterial assay techniques. The third group of miscellaneous bacteria included in Table 3 represent some of the major bacterial groups. The M.I.C. values show that 8 of the 13 bacteria tested were not sensitive to pisatin. Of the five which were sensitive, *Mycobacterium phlei* had an M.I.C. value of less than 25 μg/ml, while *Bacillus brevis*, *B. cereus*, *Cellomonas biazotea*, and *Staphylococcus aureus* showed some inhibition at the higher concentrations. At the highest pisatin concentration used, *Staph. aureus* showed no visible growth. The other bacteria within the group sensitive to pisatin showed from nil to trace amounts of growth at the maximum level of pisatin concentration used in these assays.

(*b*) *Detailed Growth Studies of Selected Fungi*

In all the experiments reported in this subsection the fungi were cultured on plates (9 cm diam.) of glucose yeast extract, prepared as described above, and inoculated centrally with mycelial disks.

In order to determine the ED_{50} value for the growth of *M. fructicola* with greater precision a detailed test was carried out over the 0 to 35 μg/ml concentration range using a dilution interval of 5 μg/ml. A 96-hr incubation period was used. The results are presented in Figure 1 as a dosage–response curve.

The ED_{50} value of pisatin for germination of *M. fructicola* is of the order of 30 μg/ml (1×10^{-4}M) while germination is fully inhibited at approximately 80 μg/ml ($2 \cdot 8 \times 10^{-4}$M) (Cruickshank and Perrin 1960). The corresponding values for mycelial growth are 10 μg/ml (3×10^{-5}M) and 25 μg/ml (7×10^{-5}M) (see Fig. 2). Thus mycelial growth of *M. fructicola* is approximately three times more sensitive to pisatin than is germination.

A comparison of the ED_{50} values of pisatin for pathogens and non-pathogens of peas (Table 1) indicated a clear difference in sensitivity to pisatin between these two groups of fungi. For a more detailed comparison of these two groups, three fungi

TABLE 2

ACTIVITY IN VITRO OF PISATIN AGAINST YEASTS

Microorganism	Minimum Inhibitory Concentration of Pisatin (μg/ml)	Inhibition of Growth at Pisatin Concentration of 100 μg/ml
Cryptococcus neoformans	50–75	No visible growth
Debaryomyces klockeri	75–100	Partially inhibited
Endomyces magnusii	>100	No inhibition
Hansenula anomola	75–100	Partially inhibited
Kloeckera austriaca	50–75	Partially inhibited
Saccharomyces cereviciae	75–100	Partially inhibited
Saccharomyces fragilis	75–100	No visible growth
Saccharomyces lactis	50–75	No visible growth
Torulopsis utilis	50–75	No visible growth

were chosen from the first group representing a pod- and leaf-infecting fungus (*Ascochyta pisi*), a pod-, leaf-, and stem-infecting fungus (*Mycosphaerella pinodes*) and a root- and stem-infecting fungus (*Fusarium solani* var. *martii* f. *pisi*). These three organisms were pathogenic towards the endocarp tissues of detached pea pods. The three fungi chosen from the group of non-pathogens represented a storage-rotting fungus (*Botrytis allii*) of onions, a pod-, stem-, and leaf-infecting fungus (*Colletotrichum lindemuthianum*) of French beans, and a leaf-, stem-, and root-infecting fungus (*Leptosphaeria maculans*) of crucifers. They were non-pathogenic towards the endocarp tissues of pea pods. In this experiment the pisatin concentrations were 0, 20, 40, 60, 80, and 100 μg/ml. The incubation period was 8 days.

The results shown in Figure 2 emphasize further the differential sensitivity of pathogens and non-pathogens of peas towards pisatin. It is seen in the case of the two taxonomically related pea pathogens (*A. pisi* and *M. pinodes*) that the curves are of similar shape. Essentially there is an initial growth inhibition in each case, and then very little further effect with increase in pisatin concentration. In the case

of the third pea pathogen (*F. solani* var. *martii* f. *pisi*) there is increasing inhibition with increasing dosage. However, even at 100 μg/ml growth has not been inhibited by more than 40%. The three fungi non-pathogenic towards peas form another quite distinct group. The slope of their dosage–response curves varies according to the fungus but in each case the slopes are highly significantly different from those

TABLE 3

ANTIBACTERIAL SPECTRUM OF PISATIN IN VITRO

Microorganism	Minimum Inhibitory Concentration of Pisatin (μg/ml)	Inhibition of Growth at Pisatin Concentration of 100 μg/ml
Phytopathogens		
Pseudomonas angulata	>100	*
Pseudomonas pisi	>100	*
Pseudomonas solanacearum	>100	*
Xanthomonas campestris	>100	*
Xanthomonas phaseoli	>100	*
Rhizobium species†		
Rhizobium trifolii (SU297)	<25	**
Rhizobium leguminosarum (SU302)	50–75	**
Rhizobium leguminosarum (SU331)	25–50	**
Rhizobium phaseoli (CC511)	25–50	**
Rhizobium sp. ex *Lotus uliginosis* (CC806)	<25	**
Rhizobium meliloti (Rothamsted AH2)	25–50	**
Miscellaneous bacteria		
Aerobacter aerogenes	>100	*
Bacillus brevis	25–50	**
Bacillus cereus	25–50	**
Bacillus pumilus	>100	*
Cellomonas biazotea	25–50	**
Escherichia coli	>100	*
Micrococcus lysodeikticus	>100	*
Mycobacterium phlei	<25	**
Nocardia dermatonomus	>100	*
Pseudomonas aeruginosa	>100	*
Staphylococcus aureus	50–75	***
Staphylococcus epidermidis	>100	*
Streptococcus faecalis	>100	*

* No inhibition. ** Zero to trace of growth. *** No visible growth.

† Australian Collection No. given in parenthesis.

of the pea-pathogen group and in all cases inhibition is greater than 95% at the pisatin concentration of 100 μg/ml.

In the next experiment (illustrated in Fig. 3) the same conditions applied as in the experiment just described. However, in this experiment the sensitivity of two strains of *A. pisi* were compared, namely a strain isolated from pea pods (*A. pisi*)

and a strain isolated from broad beans (*A. pisi* var. *fabae*) not pathogenic towards pea pods. The difference in sensitivity in culture of these two strains towards pisatin corresponds to the difference in their pathogenicity towards pea pods.

The results in Figure 1 show that pisatin at high concentrations does affect the growth of pea pathogens in culture, even though these fungi are not as sensitive as are other fungi. In the final experiment the growth rate of one of the leaf- and pod-infecting fungi (*A. pisi*) and the root- and stem-infecting fungus (*F. solani* var.

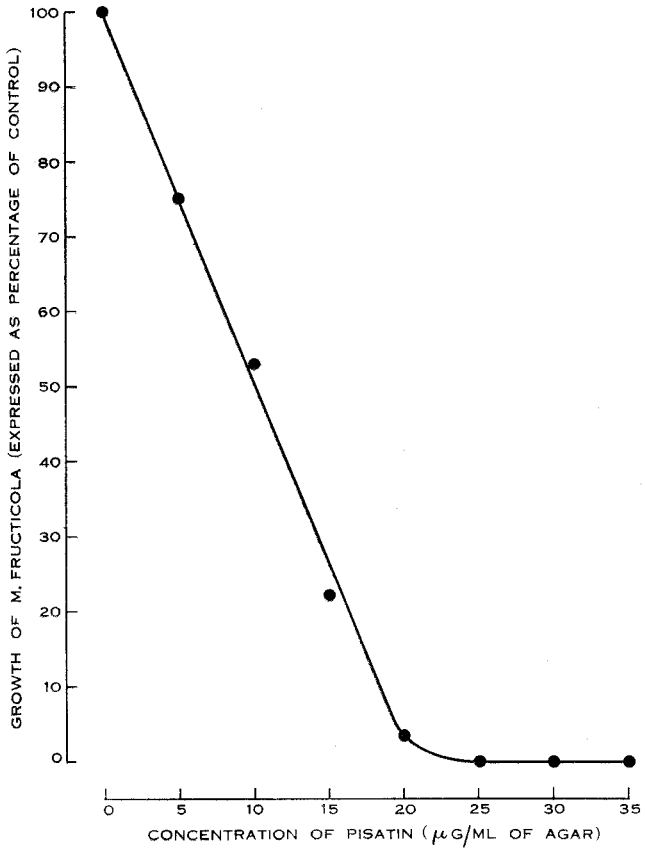


Fig. 1.—Dosage–response curve of *Monilinia fructicola* to pisatin. Radius of control colony = 25 mm.

martii f. *pisi*) were compared on agar with and without pisatin at 100 μg/ml. The results presented in Figure 4 show that in the former case the inhibition of growth is confined to the first 3 days of the incubation period. In the latter case (*F. solani* var. *martii* f. *pisi*) the effect of pisatin on the growth rate increased with time over the complete incubation period of 8 days.

IV. Discussion

The primary objective of the fungal assays was to determine the relative toxicity of pisatin to a range of fungi, in an attempt to define its role as a naturally

occurring defence compound in infected tissues of *P. sativum*. The concentration of free pisatin within infected host cells is not known. It may not, in fact, be important as many fungi grow intercellularly and hence never actually penetrate the protoplasts of their host cells. However, as pisatin is a non-ionic neutral molecule, a simple equilibrium between the pisatin concentrations in diffusate solutions on pea pod endocarp (Cruickshank and Perrin 1961) and in the liquid films lining the intercellular spaces of infected endocarp tissues can be assumed. The maximum level of pisatin

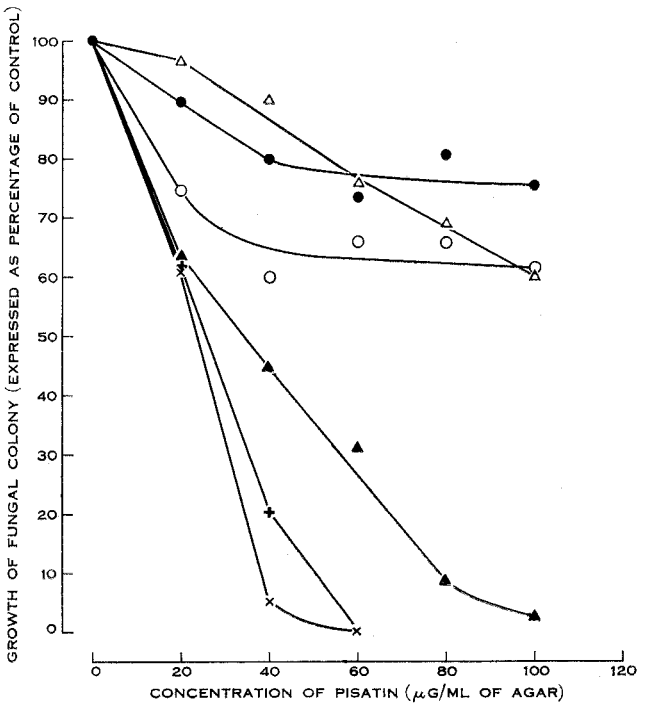


Fig. 2.—Dosage–response curves of three pathogens and three non-pathogens of peas to pisatin. Radii (mm) of control colonies are as follows:

● *Ascochyta pisi* 20 ▲ *Botrytis allii* 66
○ *Mycosphaerella pinodes* 21 + *Colletotrichum lindemuthianum* 16
△ *Fusarium solani* var. *martii* f. *pisi* 32 × *Leptosphaeria maculans* 12

used in the tests, namely 100 μg/ml approximates to the known maximum concentration of pisatin in diffusate solutions (Cruickshank and Perrin, unpublished data). If the above assumption is correct, it also approximated the concentration of the extracellular pisatin in infected tissues of the pea pod endocarp. The significance of the results would not be affected unless the assumptions made were grossly in error.

From the many reports which have attempted to give an explanation of the mechanisms underlying disease resistance in plants, especially the recent reviews by Allen (1959), Müller (1959), and Uritani and Akazawa (1959), it is clear that the

phenomenon is not a static one. Pisatin is formed as a response to inoculation of the tissues of pea plants (Cruickshank and Perrin 1960) with a wide range of fungi. The conditions under which it is formed probably involve the interaction of metabolites or enzymes or both of these from both the host plant and the fungus. It is clear from these considerations that assays in culture of the toxicity of any one toxin such as pisatin can never give a complete explanation of the complex *in vivo*

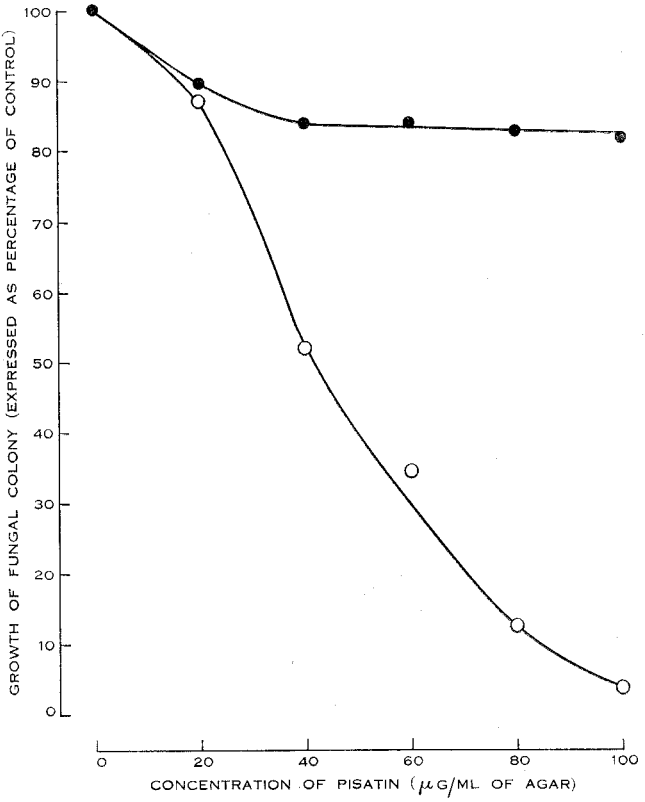


Fig. 3.—Comparison of the dosage–response curves of *Ascochyta pisi* (●) and *A. pisi* var. *fabae* (○) to pisatin. Radii of control colonies are 21 and 32 mm respectively.

phenomenon. They can, however, present a broad picture of the primary pattern of activity of the toxin. Assays of the toxicity of pisatin towards mycelial growth were used in the present studies. They were considered more realistic than spore-germination assays when considered in relation to disease resistance as a post-infectional phenomenon. The greater toxicity of pisatin towards mycelial growth may also be of significance here. It must, however, be remembered that pisatin is not formed until after spore germination has occurred. Its affect on germination in nature would thus be secondary in terms of the spores which stimulate its formation.

In the original statement of the phytoalexin theory (Müller and Börger 1940) phytoalexins were postulated to be non-specific, in the immunological sense, in their

effect on fungi. Uehara (1960) has perpetuated this idea in a recent biological paper. It is suggested, that with the additional data now available on the chemistry (Perrin and Bottomley 1961) and antimicrobial spectrum of a compound of the phytoalexin* class, that the immunological usage of specificity is no longer valid. The term specificity is not used in the subsequent discussion, as defence mechanisms in plants appear to be based on the formation of low molecular weight diffusable antibiotics and not high molecular weight antibodies as in immunological responses in animal tissues.

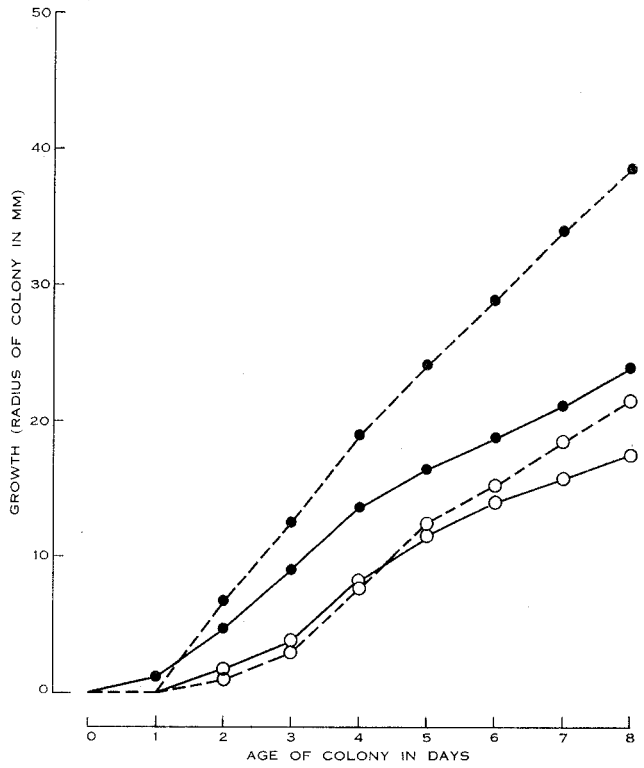


Fig. 4.—Effect of pisatin (100 μg/ml) on growth rate of two pea pathogens:

○——○ *Ascochyta pisi* + pisatin. ●——● *A. pisi*—control.
○ - - - ○ *Fusarium solani* var. *martii* f. *pisi* + pisatin.
● - - - ● *F. solani*—control.

Differential sensitivity between fungi to a phytoalexin was first demonstrated by Müller (1958) using diffusate solutions from French bean pods (*Phaseolus vulgaris* L.). The results of Condon and Kuć (1960) who studied the antifungal activity of a compound isolated from carrot (*Daucus carota* L.) inoculated with *Ceratostomella fimbriata* (Ell. & Halst.) Elliot (syn. *Ceratocystis fimbriata* Ell. & Halst.) did not confirm Müller's results. Phytoalexin action has been claimed for ipomeamarone

* "Alexin" is used here with its original Greek meaning—a warding-off compound. There is no implied or expressed association with modern immunological usage.

(Akazawa 1960) which occurs in the diseased tissue of sweet potato infected with *C. fimbriata*. However, the antifungal spectrum of this compound does not appear to have been published.

The only compounds of significance to defence reaction in plants, in the phytoalexin sense, for which antimicrobial spectra covering representative groups of microorganisms are available, are orchinol (Gäumann and Kern 1959; Gäumann, Nuesch, and Rimpau 1960) and pisatin. As the assay conditions used by Gäumann *et al.* and those used in the present work were different, it is impossible to compare the antimicrobial spectra of orchinol and pisatin in detail; nevertheless, an examination of the two spectra does show the following similarities and differences: both compounds have broad antifungal spectra and judged by their antifungal efficiencies must be termed "weak antibiotics". The antibiotic action of orchinol according to Gäumann, Nuesch, and Rimpau (1960) is "hardly specific", however, their data show considerable differences in sensitivity among mycorrhizal and soil fungi. In the case of the mycorrhizal fungi most of the selective toxicity could be related to the host reactions of the orchids tested, but the results could not explain the resistance of *Orchis militaris* to the soil fungi tested.

The selective toxicity of pisatin towards fungi is very well defined. Fungi pathogenic towards the tissues of *P. sativum*, irrespective of whether they are pod-, leaf-, stem-, or root-infecting, are relatively insensitive to pisatin, while fungi which are non-pathogenic towards the tissues of *P. sativum*, are in general highly sensitive to pisatin. This pattern of selective toxicity would be expected if pisatin is the primary factor responsible for the resistance of the tissues of *P. sativum* to most fungi.

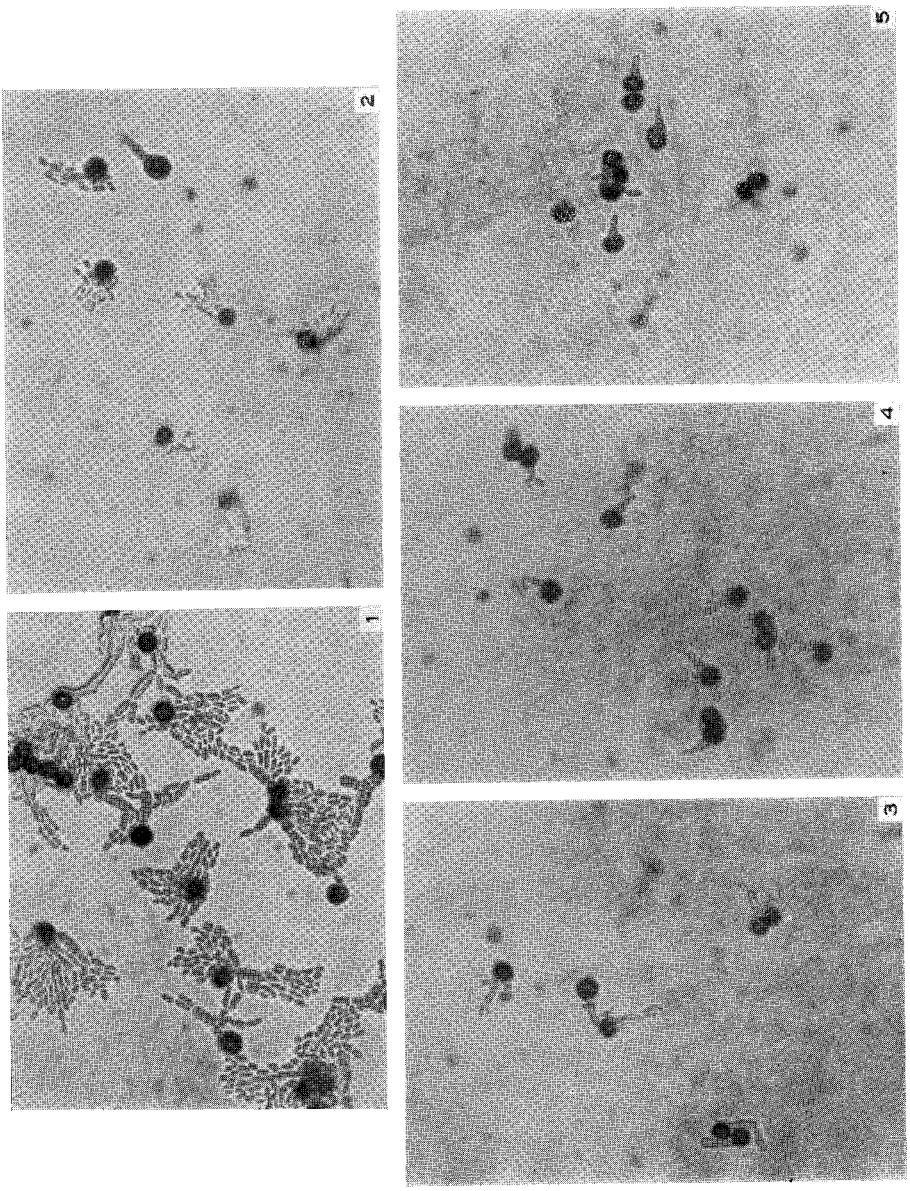
Bacteria as a group do not appear to be very sensitive to either orchinol or pisatin; however, within this group some sensitive species occur.

The relatively weak antibiotic activity of pisatin when considered along with some of its physical properties (Cruickshank and Perrin 1961) reduce interest in the compound as a therapeutant. However, natural or synthetic analogues of pisatin, or other phytoalexins, may provide a new source of biologically active molecules, a few of which may find a role in plant or animal chemotherapy.

The implications of the differential sensitivity of pathogens and non-pathogens of peas towards pisatin are interesting. It seems possible that the pattern of pathogenicity of fungi is closely associated with their sensitivity to the phytoalexin which they cause the host to produce. With the present limited knowledge of parasitism it is impossible to set out a hypothesis to cover the details of mechanisms of disease reaction which would have no exceptions. However, the differential sensitivity reported in this paper appears necessary if the differences in pathogenicity of the various fungal parasites to peas are to be explained on the basis of the phytoalexin theory.

V. Acknowledgments

The author wishes to acknowledge the cooperation of the mycologists and bacteriologists from other research institutes who supplied cultures of microorganisms used in the assays, and to thank Dr. F. J. Bergersen for assistance with the assays of species of *Rhizobium* on defined liquid media.



Figs. 1–5.—Effect of pisatin on the germination and growth of *Ustilago bullata*. Spores dusted on to agar surface only. Pisatin concentrations respectively 0, 25, 50, 75, and 100 μg/ml agar.

VI. References

AKAZAWA, T. (1960).—*Arch. Biochem. Biophys.* **90**: 82–9.

ALLEN, P. J. (1959).—In "Plant Pathology". Vol. 1. pp. 435–67. (Ed. J. G. Horsfall and A. E. Dimond.) (Academic Press Inc.: New York.)

ANON. (1960).—"British Commonwealth Collections of Micro-organisms." (H.M.S.O.: London.)

BERGERSEN, F. J. (1961).—*Aust. J. Biol. Sci.* **14**: 349–60.

CONDON, P., and KUĆ, J. (1960).—*Phytopathology* **50**: 267–70.

CRUICKSHANK, I. A. M., and PERRIN, DAWN R. (1960).—*Nature* **187**: 799–800.

CRUICKSHANK, I. A. M., and PERRIN, DAWN R. (1961).—*Aust. J. Biol. Sci.* **14**: 336–48.

GÄUMANN, E., and KERN, H. (1959).—*Phytopath. Z.* **36**: 1–26.

GÄUMANN, E., NUESCH, J., and RIMPAU, R. H. (1960).—*Phytopath. Z.* **38**: 274–308.

MÜLLER, K. O. (1958).—*Aust. J. Biol. Sci.* **11**: 275–300.

MÜLLER, K. O. (1959).—In "Plant Pathology." Vol. 1. pp. 469–519. (Ed. J. G. Horsfall and A. E. Dimond.) (Academic Press Inc.: New York.)

MÜLLER, K. O., and BÖRGER, H. (1940).—*Arb. Biol. Reichsanst. Land-u. Forstwirtsch. Berl.* **23**: 189–231.

PERRIN, DAWN R., and BOTTOMLEY, W. (1961).—*Nature* **191**: 76–7.

UEHARA, K. (1960).—*Saijo Hiroshima Agric. Coll.* B **1**: 7–10.

URITANI, I., and AKAZAWA, T. (1959).—In "Plant Pathology." Vol. 1. pp. 349–90. (Ed. J. G. Horsfall and A. E. Dimond.) (Academic Press Inc.: New York.)

WHIFFEN, A. J. (1948).—*J. Bact.* **56**: 283–91.