# STUDIES ON PHYTOALEXINS

## IX. PISATIN FORMATION BY CULTIVARS OF PISUM SATIVUM L. AND SEVERAL OTHER PISUM SPECIES

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*Summary*

The qualitative formation of pisatin by 58 named cultivars and nine numbered lines of *P. sativum* and *P. arvense* and three other species of *Pisum* following inoculation by *Monilinia fructicola* was established.

Quantitative studies of pisatin formation using host–parasite combinations of several pea cultivars and several strains of *Ascochyta pisi* showed that where one strain of *A. pisi* was used to inoculate several cultivars of peas, the concentration of pisatin varied with the variety. On the other hand, where one pea cultivar was inoculated with several strains of *A. pisi* the concentration of pisatin was related to the strain of *A. pisi*.

## I. INTRODUCTION

The central role of pisatin as a compound causally involved in the immunity of the tissues of the garden pea (*Pisum sativum* L.) to fungi non-pathogenic to this host plant has been previously reported (Cruickshank and Perrin 1961; 1963; 1965). The qualitative formation of pisatin is recorded in this paper for a wide range of cultivars and numbered lines of *P. sativum* and *P. arvense* L. and several other species of *Pisum*. Quantitative evidence on the concentrations of pisatin formed following inoculation and infection of some selected pea cultivars with several strains of *Ascochyta pisi* Lib. is reported and discussed.

## II. MATERIALS AND METHODS

The host material consisted of detatched half pea pods (class 2 and 3 host material —see Cruickshank and Perrin 1963, Table 1). The pea cultivars and *Pisum* species tested qualitatively for pisatin formation were grown in the glasshouse and inoculated with *Monilinia fructicola* (Wint.) Honey. The pea cultivar Little Gem was used for the experiments involving the comparison of strains of *A. pisi* as inocula. The Canberra strain of *A. pisi* served as inoculum in the experiment designed to compare the relative production of pisatin by several cultivars of peas. The latter were selected on the basis of uniformity of maturity of pods, and their reported disease reaction to *A. pisi* (Wark 1950; Brewer 1960; Lyall, personal communication). They were grown in a field trial arranged as a simple lattice with five replications. Equal numbers of

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pods were picked from each replicate of each variety on the same day and stored at 4°C overnight prior to use. Replicates of each cultivar were bulked and a sample of 50 half-pods taken for inoculation.

For each of the six species of *Pisum* the identification of pisatin was established from its full ultraviolet absorption spectrum ($\lambda_{max.}$ in ethanol 280, 286, and 309 m$\mu$; ratio of optical density at 309 m$\mu$ to optical density at 286 m$\mu$ 1·47), its quantitative

TABLE 1

CONCENTRATION OF PISATIN FORMED BY PEA CULTIVARS INOCULATED WITH THE CANBERRA STRAIN OF ASCOCHYTA PISI

| Pea Cultivar | Mean Concentration of Pisatin (μg/g of endocarp fresh wt.) | | | | | | Disease Reaction of Host Tissues |
|---|---|---|---|---|---|---|---|
| | Diffusate* | | Endocarp | | Total | | |
| | Experimental Values | Log Transformed Data | Experimental Values | Log Transformed Data | Experimental Values | Log Transformed Data | |
| M. U. 9 ex Tibet | 96·9 | 1·97 | 212·5 | 2·31 | 309·5 | 2·49 | Highly susceptible |
| Little Gem | 229·9 | 2·33 | 315·0 | 2·49 | 545·0 | 2·73 | Highly susceptible |
| Collegian | 282·9 | 2·44 | 342·5 | 2·53 | 625·4 | 2·79 | Highly susceptible |
| Greenfeast | 374·8 | 2·55 | 312·0 | 2·49 | 686·8 | 2·82 | Susceptible |
| Veritable St. Laurent | 359·1 | 2·50 | 467·5 | 2·61 | 826·6 | 2·92 | Susceptible |
| Sans Par Chemin de 40 Jours | 214·2 | 2·33 | 607·5 | 2·78 | 821·7 | 2·91 | Susceptible |
| O.A.C. 181 | 444·1 | 2·64 | 605·0 | 2·78 | 1049·5 | 3·01 | Semi-resistant |
| Early Blue | 239·6 | 2·34 | 932·5 | 2·94 | 1172·1 | 3·05 | Semi-resistant |
| A-100 | 315·2 | 2·42 | 882·5 | 2·94 | 1197·7 | 3·07 | Semi-resistant |
| Least significant difference for transformed data | | | | | | | |
| 5% level | | 0·28 | | 0·19 | | 0·17 | |
| 1% level | | 0·38 | | 0·26 | | 0·22 | |

* Mean fresh weight of endocarp covered by 1 ml of spore suspension is 68 mg.

conversion to anhydropisatin ($\lambda_{max.}$ 339 and 358 m$\mu$), and photochemical change to a phenol ether ($\lambda_{max.}$ 269 and 319 m$\mu$) (Perrin and Bottomley 1962). *A. pisi* was cultured on oatmeal agar (Ainsworth and Bisby 1961). The culture of *M. fructicola*, the preparation of spore suspensions, inoculation and incubation of host tissues, and collection of diffusates and their chemical assay have already been described (Cruickshank and Perrin 1963). The extraction and assay techniques used for the estimation of pisatin concentration in endocarp tissues were similar to those used by Cruickshank and Perrin (1965). Triplicate or quadruplicate samples were taken for analysis and the data statistically analysed after logarithmic transformation.

The disease reaction of the detached pea pod tissues was assessed from observations on host pigmentation, mycelial growth, and sporulation of *A. pisi* 7–8 days after inoculation.

## III. Results

Pisatin was formed by 58 named cultivars and nine numbered lines of *P. sativum* and *P. arvense* and three other species of *Pisum* following the inoculation of their pods with *M. fructicola* (see Appendix). This survey showed that the capacity to form pisatin following fungal stimulation was a general characteristic of the pea cultivars and species of *Pisum* tested. Quantitative studies of pisatin formation by a smaller group of pea cultivars (Table 1), following inoculation with a strain of *A. pisi*, showed that the concentration of pisatin was dependent on the cultivar involved.

Table 2

CONCENTRATION OF PISATIN FORMED BY THE PEA CULTIVAR LITTLE GEM FOLLOWING INOCULATION WITH FIVE STRAINS OF ASCOCHYTA PISI

| Strain of *A. pisi** | Mean Concentration of Pisatin (μg/ml of diffusate) | Disease Reaction of Host Tissues |
|---|---|---|
| Canberra strain | 67·5 | Susceptible |
| Canadian strain I | 88·6 | Semi-resistant |
| Canadian strain II | 103·6 | Semi-resistant |
| Canadian strain III | 76·8 | Susceptible |
| Canadian strain IV | 58·1 | Susceptible |
| Water control | 4·9 | |
| Least significant difference | | |
| 5% level | 8·0 | |
| 1% level | 11·5 | |

* 48 hr incubation period at 20°C.

Differences in pisatin concentration between individual cultivars did not, in all cases, reach significance; however, the difference between the lowest and highest concentrations formed was highly significant when either the values for the endocarp or for total pisatin were considered. The cultivars in which the lowest and highest concentrations of pisatin were formed were those which were most susceptible and least susceptible to *A. pisi* (Table 1).

The results of preliminary studies on the concentration of pisatin formed by the cultivar Little Gem, following inoculation with five strains of *A. pisi* (Wallen 1957), are presented in Table 2. Observations on the disease reaction of detatched pods of Little Gem to the five strains of *A. pisi* indicated that this cultivar was more susceptible to the Canberra strain and Canadian strains III and IV, than to Canadian strains I and II.

Canadian strains II and IV were selected for a detailed time course study of the relationship between these two strains of *A. pisi* and the pea cultivar Little Gem in relation to pisatin formation. The results (Table 3) indicated significant differences

TABLE 3

RELATIONSHIP BETWEEN LENGTH OF INCUBATION PERIOD AND PISATIN CONCENTRATION FOLLOWING INOCULATION OF THE PEA CULTIVAR LITTLE GEM WITH TWO STRAINS OF ASCOCHYTA PISI

| Time (hr) | Mean Concentration of Pisatin ($\mu$g/g fresh weight of endocarp) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Diffusate* | | | | Endocarp | | | | Total | | | |
| | Strain II | | Strain IV | | Strain II | | Strain IV | | Strain II | | Strain IV | |
| | Experimental Value | Log Transformed Data | Experimental Value | Log Transformed Data | Experimental Value | Log Transformed Data | Experimental Value | Log Transformed Data | Experimental Value | Log Transformed Data | Experimental Value | Log Transformed Data |
| 12 | 23·9 | 1·37 | 22·7 | 1·36 | 27·2 | 1·43 | 32·7 | 1·49 | 51·1 | 1·71 | 55·4 | 1·74 |
| 24 | 195·5 | 2·29 | 176·3 | 2·24 | 169·5 | 2·23 | 135·2 | 2·12 | 365·0 | 2·56 | 311·5 | 2·43 |
| 36 | 375·8 | 2·57 | 381·4 | 2·57 | 400·7 | 2·58 | 317·2 | 2·50 | 776·5 | 2·89 | 698·6 | 2·82 |
| 48 | 764·7 | 2·88 | 601·5 | 2·77 | 817·7 | 2·90 | 447·0 | 2·64 | 1582·4 | 3·20 | 1048·5 | 3·02 |
| 60 | 707·0 | 2·84 | 608·9 | 2·78 | 941·5 | 2·97 | 595·0 | 2·77 | 1648·5 | 3·22 | 1203·9 | 3·08 |
| 72 | 743·0 | 2·87 | 442·4 | 2·64 | 1193·5 | 3·07 | 673·5 | 2·83 | 1936·5 | 3·29 | 1115·9 | 3·05 |
| Least significant differences for transformed data | | | | | | | | | | | | |
| 5% level | | 0·11 | | | | 0·15 | | | | 0·08 | | |
| 1% level | | 0·14 | | | | 0·20 | | | | 0·10 | | |

* Mean fresh weight of endocarp covered by 1 ml of spore suspension is 68 mg.

in the concentration of pisatin between the two treatments. Where endocarp values only were considered, the differences reached significance at 48 hr after inoculation. If total pisatin was considered, a significant difference in pisatin concentration between strain treatments was apparent at 24 hr incubation.

## IV. Discussion

Reports of the detection and to a lesser degree the isolation, characterization, and biological significance of several compounds tentatively classified as phytoalexins have been reviewed recently (Cruickshank 1963, 1965). The host–parasite combinations have involved chiefly non-pathogens of the host plants studied.

Uritani and Akazawa (1955) and Akazawa and Wada (1961) in studies on the host–parasite combination sweet potato–*Ceratocystis fimbriata* (Ell. & Hals.) Elliot have reported some degree of correlation between the concentration of the phytoalexin-like compound ipomeamarone synthesized in the host and the degree of host resistance against this pathogen. Similar results have been reported (Uehara 1958) in relation to the production of an unidentified phytoalexin-like compound and four cultivars of wet rice inoculated with *Piricularia oryzae* Cav. A negative correlation between concentration of a phytoalexin-like, spore-inhibition substance and varietal reaction has been reported briefly by Nishimura (1964) based on studies of the interaction between *Helminthosporium victoriae* Meehan & Murphy and oat leaves.

In most previous papers in this series where non-pathogens of peas have been studied, the importance of the drop–diffusate technique (Müller 1956) has been emphasized in relation to the quantitative aspects of pisatin concentration and control of fungal growth. In this study, in which a fungal pathogen of peas has been used, diffusate analysis did not give results which appear consistent with the host reactions observed. This result was not unexpected. In the case of non-pathogens, growth of the fungal species was limited to germ tube development and the occasional penetration of a host cell in the surface cell layer. On the other hand, where the pathogen *A. pisi* was used its germ tubes penetrated and the mycelium extensively permeated the host tissues. The statistical analysis of the pisatin concentration data (Tables 1 and 3) show that the combined diffusate plus endocarp values gave results with the lowest relative replicate variability and the best discrimination between the less and the more susceptible cultivars. Experimentally, the combined values represent the total pisatin concentration of the tissues involved.

Considerations of the total pisatin concentration data (Tables 1 and 3) have shown that where one strain of *A. pisi* was used to inoculate several cultivars of peas, the concentration of pisatin varied with the cultivar. On the other hand, where one cultivar was inoculated with two strains of *A. pisi* the concentration of pisatin was shown to be related to the strain of *A. pisi* used as inoculum.

The phytoalexin theory of disease resistance in plants (Müller and Börger 1940) postulated that the basis of differentiation between resistant and susceptible cultivars is the speed of formation of phytoalexin. The trends in relation to rate of pisatin formation in the experiments reported above are consistent with this theory. Differential sensitivity to pisatin among strains of *A. pisi* may also be important in relation to disease expression (Cruikshank 1963). At present, technical difficulties concerned

with the solubilization of pisatin *in vitro* at concentrations comparable to those found *in vivo* (Table 1) prevent adequate testing of strains of *A. pisi* in this respect. For the purposes of this paper it has been assumed that the strains of *A. pisi* are equally sensitive to pisatin.

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## Appendix

### Cultivars of Pisum spp. tested for pisatin formation following inoculation with Monilinia fructicola

(*a*) *Cultivars and Lines of* P. sativum *and* P. arvense

Admiral Beatty, Arthur, Autocrat, Blue Imperial, Blue Prussian, Bountiful, Canner's Perfection, Carrington Gem, Charles 1st, Churchill, Dark-skinned Perfection, Deep Green Perfection, Edelkerna, Edelkerna Early, Edelkerna Gold, Early Blue, Emitale, English Wonder, Engress, Exquisite, Feltham Forward, Fin de Gourmets, Graue Bunt Bluhende, Green Crop, Greenfeast, Greenfeast (W.R.), Harrison's Glory, Holdfast, Kelvedon Wonder, Kelvedon Champion, Laxonian, Laxton's Progress, Liberty, Little Marvel, Little Gem, Lord Chancellor, Mammoth Pod, Marathon, New Era, Olympic, Onward, Onward (W.R.), Partridge, Peter Pan, Primo, Prince of Wales, Profusion, Quarante Deudre Sarcelles, Queen, Rondo, Smallton, Valley, Victory Freezer, Weibull's No. 613, Weibull's Stral. No. 700, White Prolific, Wisconsin Early Sweet, M. U. 9* ex Tibet, M. U. 25 ex India, M. U. 33 ex Abyssinia, M. U. 212 ex Russia, M. U. 216 ex Finland, M. U. 509 ex Algeria, M. U. 549 ex Sweden, O. A. C. 181, A-100.

(*b*) *Other* Pisum *Species*

*P. elatius* Stev., *P. abyssinicum* A. Br., *P. fulvum* Sibth & Sm.

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