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Preliminary investigations on PP 675 activity against pea mildew

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PP 675 (5-butyl-2-dimethylamino-4-hydroxy-6-methylpyrimidine)* an experimental systemic fungicide was developed by I.C.I. Plant Protection Limited. Anti-mildew activity of this fungicide was demonstrated especially against *Sphaerotheca fuliginea* (Schlechtendal ex Fr.) Pollaci on cucumbers and melons and against *Erysiphe cichoracearum* DC on *Chrysanthemum* and *Cineraria* species. Lower systemic fungicidal activity was found against *Erysiphe graminis* DC., *Podosphaera leucotricha* (Ellis et Everhart) Salmon, as well as against *Sphaerotheca pannosa* (Wallroth ex Fries) Léveillé. No activity was noted against *Uncinula necator* Burrie on grapes. (Plant Protection Techn. Data Sheet... 1968).

To the author's knowledge, the activity of this fungicide against *Erysiphe pisi* DC. ex St-Am. has not been investigated.

Pea mildew does not seem to be of such economical importance as e.g. cucumber mildew (Butler, Jones 1955).

In certain circumstances, however, this disease may also cause relatively high losses (Blumer 1967), especially in the valuable new pea varieties in seed breeding plantations or in intensive pea production under glas, etc. In such cases application of a systemic fungicide may be very profitable.

In the present paper results of preliminary investigations are described, concerning the activity of PP 675 against powdery mildew of pea.

ACTIVITY OF PP 675 AGAINST PEA MILDEW

Influence of PP 675 on powdery mildew spores germination

The observations were made on 1—2 day-old spores of *Erysiphe pisi* DC. ex St-Am., *E. graminis* DC. and on spores of *E. betae* Weltzien, according to Zaracovitis method (1964) and the author's agar-disc technique (Gorska-Poczopko, 1971).

* now „Milcurb".

The attempts with *Sphaerotheca fuliginea* were unsuccessful, because of too low a per cent of spores germination "in vitro", specific to this fungus. The results of spore germination tests are included in Table 1.

Table 1

Influence of PP 675 on powdery mildew conidia germination

Method	Concentration of PP 675 in ppm of act. subst.	Condidia germination in per cent (average from 5 replications)		
		E. graminis	*E. pisi*	*E. betae*
Zaracovitis slide-germination test	0 (control)	57.7	65.9	—
	12.5	34.9	28.5	—
	25.0	33.0	12.0	—
	50.0	5.3	1.7	—
Agar-disc test	0 (control)	90.8	82.4	75.4
	12.5	62.8	17.0	—
	25	45.8	7.0	—
	50	22.2	2.2	3.7

PP 675 seems to possess a relatively high direct activity against conidia of *Erysiphaceae*, inhibiting their germination. This activity expressed itself in the following ways as regards various powdery mildew species:

1. The conidia of *Erysiphe pisi* germinate with one germ tube. Under the influence of PP 675 not only complete inhibition of germination was observed, but also shortening of the germ tubes (Table 2). Some conidia formed abnormally thick, deformed germ tubes.

Table 2

Shortening of germ tubes of conidia of *Erysiphe pisi* as effect of PP 675 (Observations on agar discs, after 26 hours)

№	Concentration of PP 675 in ppm of act. subst.	Per cent of germ tubes of length				
		0 μ (no germination)	Up to 35 μ	Up to 70 μ	Up to 100 μ	Longer than 100 μ
1	0 (control)	15.2	0.0	0.0	0.0	84.8
2	15	45.9	0.0	2.3	0.0	52.8
3	30	82.1	4.0	4.8	8.0	1.1
		(Germ tubes sometimes abnormally thick)				

2. The conidia of *Erysiphe graminis* germinate mostly with 4 germ tubes at once. Under the influence of PP 675 the proportion of conidia germinating with 3, 2 and 1 germ tubes increased (Table 3).

Table 3

Reduction of number of germ tubes in conidia of *Erysiphe graminis* due to PP 675 (Observations on agar discs after 24 hours)

№	Concentration of PP 675 in ppm of act. subst.	Number of germ tubes (in per cent)				
		0 (no germination)	one germ tube	two germ tubes	three germ tubes	four germ tubes
1	0 (control)	6.6	8.9	3.3	0.0	81.2
2	15	51.9	25.5	6.6	1.9	6.7
3	30	68.6	27.3	2.2	9.3	0.0

3. The conidia of *Erysiphe betae* formed appressoria mostly on elongated germ tubes. PP 675 provoked the formation of appressoria at the very beginning of germination, nearly without germ tube.

As on the "left-over" agar discs very abundant microflora of other fungi developed, even at concentrations inhibiting almost completely germination of powdery mildew conidia, the activity of the fungicide PP 675 seems to be restricted to the powdery mildews conidia.

Influence of PP 675 on pea powdery mildew growth on pea plants

Experiments were made on 10—16-day-old plants of pea var. Big Ben by way of root application (plants removed from soil, roots rinsed with water, immersed in water solutions of the fungicide) and on discs cut from pea-leaves in leaf-disc tests (Dekker, Oort 1964). Inoculation of both plants and leaf-discs was performed "by contact", i.e. by touching them with diseased pea leaves, covered with conidia of *Erysiphe pisi*. Inoculated plants or discs were incubated under normal greenhouse conditions (temp. ± 18—26°C, humidity 70—80%).

At first experiments were performed in order to establish the suitable concentrations and duration of treatment.

The most suitable concentration was about 15 ppm. Concentrations lower than 10 ppm were inactive, those higher than 20 ppm involved phytotoxicity symptoms: marginal brown spots on leaves of plants treated by root application, central spots on leaf discs. The most approporiate period of treatment was 4 days.

Miscroscopic observations on pea leaf-discs demonstrated that even at concentrations of fungicide as high as 80 ppm, single infections took place. However, the mycelium developed in such cases was mostly deformed, 5—7 times thicker than normal, usualy without fructification, string-like, in strands, running mostly in one direction across the plant--discs. This phenomenon seems to correspond with the germination abnormalities mentioned above.

When investigating the systemic activity of the fungicide it is of great interest to demontrate whether its action is only prophylactic or also curative. In other words, whether the fungicide is able only to protect the host against pathogen penetration, or wheather it can actively destroy the already established pathogen. This is closely connected with the application moment (pre- or post-infection). Experiments were performed to elucidate this problem.

In two coordinated experiments, one on leaf-discs, another by root application, the following constant parameters were applied: concentration of fungicide (15 ppm of act. subst.), duration of treatment (4 days) and infection moment. The only changing factor was the beginning of treatment: 6, 4 and 2 days before infection, on the infection day, 2 and 4 days thereafter.

Both experiments were started on the same day. On the first day of the experiment only the replications of the first combination were treated with fungicide, the others received water. Every two days the water in subsequent combination was replaced by the fungicide. Consequently after 4 days treatment the fungicide was again replaced by water. All combinations were infected on the same day. On the 10th day after infection all combinations were scored as follows:

Leaf-discs:

Infection index:

0 — no visible infection
1 — few small spots of mildew
2 — discs covered up to $^1/_4$ with mycelium
3 — discs covered up to $^1/_2$ with mycelium
4 — discs covered in more than $^1/_2$ with mycelium

Root application

Infection index:

0 — no visible infection
1 — small spots on 1—2 leaves
2 — spots noticeable on a few leaves
3 — up to 50 per cent of leaves spotted
4 — more than 50 per cent of leaves spotted

As a result of the above mentioned experiments a very regular sequence of infection grades was observed (see Table 4).

Table 4

Influence of the moment of treatment with PP 675 on *Erysiphe pisi* mycelium growth on pea plants

№	Beginning of treatment	Infection index	
		Leaf discs	Root application
1	6 days before infection	3.0	4.0
2	4 „ „ „	3.0	3.9
3	2 „ „ „	2.8	0.0
4	On infection day	1.0	0.0
5	2 days after infection	0.6	0.0
6	4 „ „ „	1.8	0.8
7	Control — without treatment	3.9	4.0

It was found, that on plants with root application some phytotoxicity symptoms appeared, in the from of small marginal brown spots, especially on the upper leaves. These appeared in all combinations, except 6 (treatment four days after inoculation) and of course not in the control. Microscopic inspection of leaf-discs showed an aboundant fungal fructification in all combinations with pre-infection treatments. In combinations 4 ("O days") and 6 (four days after inoculation) fructification of fungus was scarce. In combination 5 (two days after infection) fructification of the pathogen was almost completely arrested.

The results obtained seem to indicate two separate phenomena:

1. It may be presummed that the fungicide PP 675 has a curative influence at least as regards *Erysiphe pisi* on pea.

2. Its life in pea plants is very short; not more than two days in leaf-discs and a little longer in plants after root application.

The above mentioned results encouraged the author to start a new experiment with root application on pea plants, potted in sand.

Pea seeds var. Korosa were sown in sand in pots, 5 plants in each pot. At the age of 10 days the plants were inoculated as usual with conidia of *Erysiphe pisi* and than divided into 4 combinations, each consisting of 3 pots:

1. control (without treatment)
2. one treatment, on the day of inoculation
3. two treatments: first as above, second — 5 days, thereafter
4. three treatments: two as above, third — 7 days after second.

Each pot was watered with 100 ml of PP 675 solution, containing 15 ppm of active substance. Between the treatments the plants were normally watered with tap water.

On the fourth day after the last treatment the infection index of plants was estimated according to the scale used in root application experiments.

The results are presented in Table 5.

Table 5

Influence of repeated PP 675 treatments on *Erysiphe pisi* growth on potted pea plants

№	Treatment frequency	Infection index	Notes
1	Control (without treatment)	2.6	
2	1 treatment	0.6	
3	2 treatments	0.1	Phytotoxicity on single plants
4	3 treatments	0.0	Phytotoxicity on all plants

In the course of experiments the following observations were made:

At the time of the second treatment about 1/10 of leaves on the control plants were spotted with mildew. On some treated plants single spots were visible.

In microscopic observations again the same deformations in the form of mycelium "strings" were observed, running along the leaves.

UPTAKE AND DISTRIBUTION OF PP 675 IN PEA PLANTS

The systemic activity of PP 675 against pea mildew demonstrated on leaves of pea plants after root treatment, seems to be the best evidence of the uptake of fungicide.

Nevertheless some attempts were made to demonstrate this uptake by means of spectrophotometry, thin layer chromatography and also biologic indirect methods.

In Plant Protection Technical Data Sheets, concerning this fungicide (1968) a rather complicated way of fungicide extraction from plant sap was described.

A rather simple extraction method used in another work, proved to be of use in obtaining such an amount of fungicide from pea plants sap, which could be easily detected both by spectrophotometric and chromatographic methods.

The following extraction methods were used ways: 20-day-old pea plants were fed for 4 days by roots with aqueous fungicide solution containing 15 ppm of active substance of PP 675.

The roots and lower part of stems including cotyledons were removed, the plants were homogenised with 70 per cent ethanol and homogenate was filtered. Ethanol was then evaporated in vacuum and the remaining plant sap with fungicide was centrifuged. The supernatant was shaken with equal amount of light petroleum ether four times. Petroleum ether was evaporated in vacuum, and dry material was redissolved in ethanol. In spectrophotometer examination extracts from treated, both infected and noninfected plants showed maximum light absorption at about 303 mμ, characteristic for the fungicide.

In thin layer chromatographic examination extracts from treated plants gave spots visible in UV light of the same Rf value as spots of 100 ppm of PP 675 samples.

PP 675 uptake was checked also by indirect biological methods. Sap from treated with PP 675 (15 ppm a.s., 2 days) and untreated pea plants was mixed with equal parts of glucose agar medium and examined on agar discs, inoculated with conidia of *Erysiphe pisi*. The following results were obtained (Table 6):

Table 6

Influence of sap from pea plants, treated with PP 675 on germination of *Erysiphe pisi* conidia on agar discs

№	Kind of pea-sap added to glucose medium	Germination of conidia in per cent
1	Sap from untreated plants (control)	85.1
2	Sap from treated plants	18.4
3	Sap from untreated plants, 50 ppm of PP 675 added	3.8

Consequently another attempt was made to test the distribution of fungicide in pea plants.

Pea plants 10 days old were removed from soil, the roots were rinsed with water and immersed in 15 ppm concentration fungicide water solution. After 12 hours the plants were removed from the fungicide, the roots very well rinsed with water and homogenised. Separate ethanol extracts were made from roots, stems and leaves. In tests on agar discs, inoculated with *Erysiphe pisi* and *Erysiphe betae* the following results were abtained (Table 7):

Owing to the lack of quantitative determination of the of fungicide, the data presented in Table 7 can be considered only as comparative.

Table 7

Influence of extracts of parts of pea plants, treated with PP 675 on germination of mildew conidia on agar discs

№	Glucose agar with addition:	Germination of conidia in per cent	
		Erysiphe pisi	*Erysiphe betae*
1	None (control$_1$)	71.4	75.4
2	Ethanol (control$_2$)	65.9	—
3	Extract from roots of plants treated with PP 675	23.7	20.9
4	Extract from stems of plants treated with PP 675	20.1	18.0
5	Extract from stems of plants treated with PP 675	12.5	4.8
6	PP 675 up to 50 ppm of a.s.	—	3.7

It seems, however, that even after so short a period of exposure a considerable part of PP 675 was stored in leaves.

CONCLUSIONS

1. In *in vitro* investigations of PP 675 showed a marked activity inhibiting *Erysiphe pisi* conidia germination. Since other powdery mildews were similarly inhibited in contrast to other fungi, PP 675 seems to exert a very selective activity against powdery mildews.

2. PP 675 showed even higher anti-mildew, stystemic activity "in vivo" on pea leaf-discs and after root application to young pea plants.

3. The activity of PP 675 against mildew seems to be connected with the deformation of germ tubes and mycelia owing to treatment of pea plants with this fungicide.

4. The effect of PP 675 seems to be of curative value. The fungicide is capable of depress or even stop the infection process already in progress.

5. PP 675 is easily absorbed by pea plants, rapidly transported, probably stored in leaves and also quickly disintegrated. Its activity in pea plants is of rather short duration.

6. Some restrictions in the use of PP 675 in pea protection against powdery mildew may be necessary, as some phytotoxicity symptoms were observed due to this fungicide. In eventual further experiments this point must be taken into consideration.

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REFERENCES

Blumer S., 1967, Echte Mehltaupilze (*Erysiphaceae*), Jena.

Butler E., Jones S. G., 1955, Plant Pathology, London.

Dekker J., Oort A. J. P., 1964, Mode of action of 6-azauracil against powdery mildew. Phytopathology, 54, 7, 815—818.

Gorska-Poczopko J., 1971, An Agar-disc method for testing the germination of conidia of *Erysiphaceae*, Acta Mycol. 7: 157—158.

ICI Plant Protection Technical Data Sheet, PP 675 — a systemic fungicide. 1968. Fernhurst, Haslemmare, Surrey-England (March 1968).

Zaracovitis C., 1964, Factors in testing fungicides against powdery mildews I. A slide-germination method of evaluating protectant fungicides. Ann. apl. Biol. 54, 361—374.

Wstępne badania nad skutecznością PP 675 w zwalczaniu mączniaka właściwego grochu

Streszczenie

W badaniach laboratoryjnych fungicyd PP 675* skutecznie hamował kiełkowanie zarodników *Erysiphe pisi* DC. ex St-Am. W badaniach szklarniowych fungicyd ten szybko przenikał do siewek grochu zarówno przy podawaniu dokorzeniowym, jak w teście krążków liściowych i hamował rozwój *E. pisi*, wywołując charakterystyczną deformację strzępek.

Działanie PP 675 miało charakter zarówno profilaktyczny jak i kuratywny.

Metodą spektrofotometrii i chromatografii cienkowarstwowej wykazano obecność PP 675 w ekstraktach z liści i łodyg grochu, którego korzenie traktowane były wodnym roztworem związku o stężeniu 15 ppm. Ekstrakty te dodawane do agaru obniżały procent kiełkujących zarodników *E. pisi* w testach krążków agarowych.

W doświadczeniu wazonowym podlewanie siewek grochu 100 ml PP 675 o stężeniu 15 ppm na wazon chroniło rośliny przed porażeniem przez *E. pisi*, ale przy dwu- i trzykrotnym powtórzeniu zabiegu powodowało pojawienie się objawów fitotoksyczności.

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