Neth. J. Pl. Path. 72 (1966): 274–278

A MICROSCOPIC STUDY OF POWDERY MILDEW ON BARLEY AFTER APPLICATION OF THE SYSTEMIC COMPOUND WEPSYN[1]

Microscopisch onderzoek van meeldauw op gerst na toediening van de systemische verbinding wepsyn

J. F. C. MAGENDANS and J. DEKKER
Laboratory of Phytopathology, Agricultural University, Wageningen

5-Amino, 3-phenyl, 1-bis (dimethylamido) phosphoryltriazole 1, 2, 4 (wepsyn) was applied to the roots of barley seedlings, var. 'Balder' in concentrations of 10, 25, 62 and 156 ppm, and the infection process of *Erysiphe graminis* f.sp. *hordei* on these plants studied microscopically. During the first few hours after inoculation a retardation of the germination occurred at the two highest concentrations, thus before the fungus had established contact with the host protoplasm. Penetration and formation of a lignituber was retarded at all wepsyn concentrations. At 10 and 25 ppm the formation of primary haustoria was reduced about thirty fold in relation to that on control plants. Mycelium that developed was often stunted. At higher wepsyn concentrations almost no haustoria were formed. Some leaf tip necrosis occurred, especially at the higher concentrations of wepsyn.

INTRODUCTION

Wepsyn is the brand name for 5-amino, 3-phenyl, 1 bis (dimethylamido) phosphoryltriazole 1, 2, 4. Systemic action against powdery mildew on barley, *Erysiphe graminis* f.sp. *hordei*, was first reported by KOOPMANS (1960). By administration of a 31.5 ppm solution of this compound to the roots of barley seedlings, he obtained 95% protection of the leaves against the disease.

This paper reports on microscopical observations of powdery mildew development on wepsyn-treated barley plants. The results are compared with those obtained with a few other compounds systemically active against powdery mildew.

MATERIALS AND METHODS

The chemical wepsyn (WP 155, 100% technical substance) was a gift from Dr. A. TEMPEL from Philips Duphar at 's-Graveland.

The barley variety used in the experiments was 'Balder'. Fifteen days old seedlings were placed with their roots in aqueous suspensions containing 10, 25, 62 and 156 ppm of wepsyn and in water. Sixteen seedlings per treatment were inoculated with conidia of *E. graminis* f.sp. *hordei*. This was carried out by dusting the conidia over the underside of the second leaf, which had been stretched out on hardboard to ensure a more or less even distribution. At certain times after inoculation leaves from treated and control plants were fixed with F.P.A. (formalin-propionic acid-alcohol 70%, 5:5:90 v/v). Tangential sections of the leaf surface, made with a razor, were stained with cotton blue in lactophenol and microscopically examined. Usually 300 germinated conidia per treatment were counted, 150 on the lower half and 150 on the upper half of the leaf.

[1] Accepted for publication 12 April, 1966.

Powdery mildew symtoms appeared on the control plants after four days, and soon thereafter also on plants treated with 10 and 25 ppm wepsyn. No symptoms developed on plants treated with 62 and 156 ppm of wepsyn.

At wepsyn concentrations of 10 and 25 ppm no phytotoxicity was observed during the first six days of the experiment, but thereafter slight leaf tip necrosis occurred. At 62 and 125 ppm phytotoxicity symptoms developed earlier, and at the latter concentration leaf necrosis even started after two days and wilting after four days.

At various periods after inoculation, namely $3\frac{1}{2}$, 8, 12, 24 56 hours and 4, 7 and 10 days, a few leaves of each treatment were fixed in FPA, and later examined microscopically. Most attention was paid to the leaves taken at $3\frac{1}{2}$, 24, 56 and 96 hours after inoculation.

Germination

Three and a half hours after inoculation 78% of the conidia on untreated control plants had already germinated with a small, thin-walled hypha. Some concentration of host plasma was often observed at the spot where the tip of this hypha touched the epidermal cell wall (Fig. 1). During the first day after inoculation usually more than one germ tube appeared; 90% of the germinating conidia formed two germ tubes, which always differed in size. Sometimes up to four or even more germ tubes were formed. By the day following inoculation 98% of the conidia had germinated.

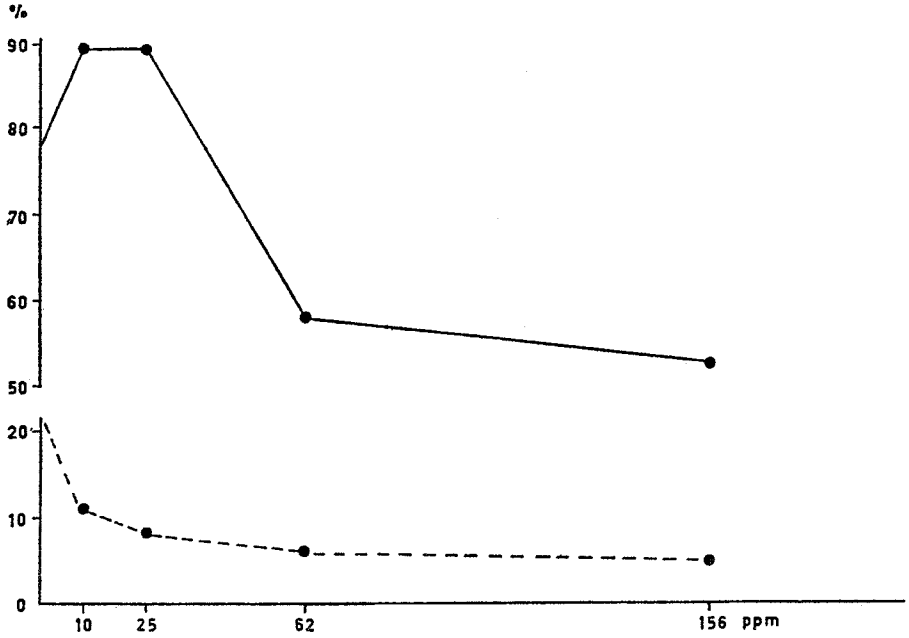

FIG. 1. Concentration of host cytoplasm below the tip of the germination hypha, before penetration of the epidermis has started, $3\frac{1}{2}$ hours after inoculation.

Concentratie van cytoplasma in de waardplant onder het uiteinde van een kiemhyfe, vóór penetratie in de epidermis begonnen is, $3\frac{1}{2}$ uur na inoculatie.

On wepsyn-treated plants conidia germinated in the same way as just described. A marked difference, however, could be observed in the speed with which germination took place. On plants treated with 62 and 156 ppm wepsyn, respectively 58 and 53% of the conidia had germinated after three and a half hours, against 78% on the control plants (Fig. 2). After 24 hours these percentages had risen to 92 and 78 on the wepsyn-treated plants, against 98% on the control plants. These last percentages did not alter significantly in the next few days. It appears therefore that at 62 ppm wepsyn the germination of conidia was significantly retarded, but that the number of germinating conidia was scarcely reduced; at 156 ppm, however, there was a small permanent reduction of the number of germinated conidia. At the lower concentrations of 10 and 25 ppm wepsyn there appeared to be a slight stimulation of germination.

The retardation of germination during the first hours after inoculation is shown not only by the number of germinated conidia, but also by the percentage showing more than one (2–4) germ tube. Three and a half hours after inoculation these percentages were 22, 11, 8, 6 and 5 at the concentrations 0, 10, 25, 62 and 156 ppm wepsyn respectively (Fig. 2). After 24 hours, however, these differences had disappeared; in all treatments more than 97% of the germinating conidia showed more than one germ tube. From these data it may be concluded that the germination of conidia on barley leaves is influenced by wepsyn treatment of the plants. In other words the fungus is influenced, during germination, by the treated plant and before contact with the host cytoplasm has been established.

Penetration and formation of haustoria

Infection of control plants occurs as follows. The smaller of the usually two germ tubes soon stops growth; occasionally a halo or even a lignituber is observed, but a haustorium is never formed. The larger germ tube grows along the border between two epidermal cells, and soon forms a septum. The end cell contains a nucleus and is rich in plasma. It forms, usually at its end, a small apressorium, beneath wich a halo and a lignituber appear in the same way as described for wheat powdery mildew (DEKKER & VAN DER HOEK-SCHEUER, 1964). The lignituber is not lignified, since no red colour appears after treatment with phloroglucinol and hydrogen chloride. In contrast to the mycelium, it is clearly stained with reso blue, which indicates the presence of callose. When the lignituber is pierced by the penetration hypha, formation of a haustorium starts. If the fungus fails to penetrate the lignituber, which may happen quite often, a new outgrowth from the end cell of the germ tube appears, with formation of a second halo and lignituber. In this way several lignitubers may be formed by one fungal end cell before penetration succeeds (Fig. 3). After formation of a haustorium, a rapid development of the fungus takes place with abundant growth of mycelium, and secondary lignitubers and haustoria.

In order to follow the infection process on wepsyn-treated plants in comparison with that on untreated plants, counts were made of the number of lignitubers and haustoria formed by the germination hyphae, i.e. the so-called primary lignitubers and haustoria. In Table 1 these numbers are expressed as percentages of the number of germinated conidia.

From this table it may be concluded that at the lower concentrations of wepsyn the formation of lignitubers is merely retarded, whereas at the higher con-

TABLE 1. Formation of primary lignitubers and haustoria on wepsyn-treated barley seedlings, expressed as a percentage of the number of germinating conidia; 250 germinating conidia were counted per treatment.

Vorming van primaire lignitubers en haustoriën op met wepsyn behandelde gerstezaailingen, uitgedrukt in percentage van het aantal kiemende conidiën; per object werden 250 kiemende conidiën geteld.

ppm wepsyn	Primary lignitubers after		Primary haustoria after	
	24 hrs	56 hrs	24 hrs	56 hrs
0	177.3	184.3	31.0	38.8
10	141.0	216.6	1.3	1.3
25	89.6	171.0	0.0	1.3
63	59.6	118.6	0.0	0.3
156	59.3	80.0	0.0	0.3
ppm wepsyn	*24 uur*	*56 uur*	*24uur*	*56 uur*
	Primaire lignitubers na		*Primaire haustoriën na*	

centrations it is definitely reduced. The formation of haustoria is much more reduced, namely from 38.8 on control plants to 0.3–1.3 on wepsyn-treated plants. It would appear that the principal barrier against development of the fungus on wepsyn-treated plants lies at this stage (Table 1). The few primary haustoria that do form on wepsyn-treated plants are for the most part normal in shape. On control plants as well as on wepsyn-treated plants about one third of the number of haustoria show abnormalities: a wrinkled surface, clotted protoplasm or irregular staining with cotton blue. At 10 and 25 ppm wepsyn, there is a retardation in the rate of development of mycelium and secondary haustoria, but on plants treated with 62 ppm of wepsyn no secondary haustoria are formed at all. When mycelium of powdery mildew develops on wepsyn-treated plants, a stunted growth may often be observed, showing short, thick cells with irregular bumps. In the curves along the cell wall and along the septa these bumps contain a refractive deposit. Many hyphae cease growth after formation of these bumps. Stunted growth of the mycelium occurs increasingly at higher concentrations of wepsyn.

DISCUSSION

From the microscopical observations it appears that on wepsyn-treated barley plants the main barrier to the development of the powdery mildew fungus lies at the stage of haustorial formation. In this respect the systemic action of wepsyn resembles that of procaine hydrochloride, griseofulvin and 6-azauracil against wheat powdery mildew. The few haustoria that did form, however, were usually of normal shape, this being in contrast to the haustoria developing on wheat plants treated with the antibiotic griseofulvin (DEKKER & VAN DER HOEK-SCHEUER, 1964). At the lower concentrations of wepsyn, development of mycelium took place, but it was often stunted. It resembled to some extent the bulbous swelling of germination hyphae of cucumber powdery mildew on 6-azauracil treated plants (DEKKER & OORT, 1964).

The above mentioned deviations from normal fungal development occurred after the fungus had established intimate contact with the treated-host plant by

its germination hyphae. It was, furthermore, observed that the process of germination itself was affected even before penetration had started. The germination of the conidia was retarded on wepsyn-treated plants, as appeared from counts of the number of germinated conidia and of the number of conidia developing more than one germ tube. The observation of KOOPMANS (1960) that wepsyn only slightly reduces germination of powdery mildew conidia on glass slides, was confirmed by us. It seems therefore highly improbable that excretion of wepsyn on the surface of the leaf could account for the reduction in germination. If there is no direct effect of wepsyn on the germination of conidia on the leaf surface, there must be an indirect effect via the host plant. However, we have no experimental evidence in explanation of this phenomenon, nor of the slight stimulation of germination observed on plants treated with 10 and 25 ppm of wepsyn.

It should be noted that haustorial deviations occurred to the same extent on control plants as on wepsyn-treated plants. The appearance of abnormal haustoria can therefore not be attributed to the action of wepsyn. In this connection it should also be mentioned that on control plants only 38.8% of the germinating conidia succeeded in forming a haustorium. The barley variety 'Balder' apparently is not an ideal host for powdery mildew under the conditions of the experiment.

SAMENVATTING

Gersteplanten, ras 'Balder', werden geïnoculeerd met *Erysiphe graminis* f.sp. *hordei* en met de wortels in wepsyn-oplossingen van 10, 25, 62 en 156 ppm geplaatst. Het infectieproces werd gevolgd door op verschillende tijdstippen na inoculatie coupes te maken van gefixeerde bladeren, evenwijdig aan het bladoppervlak en deze microscopisch te bestuderen. Gedurende de eerste uren na inoculatie, dus voordat de schimmel contact gemaakt had met het cytoplasma van de waardplant, trad een vertraging van het kiemingsproces der conidiën op bij de twee hoogste wepsyn-concentraties. De penetratie en vorming van lignitubers was vertraagd bij alle wepsyn-concentraties. Vorming van haustoriën had nagenoeg niet plaats op planten behandeld met 62 en 156 ppm wepsyn. Het aantal haustoriën was sterk gereduceerd op planten behandeld met 10 en 25 ppm. Mycelium dat op deze planten gevormd werd, vertoonde vaak een gedrongen uiterlijk.

Necrose van de bladtoppen trad vooral bij de hoogste wepsyn-concentraties op.

REFERENCES

DEKKER, J. & R. G. VAN DER HOEK-SCHEUER, – 1964. A microscopic study of the wheat powdery mildew relationship after application of the systemic compounds procaine, griseofulvin and 6-azauracil. Neth. J. Pl. Path. 70: 142–148.

DEKKER, J. & A. J. P. OORT, – 1964. Mode of action of 6-azauracil against powdery mildew. Phytopathology 54: 815–818.

KOOPMANS, M. J., – 1960. Systemic fungicidal action of some 5-amino, 1 bis (dimethylamido) phosphoryl triazoles 1, 2, 4. Meded. LandbHogesch. OpzoekStns Gent 25: 1221–1226.