Neth. J. Pl. Path. 78 (1972) 258–264

Uredospore germination and germ tube penetration of Puccinia striiformis in seedling leaves of resistant and susceptible wheat varieties

R. W. STUBBS and J. M. PLOTNIKOVA[1]

Institute of Phytopathological Research (IPO), Wageningen

Accepted 25 May 1972

Abstract

Differences were observed in spore germination and germ tube penetration of race 60 of *Puccinia striiformis* on some wheat varieties. Of the five wheat varieties investigated *Triticum spelta* var. *album* was the most resistant, strongly inhibiting spore germination and retarding germ tube penetration. On the hexaploid wheat varieties the germ tube penetrated through the stomata, whereas on the tetraploid varieties it did so at the junction of two epidermal cells. There was no correlation between density or length of hairs on the leaves and the rate of spore germination and germ tube penetration.

Introduction

Resistance of a host to infection by a pathogen can be effective in the pre-infection stage as well as in the post-infection stage. The modes of resistance in the former stage, viz. the inhibition of spore germination and the resistance to penetration, have been studied for several diseases such as *Erysiphe graminis* on barley (Schulze and Fischbeck, 1969), *Phytophthora infestans* on potato (Umaerus, 1969; Umaerus and Stålhammer, 1969), *Puccinia helianthi* on sunflower (Sood and Sackston, 1970) and cereal rusts, as reviewed and evaluated by Hooker (1967).

Our numerous observations on yellow rust infection on many wheat varieties showed that wheat varieties may differ in the amount of infection foci on the leaves when exposed to high inoculum densities. The presumption that this difference might be related to differences in host resistance in the pre-infection stage, stimulated a study on the germination and penetration of yellow rust on wheats of different resistance to this pathogen.

Materials and methods

Race 60 (IPO collection culture no. 6049) was used to inoculate the following wheat varieties: *Triticum dicoccum* var. *tricoccum* (2n = 28) and *Triticum aestivum* 'Harvest Queen' (2n = 42), which are both highly susceptible to almost all yellow rust races and commonly used for the multiplication of rust cultures, *Triticum aestivum* 'Vilmorin 23' (2n = 42), a differential variety which reacts to race 60 of the fungus with a resis-

[1] Visiting scientist of the Laboratory of Plant Pathology of the Main Botanical Gardens, USSR Academy of Sciences, Moscow, USSR.

tant infection type 0–0$^+$ (chlorotic or necrotic blotches with or without sporulation), *Triticum spelta* var. *album* (2n = 42) and *Triticum dicoccoides* var. *aaronshoni* 'G.25' (2n = 28) which are both universally resistant to yellow rust as far as they have been tested (Zeven et al., 1968; Gerechter and Stubbs, 1970). The two latter varieties respond to race 60 with an almost immune infection type.

The seedlings, grown in a greenhouse at about 15 °C, were inoculated 11 days after sowing. Uredospores of race 60 were evenly dusted on the fully developed first leaves. The inoculated seedlings were placed in a dew chamber at 10 °C for 24 hours and then transferred to the original conditions. Five days after inoculation the upper parts of the leaves were cut into pieces of 1 cm length and treated according to the method described by Fokkema (1968). Germination of the spores and penetration of the germ tubes into the leaves were examined microscopically.

Results and discussion

When the inoculated seedlings were examined, infection foci in the form of minute chlorotic spots appeared on the leaves of the susceptible varieties, while the three resistant ones did not show any visible signs of infection. Microscopic observation of the upper epidermis revealed marked varietal differences with regard to spore germination and penetration (Table 1).

Table 1. Spore germination and germ tube penetration of *Puccinia striiformis* race 60 on first leaves of two susceptible and three resistant wheat varieties, 5 days after inoculation.

Variety	Response to race 60	Number of spores observed	Germination (% of total number of spores)	Penetration (% of the number of germinated spores)
Triticum dicoccum var. *tricoccum*	susceptible	227	98.0	46.7
'Harvest Queen'	susceptible	184	73.9	27.2
'Vilmorin 23'	resistant	82	62.8	17.6
Triticum dicoccoides 'G. 25'	resistant	223	65.9	10.2
Triticum spelta var. *album*	resistant	51	33.4	5.1

Tabel 1. Sporekieming en kiembuis-penetratie van Puccinia striiformis fysio 60 op kiembladeren van twee vatbare en drie resistente variëteiten, 5 dagen na inoculatie.

According to the data in Table 1 *T. dicoccum* var. *tricoccum* was the most susceptible variety and its exceptionally high spore germination and germ tube penetration agrees with our experience that this variety is more receptive for infection than the susceptible cv. Harvest Queen. *T. spelta* var. *album*, inhibiting spore germination as well as germ tube penetration, was the most resistant one and its immune reaction is expressed by a sparse distribution of the infection foci which just appear as vague minute chlorotic spots.

Varietal differences were also observed with regard to the manner of leaf penetration by the germtubes. Both on *T. dicoccum* var. *tricoccum* and on *T. dicoccoides* 'G.25' the germ tubes did not penetrate through the stomata but via the cell wall at the junction of two epidermal cells (Fig. 1). Growing over the leaf surface of these varieties

Fig. 1. Germ tube penetration of *Puccinia striiformis* race 60 at the junction of two epidermal cells of the upper leaf surface of a seedling of *Triticum dicoccum* var. *tricoccum*.

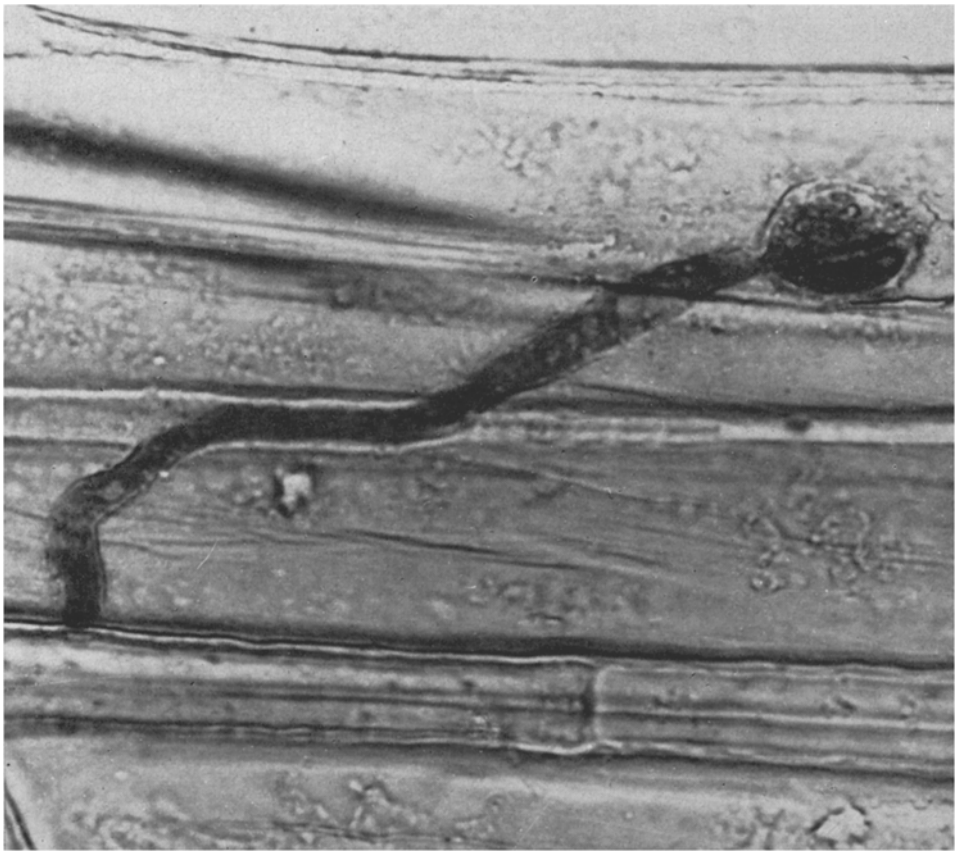

Fig. 1. Penetratie van de kiembuis van Puccinia striiformis fysio 60 op de grens van twee epidermiscellen aan de bovenzijde van een kiemblad van Triticum dicoccum var. tricoccum.

the germ tubes even seemed to avoid the stomata as if they were repelled chemically. On *T. dicoccoides* 'G.25' the germ tubes grew to a rather great length before they succeeded to penetrate. They formed several curvatures and appressorialike thickenings on the anticlinal cells walls.

On 'Harvest Queen', 'Vilmorin 23' and *T. spelta* var. *album* penetration took place through the stomata (Fig. 2). The growth of the germ tubes was generally directed towards the stomata, possibly by a chemotropic stimulus, presumed by Burrage (1969) for uredospores of *Puccinia graminis*. On 'Harvest Queen' the germ tubes grew in straight lines across the leaf surface and each tube entered a stoma of the nearest or the next row. On 'Vilmorin 23' the germ tubes grew in curves and often failed to penetrate (Fig. 3), apparentty due to stomatal exclusion as described by Caldwell and Stone (1936) and Romig and Caldwell (1964) for *Puccinia recondita* on wheat. On *T. spelta* var. *album*, also showing stomatal exclusion, some germ tubes were very

Fig. 2. Germ tube penetration of *Puccinia striiformis* race 60 through a stoma of the wheat variety 'Harvest Queen'.

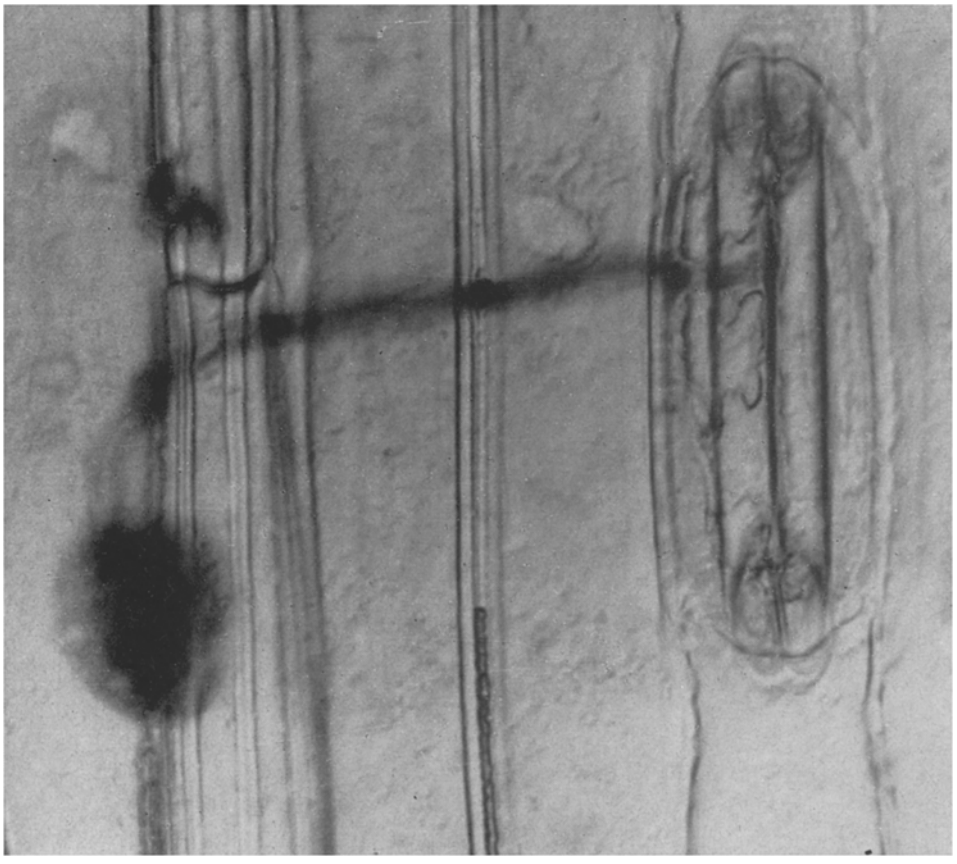

Fig. 2. Penetratie van een kiembuis van Puccinia striiformis fysio 60 door een huidmondje van de tarwevariëteit 'Harvest Queen'.

short, as if their growth were suppressed by some physical or chemical factor. This would indicate an additional factor of resistance.

The marked varietal difference in the manner of penetration of the germ tubes, viz. through the stoma or at the junction of two cells, might be an inheritable characteristic. Both *T. dicoccoides* 'G.25' and *T. dicoccum* var. *tricoccum* are tetraploid varieties, while the other three varieties on which the germ tubes penetrated through the stomata, are hexaploid. In view of this fact it would be worthwhile to investigate the manner of penetration on the diploid forms of wheat and on other tetraploid and hexaploid varieties.

Free water on the leaf surface in the form of dew droplets is essential for the germination of the uredospores of *Puccinia graminis* (Burrage, 1969), as it is with the hydrophobic uredospores of *Puccinia striiformis*. Germination of the spores and growth and penetration of the germ tubes may also be dependent on the capacity of the leaf to

Fig. 3. Germ tube growth of *Puccinia striiformis* race 60 in a closed stoma of the wheat variety 'Vilmorin 23'.

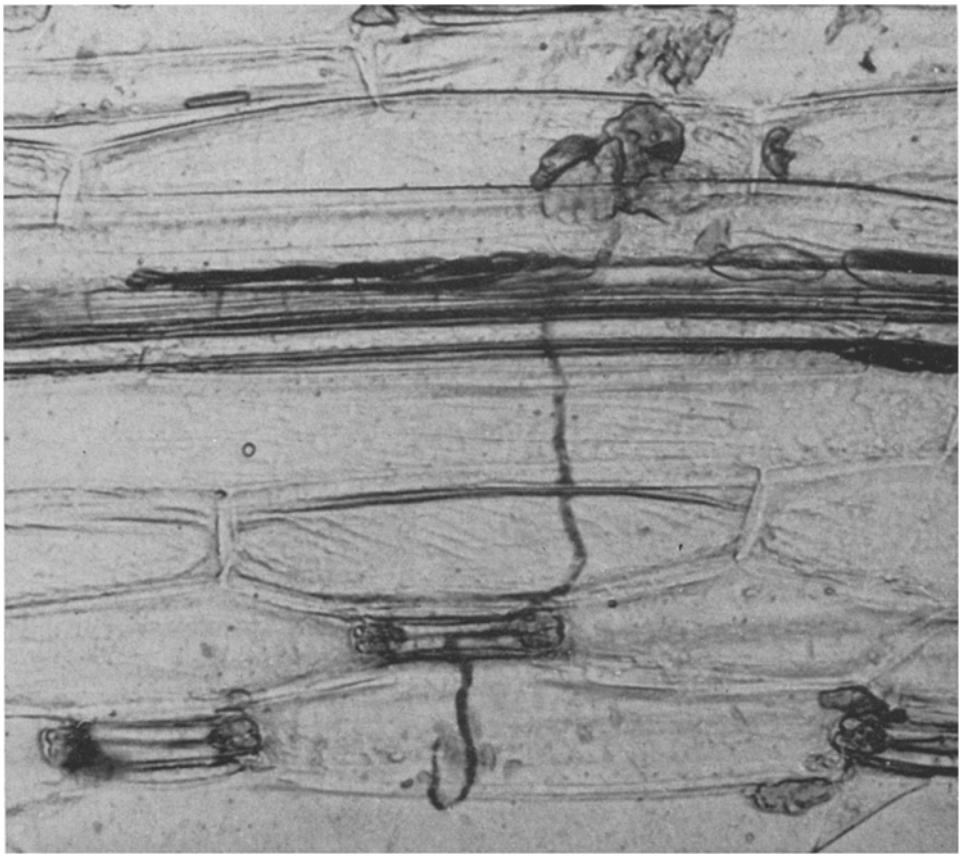

Fig. 3. Groei van een kiembuis van Puccinia striiformis fysio 60 in een gesloten huidmondje van de tarwevariëteit 'Vilmorin 23'.

Table 2. Density and length of the hairs on the leaves of the five wheat varieties used in the investigation.

Variety	Number of hairs per mm^2	Relative length
Triticum dicoccum var. *tricoccum*	26.0	very long
'Harvest Queen'	4.2	medium
'Vilmorin 23'	6.5	very long
Triticum dicoccoides 'G. 25'	31.4	long
Triticum spelta var. *album*	17.4	very short

Tabel 2. Dichtheid en lengte van de haren op het blad van de vijf in het onderzoek betrokken tarwerassen.

retain water (Burrage, 1969, 1970). As hair density and shape may be of importance in this respect, observations were made on these leaf surface characteristics (Table 2).

No correlation was found between hair density and the germination and penetration percentages. The low germination percentage on *T. spelta* var. *album* may be indirectly related to the very short hairs, which give the smooth surface of this wheat a relatively low capacity for retaining water. Both *T. dicoccoides* 'G.25' and *T. dicoccum* var. *tricoccum*, the tetraploid wheats, have relatively long hairs and a high hair density, giving them a high capacity for retaining water. Considering the great difference in resistance to penetration, the former wheat must possess some other physical or non-physical barrier.

As in our study only one rust isolate was used, no definite conclusion can be drawn on the race specifity of the obtained varietal differences with regard to the inhibition of germination and to the resistance to penetration. However, the extensive tests performed with *T. spelta* var. *album* (Zeven et al., 1968) and *T. dicoccoides* 'G.25' (Gerechter-Amitai and Stubbs, 1970) would favour the conclusion that the resistance of both wheats to penetration is independent of the race. Both cases of specific and non-specific behaviour of mildew races on barley with regard to host resistance in the pre-infection stage have been demonstrated by Schulze and Fischbeck (1969). Brown and Shipton (1964) also found that races of *Puccinia graminis* can differ in their ability to penetrate. Varietal differences in infection efficiency of *Phytophthora infestans* were considered as race non-specific by Umaerus and Stålhammer (1969) based on experiments with a single virulent race. Like Hooker (1967) we think that it is difficult to prove conclusively that a form of resistance is of the race non-specific type and that forms of resistance may be classified as this type, as long as the contrary has not yet been proved.

Samenvatting

Uredosporekieming en kiembuispenetratie van Puccinia striiformis op kiembladen van resistente en vatbare tarwevariëteiten

Waarnemingen zijn verricht over sporekieming en kiembuispenetratie van fysio 60 van *Puccinia striiformis* op vijf tarwevariëteiten. Ten opzichte van de meest vatbare variëteit *Triticum dicoccum* var. *tricoccum* gaven de overige variëteiten een duidelijke remming van sporekieming en resistentie tegen kiembuispenetratie te zien (Tabel 1), *Triticum spelta* var. *album* toonde zich in beide opzichten het meest resistent. De wijze van penetratie van de kiembuizen verschilde al naar de genetische samenstelling van de onderzochte tarwevariëteiten. Op de drie hexaploïde variëteiten bleek de kiembuis via het huidmondje binnen te dringen (Fig. 2), maar op de twee tetraploïde variëteiten via de wand tussen twee epidermiscellen (Fig. 1).

De kiem- en penetratiepercentages bleken niet gecorreleerd te zijn aan dichtheid en lengte van de haren op het blad (Tabel 2).

Acknowledgments

The hospitality extended to the second author by Prof. Dr J. G. ten Houten and all other members of the Institute of Phytopathological Research is gratefully appreciated. The research was made while the second author was in receipt of a fellowship of International Agricultural Centre.

References

Brown, J. F. & Shipton, W. A., 1964. Relationship of penetration to infection type when seedling wheat leaves are inoculated with *Puccinia graminis tritici*. Phytopathology 54: 89–91.

Burrage, S. W., 1969. Dew and the growth of the uredospore germ tube of *Puccinia graminis* on the wheat leaf. Ann. appl. Biol. 64: 495–501.

Burrage, S. W., 1970. Environmental factors influencing the infection of wheat by *Puccinia graminis*. Ann. appl. Biol. 66: 429–440.

Caldwell, R. W. & Stone, G. M., 1936. Relation of stomatal function of wheat to invasion and infection by leaf rust (*Puccinia triticina*). J. agric. Res. 52: 917–932.

Fokkema, N. J., 1968. The influence of pollen on the development of *Cladosporium herbarum* in the phyllosphere of rye. Neth. J. Pl. Path. 74: 159–165.

Gerechter-Amitai, Z. K. & Stubbs, R. W., 1970. A valuable source of yellow rust resistance in Isaeli populations of wild emmer, *Triticum dicoccoides* Koern. Euphytica 19: 12–21.

Hooker, A. L., 1967. The genetics and expression of resistance in plants to rusts of the genus *Puccinia*. A. Rev. Phytopath. 5: 163–182.

Romig, R. W. & Caldwell, R. M., 1964. Stomatal exclusion of *Puccinia recondita* by wheat peduncles and sheaths. Phytopathology 54: 214–218.

Schulze, F. W. & Fischbeck, G., 1969. Die Keimung von Konidien des Mehltaues (*Erysiphe graminis* D.C. f. sp. *hordei* Marchal) auf den Blättern anfälliger und resistenter Gersten. Z. PflZüchtung 62: 343–356.

Sood, P. N. & Sackston, W. E., 1970. Studies on sunflower rust. VI. Penetration and infection of sunflower susceptible and resistant to *Puccinia helianthi* race I. Can. J. Bot. 48: 2179–2181.

Umaerus, V., 1969. Studies on field resistance to *Phytophthora infestans*. 1. The infection efficiency of zoospores of *P. infestans* as influenced by the host genotype. Z.PlfZücht. 61: 29–45.

Umaerus, V. & Stålhammer, M., 1969. Studies on field resistance to *Phytophthora infestans*. 3. Screening of *Solanum* species for field resistance to *P. infestans*. Z. PlfZücht. 62: 6–15.

Zeven, A. C., Turkensteen, L. J. & Stubbs, R. W., 1968. Spelt (*Triticum spelta* L.) as a possible source of race-non-specific resistance to yellow rust (*Puccinia striiformis* Westend.). Euphytica 17: 381–384.

Addresses

R. W. Stubbs: Instituut voor Plantenziektenkundig Onderzoek (IPO), Binnenhaven 12, Wageningen, the Netherlands.

Julia M. Plotnikova: Laboratory of Plant Pathology of the Main Botanical Gardens, USSR Academy of Sciences, Moscow, USSR.