The Abnormal Morphology of a Very Virulent Moroccan Isolate Belonging or Related to *Puccinia hordei*

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ABSTRACT

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An isolate of a "brown rust" fungus from barley collected in Morocco combined a rare virulence spectrum, viz., virulence to *Pa3* and *Pa7*, and a relatively high aggressiveness on cv. Vada, known for its high level of partial resistance to *Puccinia hordei*. The morphology of the substomatal vesicle of this isolate differed remarkably from that of 17 isolates of *P. hordei*, including four Moroccan isolates with a more normal virulence spectrum and one Israelian isolate possessing virulence to *Pa3* and *Pa7*. The telia contained one- and two-celled teleutospores and brown paraphyses, indicating that the isolate is at least closely related to *P. hordei*.

In 1981 Parlevliet et al (13) reported the collection of two cultures of a brown rust fungus from barley in a nursery at Rabat, Morocco, with two interesting features, viz., an unusually wide virulence spectrum and a relatively short latent period on a cultivar like Vada. This cultivar possesses a high level of partial resistance to *Puccinia hordei* Otth, the causal agent of barley leaf rust.

Further study on one of these cultures (isolate 28) revealed a third interesting feature. The morphology of the substomatal vesicle (SSV) differed markedly from that of *P. hordei* isolates studied so far. Morphology of the infection structures of rust fungi of grasses and cereals shows a considerable intertaxon variation (11). The abnormal morphology of isolate 28 may indicate that the isolate represents: 1) a species not closely related to *P. hordei* but belonging to, for instance, *P. recondita* or to a species in the genus *Uromyces*; 2) an infraspecific taxon of *P. hordei*, sympatric with the typical form; 3) a morphologically abnormal form of *P. hordei* not deserving a taxonomic status; or 4) a geographic morphological variant of *P. hordei* replacing the typical form in (a part of) Morocco. This paper provides evidence for the second or third of these four alternatives.

MATERIALS AND METHODS

Rust isolates. Isolate 28 was established as monospore culture from Mor-1(13) in 1981 and was vacuum-stored in a glass vial at 4 C. Parlevliet (12) characterized the isolate for virulence spectrum and latent period on barley seedlings. The isolate was renewed annually by passage on barley cultivar Cebada Capa. A glass vial containing such 1-yr-old inoculum was opened in March 1986 to start the present study. First multiplication was on Ribari (*Pa3*) to prevent contamination with our standard isolate 121, which is avirulent for Ribari. Four isolates collected as *P. hordei* in Morocco were kindly provided by M. Reinhold Johnston (Department of Plant Pathology, Montana State University, Bozeman). These isolates were designated M1 (Marrakech), M2 (Merchouch), M3 (Rabat), and M4 (Khemi Zemara); they are also called M-isolates. Isolate 28 was compared with these four M-isolates and with isolate 121 for SSV morphology, percentage of one-celled teleutospores, and virulence spectrum.

In addition to these six isolates, 12 were checked for their SSV morphology. Code numbers and countries of origin were: 9, Kenya; 5 and 202, Israel; 13, Crete; 29, Greece; 25, Italy; 22, France; 17, 18, and 24, the Netherlands; 3, United Kingdom; and 26, Finland. Isolate 5 was derived from isolate T-46 (5), which was a gift from I. Wahl (Tel Aviv, Israel); it combines many alleles for virulence, i.a., for *Pa3* and *Pa7*.

Morphology of infection structures. Spores of isolates 28, M1 through M4, and 121 were applied to primary leaves of *Triticum dicoccum* Schubl. ("tricoccum"), incubated, sampled 40 hr after inoculation, stained, and observed as described

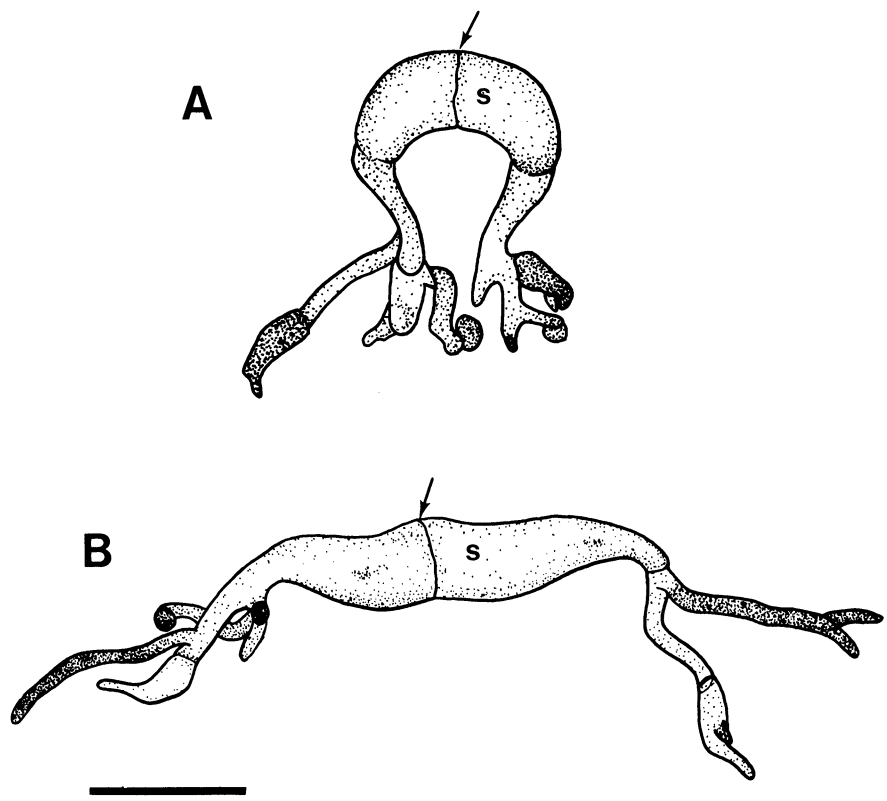

Fig. 1. Typical sporelings of two Moroccan isolates of *Puccinia hordei*, **(A)** isolate 28 and **(B)** isolate M3, depicted with the long axis of the leaf running parallel to the scale bar (= 25 μm). Arrow indicates central septum; S = substomatal vesicle.

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before (11). Thirty sporelings were observed per isolate. Representative sporelings were drawn and photographed as far as the isolates differed. The morphology of the remaining 12 non-Moroccan isolates was checked from preparations of barley seedling leaves that were used in a routine experiment.

Percentage of one-celled teleutospores. The increase of the isolates occurred in isolated greenhouse compartments on adult plants of L98 (isolates M1 through M4, 121, and 28) and of Ribari (isolate 28). The plants were inspected for the occurrence of telia. If telia were found, the contents of a few were spread on a microscope slide. Samples of at least 230 spores were screened for the percentage of one-celled teleutospores (mesospores). The percentage of mesospores was determined on samples of telia collected at different times of the year.

Virulence spectrum. The virulence spectrum of isolate 28, the four M-isolates, and isolate 121 was determined on seedlings of the differential set of cultivars (3), to which CI 1243 carrying *Pa9* was added. Isolates M1 through M4 were tested in duplicate, as two monospore cultures were derived from each of the M-isolates. The infection types were scored using the 0–9 scale of McNeal et al (10). Infection types were read 12 days after inoculation.

RESULTS AND DISCUSSION

Morphology of infection structures. The SSV morphology of isolate 28 deviates strongly from that of the standard isolate 121 as well as from that of all other isolates checked (Figs. 1 and 2). No differences in morphology of the infection structures were noticed among the other 17 isolates.

The SSV always contained a central septum, which is also a striking feature of the SSVs of regular *P. hordei* isolates (11), including M1 through M4. The septum was folded, however, so that the infection hyphae grew toward the adjacent vein. The infection hyphae tended to grow down into the mesophyll rather than in longitudinal direction close under the epidermis, as was the case in the other isolates. The morphology allowed identification of isolate 28 from the other isolates on the basis of single sporelings. The conventional morphology of the M-isolates, of which one (M3) was collected also in Rabat, refutes the fourth alternative we proposed, i.e., isolate 28 would replace the typical form in (a part of) Morocco. Apparently, both morphotypes are sympatric.

Percentage of one-celled teleutospores. On barley (*Hordeum vulgare* L.), including the wild form *H. spontaneum* C. Koch, at least three species of "brown leaf rust" fungi are reported to occur (2). By far the most common species is *P. hordei* sensu stricto, which occurs worldwide. One of the most distinctive characteristics of this organism is that it produces a high rate of one-celled teleutospores in the telia. In the Middle East, *Uromyces* species are also known to occur on *H. vulgare*, e.g., *U. viennot-bourginii* Wahl & Anikst. (1) (according to Cummins [4], synonymous to *U. turcomanicum* Katajev). This species produces only one-celled teleutospores. A third leaf rust reported to attack barley is *P. recondita* Rob. ex Desm. var. *tritici* Erikss., the brown leaf rust fungus of wheat. The pathogenicity of this species for cultivated barley, however, appears to be weak (8). The species produces only two-celled teleutospores.

Observation of the teleutospores was considered necessary to determine whether isolate 28 belongs to *P. hordei*. Isolates M1 through M4 and our standard isolate 121 produced telia on barley line L98 rather rapidly. Attempts to obtain telia of isolate 28 on L98 failed. Only on Ribari (possessing *Pa3*) were telia obtained. The telia of isolates 121 and M1 through M4 contained 63–90% one-celled teleutospores and the telia of isolate 28, 13% (Fig. 3). The fact that isolate 28 forms one- and two-celled teleutospores indicates it belongs to *P. hordei* and not to a *Uromyces* or a form of *P. recondita*. Its telia were loculate with abundant brown paraphyses, which is consistent with the view that isolate 28 belongs to *P. hordei* (4).

Virulence spectrum. The infection types of the seedlings of the differential set of cultivars are presented in Table 1. The unusually broad virulence spectrum of isolate 28 (12,13) is confirmed by the present results. The infection types on CI 1243, Sundance, and Gold were even higher than reported by Parlevliet (12). The duplo cultures from isolates M2 and M4 differed in virulence spectrum, indicating that the original isolates collected in Morocco were mixtures of races or were contaminated at a later stage. Culture M3-1 may not have been purified successfully (witness the ambiguous infection type readings). Cultures M2-2 and M3-2 had the very commonly occurring virulence spectrum also represented by isolate 121 (12). The spectrum of M4-2 is somewhat unusual because of the virulence to *Pa9* and the avirulence to *Pa5*. M1-1, M1-2, M2-1, and M4-1 shared a virulence spectrum

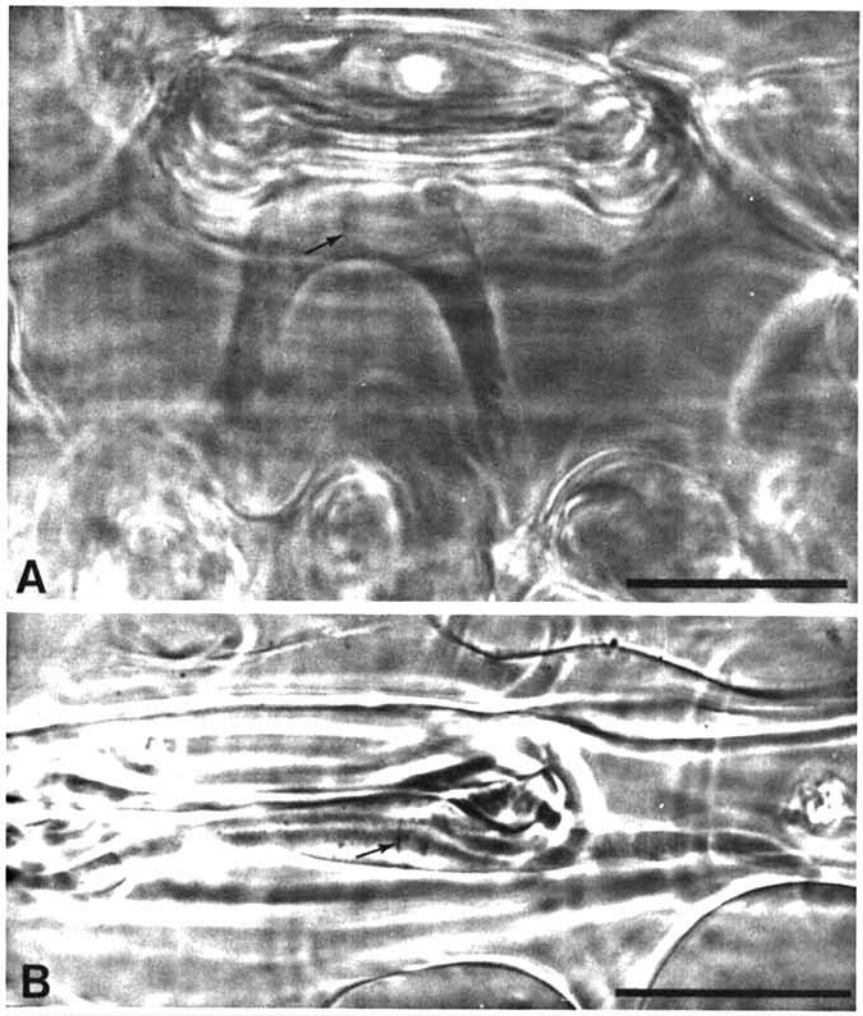

Fig. 2. Micrographs of substomatal vesicles of *Puccinia hordei* in a whole-mount preparation of a seedling leaf of *Triticum dicoccum*: **(A)** isolate 28 from Morocco and **(B)** isolate 121 from the Netherlands. Arrow indicates central septum. Scale bar = 25 μm.

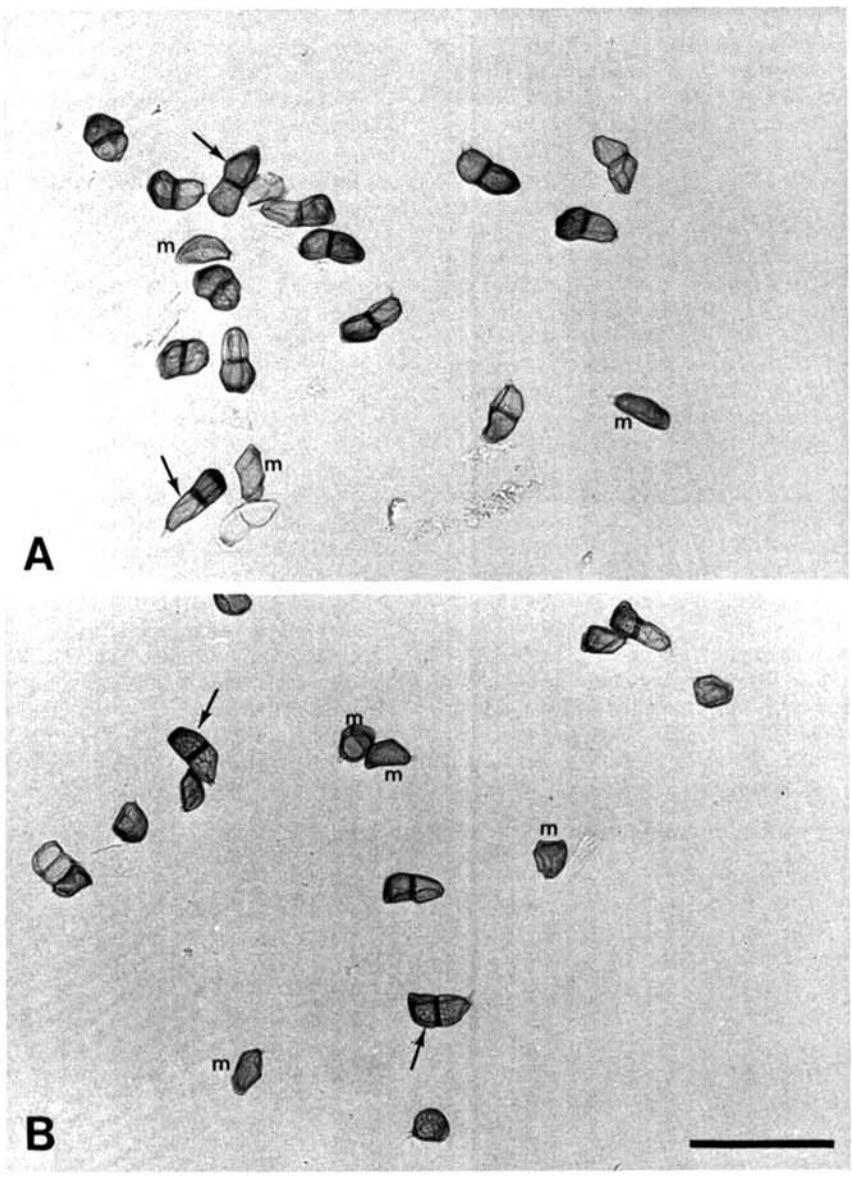

Fig. 3. Samples of spores from telia of two Moroccan isolates of *Puccinia hordei* produced on barley: **(A)** isolate 28 and **(B)** isolate M3. Two-celled (arrows) and one-celled (m, mesospore) teleutospores. Scale bar = 20 μm.

that was relatively narrow. None of the M-isolates, nor isolate 121, was virulent to *Pa3* and/or *Pa7*. We conclude that isolate 28 differs not only in morphology but also in virulence spectrum from the sympatric M-isolates.

Of the four alternatives presented in the introduction, two are still open. Isolate 28 either is just an abnormal form of *P. hordei*, not deserving a taxonomic status, or has diverged from the typical *P. hordei* and represents a distinct taxon.

We know from analysis in the barley/*P. graminis* system that major genes for resistance to one infraspecific taxon, viz., gene T to *P. graminis* subsp. *graminis* var. *graminis* from wheat, may not be effective to an other subspecific taxon, viz., *P. g.* subsp. *graminis* var. *stakmanii* from rye (6) or from oats (9). We suggest that the ineffectiveness of the *Pa* genes in barley to isolate 28 may be taken as evidence that isolate 28 is not just an abnormal form of *P. hordei* but a distinct infraspecific taxon. Other techniques, such as analysis of detergent-soluble polypeptides (7) and hybridization experiments, may (dis)prove our hypothesis.

Y. Anikster (*personal communication* 1988), however, studied barley leaf rust isolates from telia obtained from Morocco. These isolates had a virulence spectrum similar to that of isolate 28 and could be crossed with "regular" *P. hordei* isolates. The morphology of the SSVs of these isolates has not been studied yet. (Added in galley: Recently we observed that the mycelial morphology was as that of isolate 28.)

The agronomic importance of the putative taxon represented by isolate 28 is not known. Because virulence to *Pa3* and *Pa7* still is rare, it is not likely that the rust fungus is widespread. The distinctive morphology of the SSV may be used to identify suspect isolates occurring on normally highly resistant barley cul-

Table 1. Infection types[a] of seedlings of 12 barley cultivars inoculated with urediospores of *Puccinia hordei* representing six isolates[b]

Cultivar	Resistance gene	Isolate 28	Isolate 121	Isolate M1		Isolate M2		Isolate M3		Isolate M4	
				1	2	1	2	1	2	1	2
Sudan	*Pa*	8–9	6–8	8–9	8–9	8–9	7–9	6–9	7–8	6–8	8–9
Peruvian	*Pa2*	9	8	2–3	2–3	2–3	5–8	2–3/6–9	9	2–3	6–9
Ribari	*Pa3*	9	0–1	0–1	0–1	0–1	0–1	0–1	0	0–1	0–1
Gold	*Pa4*	8–9	9	9	8	8–9	9	8–9	9	8–9	9
Quinn	*Pa2* + *Pa5*	9	6–8	1(3)	1–2(3)	1	8–9	2–3/8–9	7	1–2	2–3
Bolivia	*Pa2* + *Pa6*	9	6–8	2(3)	2	2–3	6–8	2–3/8–9	9	2–3	7–9
Cebada Capa	*Pa7*	9	2	1–2	1–2	1–2	2	2	2	2	2
Egypt IV	*Pa8*	9	7–8	7–8	6–8	6–8	6–8	6–9	8	7–8	6–8
CI 1243	*Pa9*	6–7	3–5	2	2(3)	2	3–5	2–3	2–3	2	8–9
Sundance	*Pax*?	6–7	8–9	7–8	6–8	6–8	7–9	6–9	8–9	7–9	8–9
Armelle	*Pa* + *Pa2*?	9	9	2–3	2(3)	2(3)	2/8–9	2–3/6–9	9	2–3	9
L94		9	9	9	9	9	9	9	9	9	9
Number of cultivars on which infection type did not exceed 6		0	3	7	7	7	3	3	3	7	3

[a] According to McNeal et al (10), where 0–6 = resistant and 7–9 = susceptible. Infection types separated by a slash occurred in substantial proportions on the same leaves, and those within parentheses occurred at low frequency on the same leaves.
[b] Isolates M1 through M4 were tested as two monospore-derived cultures each.

tivars in Morocco and in neighboring countries.

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