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## Host Status of False Brome Grass to the Leaf Rust Fungus *Puccinia brachypodii* and the Stripe Rust Fungus *P. striiformis*

Mirko Barbieri, Dipartimento di Scienze Agrarie e degli Alimenti, Università degli studi di Modena e Reggio Emilia, Via Amendola 2, Pad. Besta, 42100 Reggio Emilia, Italy; Thierry C. Marcel, Laboratory of Plant Breeding, Graduate school for Experimental Plant Sciences, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands; INRA-AgroParisTech, UMR1290 BIOGER-CPP, Avenue Lucien Brétignières BP01, 78850 Thiverval-Grignon, France; and Rients E. Niks, Laboratory of Plant Breeding, Graduate school for Experimental Plant Sciences, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

### Abstract

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Purple false brome grass (*Brachypodium distachyon*) has recently emerged as a model system for temperate grasses and is also a potential model plant to investigate plant interactions with economically important pathogens such as rust fungi. We determined the host status of five *Brachypodium* species to three isolates of *Puccinia brachypodii*, the prevalent rust species on *Brachypodium sylvaticum* in nature, and to one isolate each of three *formae speciales* of the stripe rust fungus *P. striiformis*. Two *P. striiformis* isolates produced sporulating lesions, both in only one of the tested interactions, suggesting a marginal host status of *B. distachyon*. *P. brachypodii* formed sporulating uredinia on the five *Brachypodium* species tested, and a range of reactions was

observed. Surprisingly, the *B. sylvaticum*-derived rust isolates were more frequently pathogenic to *B. distachyon* than to their original host species. The *B. distachyon* diploid inbred lines, developed and distributed as reference material to the *Brachypodium* research community, include susceptible and resistant genotypes to at least three of the four *P. brachypodii* isolates tested. This creates the opportunity to use *B. distachyon/P. brachypodii* as a model pathosystem. In one *B. distachyon* accession, heavy infection by the loose smut fungus *Ustilago bromivora* occurred. That pathogen could also serve as a model pathogen of *Brachypodium*.

False brome grass (*Brachypodium* P. Beauv.) is a genus of temperate wild grasses belonging to the Brachypodieae tribe, which is evolutionarily related to the Triticeae, Avenae, Bromaeae, and Poeae (12,25). Within this genus, purple false brome (*Brachypodium distachyon* (L.) Beauv.) has recently emerged as a model system for temperate grasses, including small grain cereals like wheat and barley and herbaceous energy crops like switchgrass (*Panicum virgatum* L.) (12). *B. distachyon* has a suite of characteristics qualifying it to become an important model plant: a mostly diploid nature, small plant size, self-fertility, short life-cycle, and small genome size. Besides its physical and genomic attributes, several molecular studies indicated that *B. distachyon* is phylogenetically closer to temperate grasses and cereals than rice, which has been considered to be the major resource for cereal genomics research up to now (5,7,8,39).

Since *B. distachyon* was proposed as a model in 2001 (12), genomic tools and resources have been developed and are being made available to the research community (16,37): germplasm resources, inbred lines, and segregating populations (15,16,38,41), transformation protocols (29,34,38,40), a collection of T-DNA lines (33), a virus-induced gene-silencing protocol (11), BAC li-

braries (20–22), a BAC-based physical map (14,19) integrated with an SSR-based genetic linkage map (17), and the genome sequence of the diploid inbred line Bd21 (23).

*B. distachyon* is a suitable model plant to investigate the molecular basis of plant–pathogen interactions because it has been reported to be a host for a range of commercially relevant fungal pathogens of cereals and grasses (16). An early characterization of a *Brachypodium*–pathogen interaction involves the fungus *Magnaporthe grisea*, the causal agent of rice blast disease (12,31). Screening of 21 *B. distachyon* accessions with *M. grisea* isolates indicated different degrees of susceptibility and resistance (31). *B. distachyon* accessions were also challenged with cereal rust species (12). Isolates of *Puccinia hordei* Otth and *P. triticina* Erikss. (barley and wheat leaf rust fungi, respectively) elicited brown flecking symptoms on some of those accessions, but no occurrence of uredinia was observed. In contrast, when challenged with isolates of *P. striiformis* Westend (stripe rust), the plants displayed a range of responses, from brown flecking to uredinial formation in necrotic areas (12). Wilson and Henderson (42) mentioned unconfirmed reports of stripe rust occurring on *B. sylvaticum* in Britain. They presumed that such reports might be erroneous because of possible confusion with *P. brachypodii*, which also produces uredinia in lines.

A large number of *Puccinia* species have been reported to produce uredinia on at least one *Brachypodium* species. In addition to stripe rust, the list includes *P. graminis*, *P. agropyricola*, *P. coronata*, *P. recondita*, *P. corteziana*, *P. brachypodii*, and *P. brachypodii-phoenocoides* (10,13). Reports on the formation of uredinia by these cereal and grass rusts were predominantly based on greenhouse/climate room experiments (12,13), and have not been supported by reports of the occurrence of those rust species on *Brachypodium* in nature. In wild populations of *Brachypodium*, the prevalent rust species is *Puccinia brachypodii* Otth (10,32,42). In

Corresponding author: Rients E. Niks, E-mail: rients.niks@wur.nl

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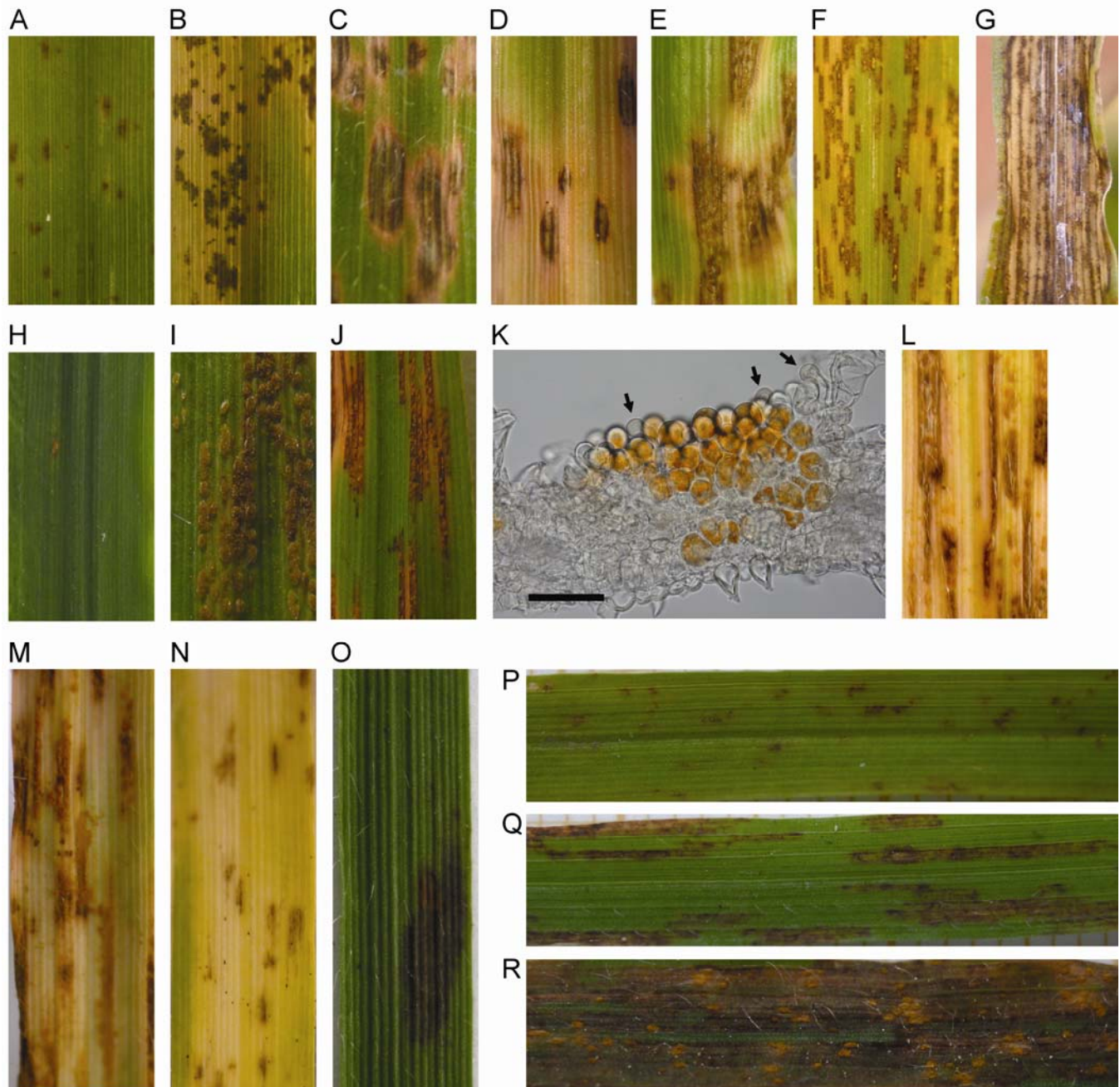
Europe, *P. brachypodii* is commonly found on populations of slender false brome, *B. sylvaticum*, and tor-grass or heath false brome, *B. pinnatum* (18,32,42). *P. brachypodii* forms aecia on *Berberis*. If the host range of *P. brachypodii* also comprises *B. distachyon*, it would be an excellent model system to study plant–pathogen interactions.

In this study, we assessed the host status of five different *Brachypodium* species (mainly *B. sylvaticum* and *B. distachyon*) to three isolates of *P. brachypodii* collected from *B. sylvaticum*, and

we describe the range of responses to infection. We also determined the ability of four *P. brachypodii* isolates to cross-infect each other's source plants. We also determined the host status of these false brome grass species to one isolate each of three *formae speciales* of *P. striiformis*.

## Materials and Methods

***Brachypodium* germplasm collection.** The plant collection primarily consisted of 40 accessions belonging to five *Brachypodium*



**Fig. 1.** Examples of phenotypes observed on *Brachypodium* accessions after inoculation with *Puccinia brachypodii*. **A**, Necrotic spots without uredinia, (PI268222 / H-Ki). **B**, Small necrotic patches without uredinia (PI1610793 / H-Ki). **C**, Large necrotic patches without uredinia (PI 239714 / F-FI). **D**, Necrotic patches and reddish chlorosis without uredinia (PI226629 / F-FI). **E**, Necrotic patches and yellow chlorosis associated with abundant sporulation (Bd21 / F-FI). Similar phenotypes were obtained with isolates H-Ki and NL-De. **F**, Uredinia associated with necrotic stripes and yellow chlorosis (PI318962 / NL-De). **G**, Collapsed tissue without uredinia (PI268222 / NL-De). **H**, Few compatible uredinia on a green background (PI 269842 / F-FI). **I**, Abundant sporulation on a green background (Bd3-1 / F-FI). Phenotypic characteristics of *P. brachypodii*. **J**, Leaf of *B. sylvaticum* showing typical infection by *P. brachypodii*, with darkening of plant tissue in the uredinal lines. **K**, Cross-section of uredium of *P. brachypodii*. Arrows indicate paraphyses. Bar represents 50 µm. **L**, Straw-brown chlorotic leaf tissue of *B. distachyon* after infection by *P. brachypodii* (accession PI 254867 inoculated with isolate H-Ki). Phenotypes observed on Bd2-3 in combinations with the different *P. brachypodii* isolates. **M**, Bd2-3 inoculated with isolate H-Ki, abundant sporulation with necrotic patches and chlorosis. **N**, Bd2-3 inoculated with isolate NL-De, small necrotic patches and chlorosis with occasional sporulation. **O**, Bd2-3 inoculated with isolate F-FI, dark necrotic patches not associated with uredinia nor with chlorosis. Different phenotypes observed on *Brachypodium* accessions after inoculation with *P. striiformis*. **P**, Small necrotic flecks without uredinia (PI 233228, *Psh*). **Q**, Large necrotic flecks without uredinia (PI 239713, *Psh*). **R**, Sporulation in necrotic lesions (PI 227011, *Psh*).

species: *B. phoenicoides* (L.) P. Beauv. ex Roem. & Schult., *B. sylvaticum* (Huds.) P. Beauv., *B. distachyon* (L.) P. Beauv., *B. pinnatum* (L.) P. Beauv., and *B. rupestre* (Host) Roem. & Schult. subsp. *rupestre*. Seeds of the 40 accessions, designated by the prefix "PI" or "W", were obtained from the USDA National Plant Germplasm System (NPGS). For details, see NPGS Germplasm Resources Information Network website (<http://www.ars-grin.gov/npgs>; verified 5 September 2011).

The plant collection also included five *B. distachyon* inbred lines, designated by the prefix "Bd", of which seed was provided by Prof. David Garvin (University of Minnesota). These inbred lines were developed by single-seed descent from NPGS accessions (38).

**Rust isolate collection and maintenance.** Four field isolates of *P. brachypodii* (32) were collected from *B. sylvaticum* plants at four locations in three European countries and were labeled accordingly as H-Ki (collected in Kistapé, Tolna, Hungary; July 2006), NL-De (Delden, Overijssel, The Netherlands; May 2007), F-Fl (Fleurines, Oise, France; August 2008), and NL-Vij (Vijlen, Limburg, The Netherlands; October 2008). The first three were used to determine the host status of the false brome grass species. All four isolates were used to test the ability of the rust fungi to infect their own and each other's source plants. From each of the localities, we also collected seeds and transplanted infected plants to pots that we placed in a greenhouse. Fresh spores were initially produced and collected on the original host *B. sylvaticum* plants, and later multiplied on some of the five *B. distachyon* inbred lines. Care was taken to produce spores of each isolate in separate greenhouse compartments to prevent contamination. The rust isolates can be obtained upon request from the last author.

The three isolates of *P. striiformis* that were used to infect the plants belong to three *formae speciales*: isolate 237E141 is of the wheat-infecting form *P. striiformis* f. sp. *tritici* (*Pst*); isolate 24 is of the barley-infecting form *P. striiformis* f. sp. *hordei* (*Psh*); and the third isolate is of the form that infects California brome grass (*Bromus carinatus* Hook. et Arn.), designated here *P. striiformis* f. sp. *bromi* (*Psb*). *Pst* and *Psh* were kindly provided by the Global Yellow Rust Gene Bank (Plant Research International, the Netherlands), which is currently being transferred to Aarhus University in Denmark. We collected *Psb* along a roadside in Wageningen (Netherlands) in summer 2007.

**Plant inoculation.** *Brachypodium* seeds were planted into 10-cm-diameter pots and maintained in a greenhouse compartment at 18°C with a photoperiod of 16 h. Each pot contained a single *Brachypodium* accession represented by about three to 10 plants, depending on the seed germination rate. Potting soil was *Arabidopsis* potting mix #101759 (Lentse Potgrond B.V.).

About 5 weeks after sowing, plants were inoculated with freshly collected spores. Approximately 4 mg of *P. brachypodii* spores were applied in a settling tower that could accommodate about 14 pots per inoculation. Such an inoculum dose results in a deposition of about 240 spores per cm<sup>2</sup> (authors' personal observation). *P. striiformis* inoculations were applied at about a threefold greater dose, for two reasons: germination of this rust species may be erratic and poor, and we expected a higher general level of resistance of the *Brachypodium* accessions. The high inoculum dose resulted in a rigid test even for low levels of susceptibility. For both rust species, an approximately 10-fold greater volume of inert *Lycopersicon* spores was added to the rust spores to increase the volume and the homogeneity of spore deposition over the leaves. Glass microscope slides were placed between the plants prior to each inoculation in order to verify spore deposition and germination. After incubation at a relative humidity of 100% overnight in darkness, plants were transferred to a greenhouse compartment where the temperature was set at 18°C.

Infection experiments were conducted twice for each field isolate of *P. brachypodii* and one time for each form of *P. striiformis*. The inoculations with the different isolates/forms were performed on subsequent days within the same week in order to avoid cross-contamination while maintaining similar environmental circumstances.

For some of the accessions, seed germination was poor, resulting in no or only a few seedlings per pot. Despite this, for all accession/*P. brachypodii* isolate combinations, at least in one of the two experiments there was at least one plant available to be scored, except for one *B. distachyon*/*P. brachypodii* isolate NL-De combination. However, in the experiments with *P. striiformis*, lack of seeds or poor germination caused 12 (*Pst*), 3 (*Psh*), and 1 (*Psb*) accessions to remain untested.

**Disease assessment.** *P. brachypodii* isolates. Approximately 12 to 14 days after inoculation, i.e., when symptom expression seemed at its peak, each accession was visually inspected for disease symptoms. We observed a range of very different reactions depending on the plant accession and the rust isolate. The reaction types (qualitative description of infections) and the sporulation intensity (quantity of pustules) were recorded.

The reaction type (RT) was described as: immune or without any symptom (Im); spreading necrotic lesions such as spots (Ns, Fig. 1A), patches (Np, Fig. 1B–E), and stripes (Nst, Fig. 1F); occurrence of yellow or reddish chlorosis (Ch, Fig. 1D–F); collapse of leaf tissues (Col, Fig. 1G); or uredinia against green background (S, Fig. 1H, I).

The sporulation intensity (SI) was evaluated as: 0 = no sporulation (highly resistant); 1 = rare sporulation (resistant); 2 = sporulation mainly restricted to a few old leaves (intermediate); 3 = sporulation on many leaves (susceptible); or 4 = abundant sporulation (very susceptible).

Finally, we recorded whether individual plants of the same accession reacted differently, indicating that the accession was genetically heterogeneous.

*P. striiformis* forms. Scoring was simplified to a 0 to 3 scale: 0 = immune; 1 = small dark flecks; 2 = large dark flecks; and 3 = some sporulation. For each inoculation with a different form, a susceptible control plant was added to verify the success of the infection. Heterogeneity in the response of plants within the same accession was recorded as for the *P. brachypodii* observations.

## Results

**Heterogeneity within *Brachypodium* accessions.** *B. sylvaticum* normally has hairy, flaccid, and wide leaves (~12 mm). Eight of the 21 *B. sylvaticum* accessions had slender, more stiff and glabrous leaves and leaf sheaths, as in *B. pinnatum* and *B. rupestre*. Morphological diversity between *B. sylvaticum* accessions has been reported before (9).

In 10% of the accession/*P. brachypodii* isolate combinations, plants within the same accession showed a mixed reaction, i.e., different RT or different SI, indicating genetic heterogeneity of these accessions. NPGS accessions are population samples that are not necessarily genetically homogeneous, and such mixed reactions were to be expected. In those cases, the most common phenotype was recorded. In only five cases, the recorded phenotypes differed between the two experiments, and the accession/isolate combinations were tested a third time. The third disease experiment always confirmed the observation in one of the previous experiments, and that result was recorded for the accession/isolate combination.

**Variation in response among accessions inoculated with *P. brachypodii*.** In each inoculated batch of accessions, several accessions showed a relatively high density of necrotic or sporulating lesions, indicating that inoculum quantity administered in the settling tower was high enough to exclude the possibility of accessions escaping inoculation by chance. Therefore, differences in RT and SI can be attributed to the relative susceptibility of the *Brachypodium* accessions. Detailed information on the 45 *Brachypodium* accessions and inbred lines and on their symptoms to the three *P. brachypodii* isolates is presented in Supplemental Table 1 and summarized in Table 1.

On susceptible *Brachypodium* accessions, each *P. brachypodii* infection unit formed several uredinia (typically 3 to about 20) arranged in lines. Such a uredinium-forming lesion was usually only one or two uredinia wide (Fig. 1J), similar to the lesions

formed by *P. striiformis* on grasses but at smaller scale. Nevertheless, *P. brachypodii* can clearly be distinguished from *P. striiformis* by a more brownish color of uredinia, and by the presence of hyaline capitate filaments called paraphyses in the uredinia (Fig. 1K). *P. brachypodii* also forms an appressorium over a stoma before entering the leaf, which is not the case for *P. striiformis*. Infected leaf tissues tended to develop a dark coloration in the sporulating lesions (as observed on the original *B. sylvaticum* plants on which the rust isolates were collected, Fig. 1J), or to become straw-brown and chlorotic in most of the infected tissues on many of the *B. distachyon* accessions (Fig. 1L).

RT and SI were not closely associated. On some accessions, many uredinia developed despite abundant chlorosis and necrosis of plant tissue (Fig. 1E), while on other accessions very few but compatible uredinia appeared without any macroscopic chlorosis or necrosis (Fig. 1H).

**Differences between and within *Brachypodium* species in reaction to *P. brachypodii*.** Sporulating uredinia were observed on the five *Brachypodium* species tested with at least one of the three *P. brachypodii* isolates. Accessions of *B. pinnatum*, *B. phoenicoides*, and *B. rupestre* had resistant or intermediate phenotypes (SI 0 to 2) to all *P. brachypodii* isolates, while accessions from *B. sylvaticum* and *B. distachyon* ranged from completely resistant to highly susceptible (Table 2).

*B. sylvaticum* and *B. distachyon* differed greatly in their general level of resistance/susceptibility, as clear susceptibility was observed for only two (3%) of the *B. sylvaticum* accession/isolate combinations but for 30 (57%) of the *B. distachyon* accession/isolate combinations. The two cases of susceptibility (SI 3) on *B. sylvaticum* were reported for the same accession (PI 564896) with isolates F-FI and H-Ki, respectively. PI 564896 is one of the eight *B. sylvaticum* accessions with narrow leaf morphology. The generally higher level of resistance of *B. sylvaticum* than *B. distachyon* accessions was apparent from the fact that in 23 of the 63 combinations, the *B. sylvaticum* plants were immune (Im, SI 0), while none of the 54 *B. distachyon* accession/isolate combinations was immune. Six *B. distachyon* accessions were susceptible (SI 3 and 4) to all three isolates: PI 239713, PI 245730, PI 254867, and the three inbred lines Bd3-1, Bd18-1, and Bd21. Since Bd18-1 and Bd21 were developed by single-seed descent from PI 245730 and PI 254867, respectively (38), their similar responses were expected. The other two inbred lines Bd1-1 and Bd2-3 displayed different responses to different isolates. Bd1-1 was susceptible to isolate H-Ki (SI 4) and resistant to isolates F-FI and NL-De (SI 1). Bd2-3 was susceptible to isolate H-Ki (SI 4, Fig. 1M), resistant to isolate NL-De (SI 1, Fig. 1N), and very resistant to isolate F-FI (SI 0, Fig. 1O); this inbred line Bd2-3 resembled the disease phenotypes of PI 185133, the accession from which it had been derived (38). Among the susceptible interactions with *B. distachyon* accessions and inbred lines, quantitative differences were observed (data not shown). Indeed, even though the final number of sporulating uredinia was high, on some genotypes uredinium development was much faster and more abundant than on others.

We did not find an obvious association between putative ploidy level or origin of *B. distachyon* accessions and level of susceptibility to the rust isolates.

**Isolate-specific resistance to *P. brachypodii*.** Individual *P. brachypodii* isolates varied in pathogenicity (Table 3). Isolate H-Ki had the widest virulence spectrum compared with the other two isolates, causing susceptible reactions (SI 3 and 4) on 40% of the accessions. Isolates F-FI and NL-De had more narrow virulence spectra, with 18 and 14% susceptible interactions, respectively. Isolate-specific reactions by *Brachypodium* accessions were common in our experiments. Among the 45 accessions, 17 were resistant (SI 0 and 1) and six susceptible (SI 3 and 4) to all three isolates, the other 21 accessions were more susceptible to one isolate than to the others.

We did not find a clear accession × isolate interaction that might indicate gene-for-gene resistance. In some cases (e.g., Bd2-3 and Bd1-1), an accession scored SI 4 for isolate H-Ki and SI 0 or 1 to F-FI and/or NL-De, but none of the accessions scored SI 4 to NL-De or F-FI and SI 0 or 1 to isolate H-Ki. Accessions coming closest to the latter were *B. sylvaticum* PI 639817 and PI 204863, which showed higher infection severity with isolate F-FI (SI 2) than with isolate H-Ki (SI 0).

**Pathogenicity of *P. brachypodii* isolates on *B. sylvaticum* accessions of origin.** The high frequency of resistant accessions in

**Table 2.** Number of accessions of five *Brachypodium* species each challenged with three *Puccinia brachypodii* isolates, and the resulting disease phenotypes

Species	No. of accessions	Disease phenotype <sup>a</sup>		
		R	I	S
<i>B. pinnatum</i>	2	5	1	0
<i>B. rupestre</i>	2	6	0	0
<i>B. phoenicoides</i>	2	5	1	0
<i>B. sylvaticum</i>	21	52	9	2
<i>B. distachyon</i>	18 <sup>b</sup>	21	2	30
Total	45 (134)	89	13	32

<sup>a</sup> Number of accession–isolate combinations per disease phenotype within *Brachypodium* species. R = resistant (SI 0 and 1), I = intermediate (SI 2), S = susceptible (SI 3 and 4).

<sup>b</sup> One accession–isolate combination was missed.

**Table 3.** Numbers of resistant, intermediate, and susceptible interactions in 45 *Brachypodium* accessions each challenged with three isolates of *Puccinia brachypodii*

<i>P. brachypodii</i> isolate	Disease interaction <sup>a</sup>		
	R	I	S
H-Ki	20	7	18
NL-De	35	3	6
F-FI	34	3	8

<sup>a</sup> Number of accession–isolate combinations per disease interaction within *Brachypodium* species. R = resistant (SI 0 and 1), I = intermediate (SI 2), S = susceptible (SI 3 and 4).

**Table 1.** Percentage of reaction type/sporulation intensity combinations observed among 45 *Brachypodium* accessions each challenged with three *Puccinia brachypodii* isolates

Reaction type		Sporulation intensity <sup>a</sup>				
		0	1	2	3	4
Immune	Im	22.4				
Necrotic spots	Ns	14.9 (1A) <sup>b</sup>	11.2	3.0	2.2	
Necrotic patches	Np	9.0 (1B-D)	3.0	4.5	2.2	4.5 (1E)
Necrotic stripes	Nst	0.7	2.2	2.2 (1F)	3.0	4.5
Chlorosis <sup>c</sup>	Ch	0.7			1.5	1.5
Collapse of tissue	Col	0.7 (1G)			0.7	
Green	S		1.5 (1H)		0.7	3.0 (1I)

<sup>a</sup> Sporulation intensity: 0 = no sporulation, 1 = rare sporulation, 2 = sporulation mainly restricted to a few old leaves, 3 = sporulation on many leaves, 4 = abundant sporulation.

<sup>b</sup> In parentheses are the sections of Figure 1 showing examples of the specific phenotypes.

<sup>c</sup> Chlorosis not associated with other necrotic symptoms.

*B. sylvaticum* prompted us to check the susceptibility of the *B. sylvaticum* accessions from which the rust isolates had been collected originally. The *B. sylvaticum* plants, from which the four *P. brachypodii* rust isolates were collected, produced seeds that were harvested and sown. Germination was very low and irregular, probably because of strong dormancy. We placed the pots outdoors to expose the sown seeds to low winter temperatures (2009–2010). In spring 2010, sufficient germination was obtained to carry out an infection trial with four *P. brachypodii* isolates. We added as positive controls the five *B. distachyon* inbred lines.

The five *B. distachyon* inbred lines confirmed that Bd21, Bd18-1, and Bd3-1 were susceptible to all four isolates (Table 4), although Bd18-1 was less susceptible to isolate NL-De than before. Bd1-1 and Bd2-3 again had isolate-specific resistance. Bd1-1 was moderately susceptible to isolate H-Ki and resistant to isolates NL-De, F-Fl, and NL-Vij. Bd2-3 was susceptible to isolates H-Ki, F-Fl, and NL-Vij, but resistant to isolate NL-De. The susceptibility of this accession to isolate F-Fl was in contrast to the reaction obtained before, when this combination was highly resistant with necrotic patches.

Except for isolate F-Fl, the isolates produced compatible infections on at least some of the plants of the accessions from which they were collected (Table 4). The progeny of accession *B. sylvaticum*-NL-Vij was the only one clearly segregating for resistance and susceptibility. Accession *B. sylvaticum*-NL-De was only susceptible to isolate NL-De, and accession *B. sylvaticum*-NL-Vij was only susceptible to isolates F-Fl and NL-Vij. Most interactions of *B. sylvaticum* with rust isolates of different origin (7 out of 12) were highly resistant (SI 0) or resistant (SI 1). These data are consistent with the previous conclusion that *B. sylvaticum* has a lower frequency of susceptible accessions than *B. distachyon*, and that *P. brachypodii* tends to be specifically adapted to the local *B. sylvaticum* population.

The unexpected avirulence of isolate F-Fl to *B. sylvaticum*-F-Fl may be due to either a shift in composition of the original isolate

F-Fl during propagation on susceptible *B. distachyon* plants, and/or to segregation of resistance in the *B. sylvaticum*-F-Fl seed progeny, as observed also to some extent in accession *B. sylvaticum*-NL-Vij.

Interestingly, the results presented in Table 4 suggest a case of differential interaction (isolates NL-De and F-Fl on accessions *B. sylvaticum*-NL-De and *B. sylvaticum*-NL-Vij), and hence may indicate a gene-for-gene relationship.

**Responses of *Brachypodium* accessions to three *P. striiformis* forms.** The large majority (83 out of 119, or 70%) of *Brachypodium* accession/isolate combinations were immune to the three forms of *P. striiformis* (Table 5, Supplemental Table 2). Most of the remaining accession/isolate combinations (34 out of 119, or 29%) developed small or larger dark flecks without any sporulating uredinia (Fig. 1P, Q). Nevertheless, on one *B. distachyon* accession, PI 227011, *Psh* and *Psb* developed sporulating uredinia associated with extensive necrosis of leaf tissue (Fig. 1R). On this accession, *Pst* developed small dark spots without uredinia. Bd21 was the only one of the five *B. distachyon* inbred lines on which the three stripe rust forms developed large necrotic lesions, while the other inbred lines were predominantly immune.

**Occurrence of loose smut on PI 533015.** Infection by a loose smut fungus was observed on *B. distachyon* accession PI 533015 during seed propagation in a greenhouse compartment. At flowering, on the majority of the inflorescences, kernels were replaced by black spore masses (Fig. 2). The fungus is likely *Ustilago bromivora* (Tul. & C. Tul.) A.A. Fisch. Waldh. s. lat., which is also known under the name *U. brachypodii-distachyi* Maire (1919) (K. Vánky, personal communication). The sori of this species are restricted to the basal parts of the floral envelopes and ovaries, and do not destroy the spikelets as thoroughly as those of the close relative *U. bullata* Berk. (35) (Fig. 2). We did not try to determine the level of susceptibility to loose smut in the *Brachypodium* germ-

**Table 4.** Level of infection of four *Puccinia brachypodii* isolates on their own<sup>a</sup> and on each other's *Brachypodium sylvaticum* accessions of origin and on five *B. distachyon* inbred lines

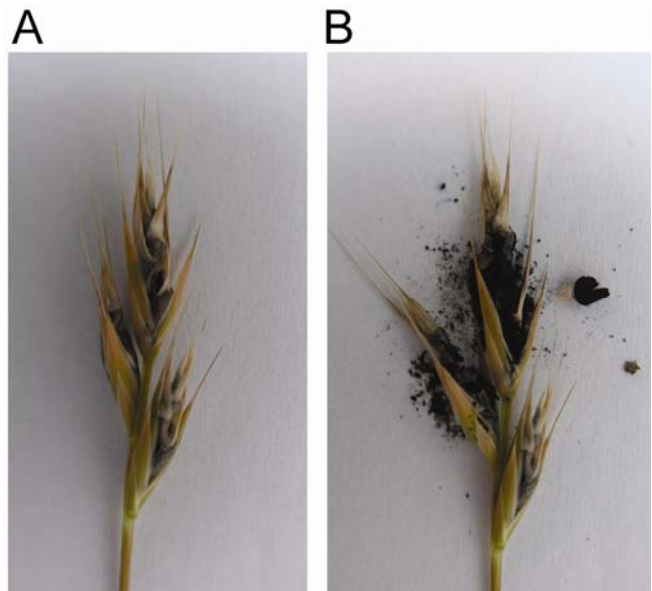
<i>Brachypodium</i> accession	Isolate of <i>P. brachypodii</i>			
	H-Ki	NL-De	F-Fl	NL-Vij
<i>B. sylvaticum</i> -H-Ki	<b>3<sup>b</sup>-Nst<sup>c</sup></b>	2-Ns	3-Nst	2-Ns
<i>B. sylvaticum</i> -NL-De	1-Ns	<b>3-S</b>	1-Ns	0-Ns
<i>B. sylvaticum</i> -F-Fl	0-Ns	3-Col + Ch	<b>1-Ns</b>	0-Ns
<i>B. sylvaticum</i> -NL-Vij	0-Ns	1-Ns	3-S	<b>Mixed<sup>d</sup></b>
Bd1-1	2-Nst	0-Ns	1-Col	1-Ns
Bd2-3	3-S	1-Np	3-Col	3-S
Bd21	4-S	4-Col	4-S	4-S
Bd18-1	3-Col	2-Col	3-Col	2-3 Col+Np
Bd3-1	3-S	3-Nst	3-Nst/Col	3-S

<sup>a</sup> Results of isolates tested on their accessions of origin are in bold.

<sup>b</sup> Sporulation intensity: 0 = no sporulation (highly resistant), 1 = rare sporulation (resistant), 2 = sporulation, mainly restricted to a few old leaves (moderately susceptible), 3 = sporulation on many leaves (susceptible), 4 = abundant sporulation (very susceptible).

<sup>c</sup> Reaction type: Ns = necrotic spots, Np = necrotic patches, Nst = necrotic stripes, Ch = chlorosis, Col = collapsed leaves, S = pustules on green background.

<sup>d</sup> One plant 0-Ns, one plant 3Nst, one plant 3-S.



**Fig. 2.** Loose smut in inflorescences of *Brachypodium distachyon* accession PI 533015 infected by *Ustilago bromivora*.

**Table 5.** Number of *Brachypodium* accessions per infection response to three *Puccinia striiformis formae speciales*

Rust form	No. of accessions tested	Infection responses <sup>a</sup>			
		0	1	2	3
<i>Puccinia striiformis</i> f. sp. <i>tritici</i>	33	19	6	8	0
<i>P. striiformis</i> f. sp. <i>hordei</i>	42	30	6	5	1 <sup>b</sup>
<i>P. striiformis</i> f. sp. <i>bromi</i>	44	34	3	6	1 <sup>b</sup>

<sup>a</sup> 0 = immune, 1 = small dark flecks, 2 = large dark flecks, 3 = (some) sporulation.

<sup>b</sup> To both rust *formae speciales*, the only accession allowing sporulation was *B. distachyon* accession PI 227011.

plasm available to us, but the isolate is available for such an experiment.

## Discussion

Since *B. distachyon* has emerged as a model grass, there is growing interest in using it to study the interaction with economically important pathogens from temperate cereals. Up to now, detailed reports on pathosystems involving *B. distachyon* or other *Brachypodium* species have been restricted to the rice blast fungus, *Magnaporthe grisea* (1,30,31). All other reports are limited to preliminary pathogen screens (16). Rusts are among the most damaging fungal diseases of temperate cereals, such as wheat and barley. *B. distachyon* displays a range of symptoms when inoculated with isolates of different rust species infecting wheat or barley (i.e., *P. triticina*, *P. hordei*, *P. striiformis*) but successful uredinium formation was exceptional and only observed on two *B. distachyon* ecotypes, ABR100 and ABR105, after infection with an isolate of *P. striiformis* f. sp. *tritici* (12). We confirmed that *P. striiformis* can infect *B. distachyon*, but full susceptibility was not found. Only the accession PI 227011 allowed limited sporulation accompanied by extensive necrosis (Fig. 1R), as observed earlier on ABR100 and ABR105 (12). Therefore, as for *P. triticina* and *P. hordei*, *P. striiformis* is unlikely to be an important pathogen of *Brachypodium* in nature, and these cereal rusts cannot be used to study host-pathogen interactions in *Brachypodium*. As an alternative, we propose to use *P. brachypodii* as a model rust species, as we established its pathogenicity on the *Brachypodium* genus, especially on *B. distachyon* and *B. sylvaticum* species.

*P. brachypodii* is a complex rust species, subdivided into various taxa, each probably specialized on different genera of grasses. The distinct mycelial morphology of these forms (26,32) suggests that they may be distinct species. *P. brachypodii* is commonly found on *B. sylvaticum*, from which we collected the four isolates studied here. We demonstrated that *B. distachyon* is a host to *P. brachypodii*. Within the *Brachypodium* genus, the two species *B. sylvaticum* and *B. distachyon* are not closely related (7), and differ greatly in plant morphology, life cycle (perennial and annual, respectively), and basic chromosome number (37). This suggests that *P. brachypodii* has co-evolved with the *Brachypodium* genus and seems not to have lost the ability to infect different species within that genus. This outcome contrasts with the diversification of leaf rusts in the barley genus *Hordeum*. The closely related leaf rust fungi *P. hordei*, *P. hordei-murini*, and *P. hordei-bulbosi* are only pathogenic on their proper species, i.e., cultivated and wild barley (*H. vulgare/spontaneum*), wall barley (*H. murinum*), and bulbous barley (*H. bulbosum*), respectively (2,3). These *Hordeum* species are nonhosts or marginal hosts (4,28) to each other's *Puccinia* species.

Among the aspects that should be considered in host range experiments are the numbers and the origins of the tested accessions of plant species, and the numbers of tested isolates of the pathogen (27). Indeed, verdicts on the host or nonhost status of a species cannot be based on observations made on a few accessions having a common provenance. In our study, *B. phoenicoides*, *B. pinnatum*, and *B. rupestre* were represented by too few accessions to be sure about their nonhost status, suggested in Table 2. Provisionally, we presume these three species to have high degrees of resistance to *P. brachypodii* isolates collected on *B. sylvaticum*.

A surprising outcome of our tests is that the isolates collected on *B. sylvaticum* had such low pathogenicity to accessions of this species (Tables 2 and 4). Obviously, fully susceptible interactions were the exception, and resistance the rule. *B. sylvaticum* should be a predominantly resistant host (27) and performed in our test as if it were a marginal host (*sensu* [28]) to the rust fungi that we collected on that species. A similar example of low pathogenicity of rust fungi to their main host species was found by Jafari (24) for *Hordeum bulbosum*-*P. hordei-bulbosi*. One wonders how a specialized pathogen persists in nature if susceptibility occurs at such low frequency. It may be relevant that *B. sylvaticum* and *H. bulbosum* are both perennial species, on which a pathogen may persist for several years. Such cases contrast with the normal situation, where

the large majority of host accessions are susceptible to any isolate of their specialized pathogen, as in the pathosystems *H. murinum*-*P. hordei-murini*, *H. vulgare*-*P. hordei*, and *Elytrigia* (syn. *Agropyron*) *repens*-*P. persistens* (R. E. Nicks, unpublished). The latter plant species is a perennial, showing that perennial plant species have not *per se* maintained high levels of resistance to their pathogens. The low level of susceptibility among the *B. sylvaticum* accessions suggests strong specialization of *P. brachypodii* populations to the locally occurring *B. sylvaticum* host populations. That hypothesis is supported by the fact that infections by three of the four *P. brachypodii* isolates were mainly restricted to the respective plant populations from which they had been collected (Table 4). Since *B. distachyon* does not occur in the wild in northern France nor in the Netherlands, the infections on the French and Dutch *B. sylvaticum* populations cannot be due to inoculum blown in fortuitously from *B. distachyon*. In the local *B. sylvaticum* populations, the rust fungus grew as a compatible pathogen rather than as a marginal infection originating from a different main host species nearby.

Although resistance is common in *B. distachyon* as well, there is sufficient susceptibility to *P. brachypodii* to consider this species a natural host. None of the combinations were immune, and 30 (57%) of the *B. distachyon* accession/rust isolate combinations were susceptible. Since the inbred lines Bd18-1 and Bd21 were developed from PI 245730 and PI 254867, respectively (38), the level of susceptibility found in *B. distachyon* could be inflated by the presence of such susceptible *B. distachyon* duplicates.

During our study, the *B. distachyon* accession PI 533015 was severely infected by the loose smut fungus *U. bromivora* s. lat. *U. bromivora* is a smut with a cosmopolitan distribution, and it is known to infect numerous *Bromus* species, but also members of the grass genera *Agropyron*, *Austrofestuca*, *Brachypodium*, *Critesion*, *Elymus*, *Festuca*, *Hordeum*, *Lolium*, *Trachynia*, and *Sitanion*. On *B. distachyon*, it was also described as a separate species under the name *U. brachypodii-distachyi* Maire. Specimens of *U. bromivora* have been collected in the Mediterranean area (Algeria, Israel, Italy, Spain, Morocco, Tunisia), Asia (Iran), and Australia (K. Vánky, personal communication). In Australia, which is the provenance of PI 533015, *U. bromivora* is widely distributed (36). Since loose smut is used as a model pathogen in maize (6), the *Brachypodium*-infecting smut is another candidate for studies on specialized biotrophic plant fungal pathogens.

Based on our results, it is promising that the five diploid *B. distachyon* inbred lines that have been developed by Vogel et al. (38) and distributed as a community resource include susceptible and resistant genotypes to at least three of the four *P. brachypodii* isolates tested (NI-De, F-Fl, and NL-Vij). Genomic tools such as polymorphic molecular markers (16,41) and segregating populations have been developed between most of the diploid inbred line combinations (16,17). Our observations offer the opportunity to study the segregation of leaf rust resistance in different *B. distachyon* populations and, eventually, to perform genetic studies on this pathosystem. Even to isolate H-Ki, for which abundant sporulation was observed on all five inbred lines, levels of susceptibility varied quantitatively from one line to the other to such a degree that we successfully mapped QTLs for resistance to *P. brachypodii* rust isolate H-Ki in a segregating population derived from Bd1-1 × Bd3-1 (M. Barbieri, T. C. Marcel, R. E. Nicks, E. Francia, M. Pasquariello, V. Mazzamuro, D. F. Garvin, and N. Pecchioni, unpublished).

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