Partial Resistance to *Uromyces appendiculatus* in Dry Edible Beans

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ABSTRACT

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Partial resistance was studied in five bean (*Phaseolus vulgaris*) cultivars that appeared to differ in their resistance to leaf rust incited by *Uromyces appendiculatus*. Inoculated leaves were sampled daily, then cleared and stained for fluorescent microscopy. The factors studied histologically were colony size, early and late colony abortion, proportion of successful penetrations, and uredial size. Cultivars Nodak and Upland had a higher percentage of both early- and late-aborted colonies than the rust-susceptible cultivars Fiesta and UI 114. Nodak and Upland had a lower proportion of successful penetrations than the susceptible cultivars at 14 days. Nodak had smaller colonies than the rust-susceptible cultivars after day 7. Uredia were counted daily to determine infection density (ID) and latent period (LP_{50}). LP_{50} did not differ significantly among the cultivars studied. The ID and number of uredia per square centimeter were significantly lower for Nodak and Upland than for the susceptible cultivars. Nodak and Pindak had smaller uredia at 14 days than the susceptible cultivars. Partial resistance was documented for Nodak and Upland. The only component of partial resistance demonstrated for Pindak was small uredia.

Rust incited by *Uromyces appendiculatus* (syn. *U. phaseoli* (Reben) Wint.) can be a devastating disease of dry edible beans (*Phaseolus vulgaris* L.) in the bean-growing regions of North Dakota, the upper Midwest, and throughout much of the world (14).

Resistant cultivars and fungicide applications have been used to control the disease (1). Major gene resistance is not permanent because the fungus is highly variable in pathogenicity and has the ability to change by mutation or sexual recombination and attack previously resistant cultivars (2,13). Repeated applications of fungicides are costly; therefore, the use of partially resistant cultivars could reduce the number of fungicide applications needed to control the disease and perhaps be more durable than major gene resistance.

Slow rusting is a type of resistance that retards disease progress in the field even if the infection type indicates a compatible interaction between the host and pathogen (5). Slow leaf rusting in wheat (*Triticum aestivum* L. em Thell.) incited by *Puccinia recondita* f. sp. *tritici* Rob. ex. Desm. has been associated with a longer latent period, reduced uredial size, and fewer uredia per square centimeter (5).

The definition for slow rusting in wheat is similar to that for partial resistance in barley (*Hordeum vulgare* L.). Partial resistance in barley to leaf rust incited by *P. hordei* Otth. has been characterized by a reduced rate of epidemic development in spite of a susceptible infection type (7). Partial resistance has been related to reduced infection densities (IDs), longer latent periods, and reduced rates of sporulation (8). Partial resistance in barley has been considered to be relatively stable compared with race specific resistance characterized by a hypersensitivelike infection type (9). Slow leaf-rusting resistance in wheat appears to be race nonspecific and durable (5).

The objective of this study was to determine if partial resistance was operative in beans against *U. appendiculatus* and, if so, which components could be used to identify cultivars with partial resistance. The components studied were latent period, ID, early abortion, late abortion, colony size, uredia per square centimeter, and uredial size. Therefore, we chose two susceptible cultivars and three cultivars that had been observed to have less rust or smaller uredia to investigate possible partial resistance of beans to *U. appendiculatus*.

MATERIALS AND METHODS

Researchers have observed less rust on dry edible bean cultivars Nodak and Upland in North Dakota than on the susceptible cultivars Fiesta or UI 114. We also observed smaller uredia on Pindak and Nodak than on the more susceptible cultivars. The bean cultivars used were UI 114 and Fiesta, known to be susceptible to *U. appendiculatus*, Nodak and Upland, observed to have less rust in the field, and Pindak, observed to have smaller uredia. The cultivar Red Kloud was used as a hypersensitive resistant control to be sure necrosis could be distinguished by the staining procedure used. Seedlings of the cultivars were grown in a glasshouse at North Dakota State University, Fargo. The diurnal temperature was 22 ± 3, and the nocturnal temperature was 15 ± 3 C. Primary bean leaves were inoculated with a suspension of spores in Soltrol 170 (Phillips Petroleum Co., Bartlesville, OK) (4 mg of spores/3 cc of Soltrol 170) and placed in a moist chamber. About the same amount of oil and urediospores (0.5 cc of the urediospore-Soltrol mixture on 10 plants) was applied to each leaf at a rate of about 1.1 spores per square centimeter with an atomizer. Inoculated plants were held at about 100% relative humidity for 24 hr. After incubation, plants were returned to the glasshouse bench at 22 ± 3 C for the duration of the experiment. The culture of *U. appendiculatus* used was designated 15C, a single-uredial isolate from a North Dakota collection. Purity was confirmed by uniformity of reactions on a set of bean differentials used by Stavely (13) inoculated with culture 15C. Genotypic differences were not observed on any of the differentials or on any of the cultivars used in this study.

Latent period was determined using the method of Parlevliet (6) by counting the uredia each day on a leaf section 8 cm^2 until no more uredia developed. The time between inoculation and 50% of uredia visible was calculated for latent period (LP_{50}). Pustule density was determined by dividing the total number of sporulating colonies on 8 cm^2 by the number of urediospores applied. Four replicates consisting of six leaves per cultivar were used for LP_{50} and ID. The experiment was designed as a randomized block with four replicates for all factors except colony and uredial size, which had only three replicates.

Two sections (1×3 cm) from the central portion of two primary leaves from two plants were collected daily until day 8 (approximate time of sporulation), then on alternate days to day 14 after inoculation. The leaf segments were prepared as whole mounts for fluorescent microscopy using a modified method of Rohringer et al (11). A 1% Uvitex (BOPT) solution (Ciba-Geigy Ltd., Manchester, UK) was used in place of Calcofluor. Samples

remained in Uvitex 3 hr. Fluorescent microscopy was used to determine colony size, early and late abortion, proportion of successful penetrations, uredial size, and necrosis of Red Kloud.

Ten penetrated stomata were used as a sample for statistical analyses. After day 2, the experimental unit consisted of 10 colonies that had penetrated the stoma but had not aborted. The length and width of the sporulating area and mycelial growth were measured each sampling day. The rate of increase in colony size in the five bean cultivars was analyzed by the Gompertz model (3). Gompertz best linearized the curve and showed a distinction between partially resistant cultivars not always detected by the logistic model. Comparisons among cultivars were made by Duncan's multiple range test. Early-aborted colonies were determined from 3 to 14 days postinoculation. Aborted colonies were slightly larger than those described by Niks (4) for *P. hordei* as colonies containing fewer than six haustorial mother cells with little or no branching. Late-aborted colonies were recorded as those that had not sporulated by day 14, the last observation date.

RESULTS

Uredia were first observed on UI 114, Fiesta, and Pindak at 7 days but not on Nodak or Upland until day 8. Only one uredium in one replicate was observed on day 7 for UI 114, Fiesta, and Pindak.

The LP_{50}s of the five bean cultivars inoculated with *U. appendiculatus* were not significantly different (Table 1). The ID was significantly lower ($P = 0.05$) for Nodak and Upland than for the *U. appendiculatus*-susceptible cultivar UI 114 (Table 1). The ID of Nodak was significantly lower than that of Fiesta. The average ID for Pindak was about midway between and not significantly different from any cultivar. The average number of uredia per square centimeter for UI 114 was significantly greater than for Upland and Nodak (Table 1). The average number of uredia per square centimeter for Fiesta was significantly greater than that for Nodak. The average number of uredia per square centimeter on Pindak was about midway between and not significantly different from any of the other cultivars.

The average number of early-aborted colonies for Nodak and Upland was significantly higher ($P = 0.05$) than for UI 114, Fiesta, and Pindak at 14 days (Table 2). A small percentage of early-aborted colonies was associated with limited necrosis. The percentage of early-aborted colonies with necrosis did not differ significantly among cultivars. All of the colonies observed for the cultivar Red Kloud were associated with bright fluorescence of cell walls and surrounding tissues, which indicated necrosis was associated with hypersensitive resistance. Bright fluorescence was not observed for the susceptible or partially resistant cultivars. There was a wide variation in the size of the necrotic area for Red Kloud, ranging from about 0.0020 to about 0.4225 mm^2 at day 12. The average number of late-aborted colonies was significantly higher ($P = 0.05$) for Nodak than for the cultivars UI 114, Fiesta, Pindak, and Upland. The average number of late-aborted colonies for Upland was significantly higher than for UI 114 at $P = 0.10$.

The proportion of successful penetration units, described as the number of colonies with uredia divided by the total number of colonies on the last observation date (day 14), was significantly lower for Nodak and Upland ($P = 0.05$) than for UI 114, Fiesta, or Pindak.

The average size of the sporulating area of Fiesta was significantly larger ($P = 0.05$) than for the other cultivars at day 14 (Table 1). The sizes of the sporulating areas of UI 114 and Upland were significantly greater than for Nodak and Pindak. When the sizes of sporulating area were averaged for days 8–14, those on Fiesta and UI 114 were significantly larger than those on Pindak and Nodak ($P = 0.05$). Size of sporulating area on Upland was significantly greater than that on Nodak. Although the uredial size increased between days 8 and 14, only day 8 was significantly lower than the other dates ($P = 0.05$). The only exception was for the cultivar Fiesta, where the uredial size at day 10 was also significantly lower than at day 12.

The average size of the colony mycelial area increased with time (Fig. 1). The largest increases occurred just before sporulation. Differences in colony size between cultivars were not significant until after day 8, when Nodak was significantly smaller ($P = 0.05$) than the susceptible cultivars. The slope of the regression line for Nodak was significantly different from that for the cultivars Pindak, Fiesta, and UI 114 ($P > 0.01$) (Fig. 2).

TABLE 1. Latent period, infection density, number of uredia per square centimeter, and uredial size of five bean cultivars inoculated with *Uromyces appendiculatus*

Cultivar	Latent period[w] (days)	Infection density[x] (%)	No. uredia/cm^2	Uredial size (mm^2 at day 14)[y]
UI 114	8.7 a[z]	8.1 a	11.6 a	0.25 b
Fiesta	7.9 a	7.9 ab	10.4 ab	0.33 a
Nodak	8.4 a	2.5 c	2.8 c	0.10 d
Pindak	8.3 a	5.7 abc	8.0 abc	0.15 cd
Upland	8.0 a	3.8 bc	5.1 bc	0.22 b

[w] Time in days between inoculation and 50% of visible uredia.
[x] (Number of colonies/cm^2)/(number of spores applied/cm^2).
[y] Average size of sporulating area of uredia at day 14.
[z] Values with the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

TABLE 2. Early abortion, late abortion, and proportion of successful units in bean cultivars 14 days after inoculation with *Uromyces appendiculatus*

Cultivar	Early abortion[w] (%)	Early abortion with necrosis (%)	Late abortion[x] (%)	Proportion successful units[y]
UI 114	16.0 a[z]	0.86 a	2.5 a	81.5 a
Fiesta	17.0 a	2.00 a	7.5 a	75.8 a
Nodak	30.5 b	2.16 a	27.5 b	41.7 b
Pindak	20.0 a	2.12 a	7.5 a	72.5 a
Upland	34.7 b	2.64 a	17.5 a	47.8 b

[w] Number of colonies with little growth/total number of infection units (av. for days 3–14).
[x] Number of colonies with no sporulation on day 14/total number of colonies.
[y] Number of colonies with uredia/total number of colonies on day 14.
[z] Values with the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

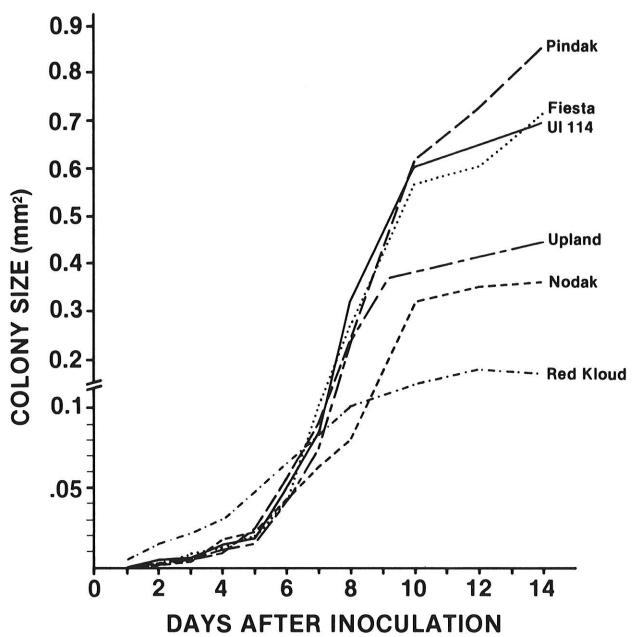

Fig. 1. Average colony area of *Uromyces appendiculatus* uredia for Pindak, Fiesta, UI 114, Upland, and Nodak and size of necrotic area of Red Kloud.

DISCUSSION

Latent period has been associated with slow leaf rusting of wheat to *P. recondita* and with partial resistance of barley to *P. hordei* (9). However, the LP_{50}s of the five bean cultivars inoculated with *U. appendiculatus* did not differ significantly in this study. The ID was significantly lower for Nodak and Upland than for UI 114. Parlevliet and Kuiper (10) found a high correlation between ID and partial resistance of barley to *P. hordei*. ID also appears to be correlated to partial resistance in Nodak and Upland. The average number of uredia per square centimeter was significantly lower for Nodak and Upland than for UI 114 and Fiesta. This information is correlated to ID because the average number of uredia per square centimeter was used to determine ID. The number of uredia per square centimeter has been associated with slow leaf rusting in wheat by Ohm and Shaner (5).

The percentage of early- and late-aborted colonies was much higher for Nodak and Upland than for the other cultivars. This probably explains why the ID of Nodak and Upland was lower than for the other cultivars studied. The higher percentage of aborted colonies was directly related to the lower proportion of successful penetrations for Nodak and Upland. Niks (4) found early abortion to be the most important component of low infectibility in barley to *P. hordei*.

Studies by Statler and Parlevliet (12) agreed with Niks (4) that early abortion was an important component of partial resistance. We (12) also found the percentage of late-aborted colonies to be significantly greater in one barley cultivar with partial resistance to *P. hordei* than in a susceptible control. It could be argued that late-aborted colonies may later develop uredia; however, we found no increase in the number of *U. appendiculatus* uredia after 14 days.

Bright fluorescence was always associated with colonies in Red Kloud. The susceptible and partially resistant cultivars did not have bright fluorescence, but a small percentage of the early-aborted colonies were associated with limited fluorescence. Limited necrosis associated with early-aborted colonies could be due to staining techniques, culture impurity, or heterogeneity and crossing over between nuclei, but the latter event is rare in rust organisms. Culture impurity is a possibility, but when culture 15C was single-spored and tested on differentials, off-type pustules were not observed on the differentials or on cultivars used in the study. The percentage of early-aborted colonies with necrosis was also not different between cultivars. Staining techniques are a possibility because the necrosis (fluorescence) observed was associated only with the colony of a few early-aborted colonies, whereas the necrosis observed in the resistant cultivar was associated with larger areas including cell walls and surrounding tissue. Niks (4) also reported a small percentage of colonies associated with necrosis for both *P. hordei* and *P. recondita*.

The average colony size increased with time. The largest increases were just before sporulation (Fig. 1). Colony sizes per unit time on different cultivars were not significantly different until after day 7, when the colony area for Nodak was significantly smaller than those of the other cultivars.

The susceptible cultivar Fiesta had the largest sporulating area followed by UI 114 and Upland at 14 days. Nodak and Pindak had much smaller sporulating areas than the other cultivars. Smaller uredia have been associated with slow rusting of wheat to *P. recondita* (5). Smaller uredia obviously produce less inoculum.

This study has documented partial resistance for Upland and Nodak with race 15C of *U. appendiculatus*. These cultivars had lower IDs and fewer uredia per square centimeter than the susceptible cultivars Fiesta and UI 114. Nodak and Upland had a higher percentage of both early- and late-aborted colonies than the susceptible cultivars, resulting in a lower proportion of successful penetrations at 14 days. Nodak had much smaller uredia than the susceptible cultivars. The only component of partial resistance demonstrated for Pindak was a smaller average uredial area than the susceptible cultivars.

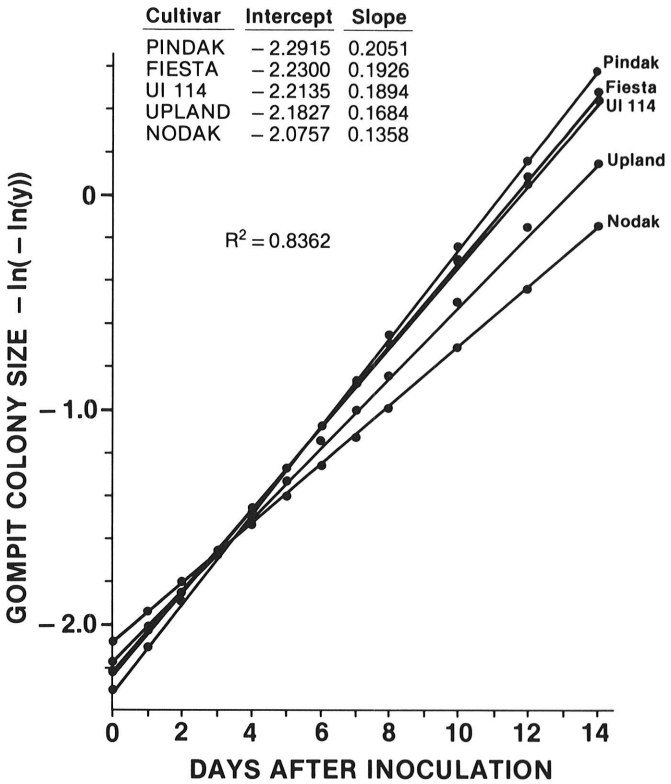

Fig. 2. Colony size of *Uromyces appendiculatus* after transforming to gompit values (Gompertz model) in five bean cultivars during a 14-day period after inoculation.

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