Three Components of Slow Leaf-Rusting at Different Growth Stages in Wheat

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ABSTRACT

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Latent period, pustule size, and number of pustules per square centimeter of leaf area of two slow-rusting [Suwon 85 and Purdue 6028A2-9-6-1, (P6028)] and two fast-rusting (Monon and Suwon 92) cultivars were compared in greenhouse and field inoculations with *Puccinia recondita* f. sp. *tritici*. The appearance of pustules was protracted on Suwon 85 and P6028 compared to Suwon 92 and Monon. Thus, the latent period for Suwon 85 and P6028 was longer by a factor of 1.2 to 1.8 compared to that of Suwon 92 and Monon. Pustule size on the two slow-rusting cultivars was four- to six-tenths of that on the two fast-rusting cultivars. Pustule size was largest on the flag leaf compared to that on the first and second leaves below the flag leaf for all cultivars. Latent period was longest and pustule size was most restricted on plants of Suwon 85 and P6028 which were inoculated in the boot stage compared to when plants were inoculated at six other plant growth stages. Suwon 85 consistently developed fewer pustules per square centimeter of leaf area than Suwon 92, Monon, or P6028 in greenhouse experiments. Leaf rust severity was less and pustule size was smaller in the field for the two slow-rusting cultivars than for Suwon 92 and Monon.

Additional key words: general resistance, horizontal resistance, nonspecific resistance, epidemiology.

Wheat researchers at Purdue University have observed for several years that the rate of leaf rust (*Puccinia recondita* Rob. ex Desm f. sp. *tritici*) development on wheat (*Triticum aestivum* L. emend. Thell.) under field conditions is slower on some cultivars than on others. Moreover, these slow-rusting cultivars appeared to retain their slow-rusting character even with shifts in races of the pathogen over a period of years. The cultivars Knox (C.I. 12798) and Monon (C.I. 13278) were developed with the mature-plant, polygenic, hypersensitive type of leaf rust resistance (1) from 'Chinese' (C.I. 6223). Eventually races of the pathogen emerged that were virulent on these two cultivars. In the field, however, leaf rust consistently has developed at a slower rate on Knox than on Monon. Stem rust of wheat (15) and crown rust of oats also have been shown to develop more slowly in the field on some cultivars than on others.

Resistance of a nonhypersensitive type could interfere with disease development (1) at any of several stages of pathogenesis and thus result in slow-rusting. Exclusion of the fungus, longer latent period, and restriction of pustule size (5, 7, 10, 13, 16) all have been shown to operate to one degree or another in oats (*Avena sativa* L.), barley (*Hordeum vulgare* L. emend. Lam.), and wheat toward their respective rust pathogens. Although slow-rusting has been recognized in wheat against *P. recondita* (1, 3, 13) in the field, the stages at which pathogenesis is retarded by this resistance have not been identified, except that some slow-rusting wheats have been shown to interfere with penetration by the fungus (3).

Identification of stages of pathogenesis at which slow-rusting is expressed will aid plant breeders in combining slow-rusting genotypes to increase the level of this resistance and to aid them in developing reliable methods of selection in segregating populations.

This study was initiated to determine at which stages of pathogenesis slow leaf-rusting is expressed in two genetically unrelated slow-rusting winter wheat cultivars, and to determine the effect of stage of plant growth on the pathogenesis of slow leaf-rusting.

MATERIALS AND METHODS

Greenhouse experiments.—Winter wheat cultivars Suwon 85 (P.I. 157600), Suwon 92 (C.I. 12666), Monon, and breeding line Purdue 6028A2-9-6-1 (P6028), a Knox type improved for Hessian fly resistance, were compared for components of slow leaf-rusting. The four cultivars were vernalized for 70 days at 2.5 C and then grown in three greenhouse experiments conducted, respectively, during January-April 1974, September-December 1974, and January-April 1975. Plants were inoculated with an aqueous (surfactant added) spore suspension of a single-spore culture of race 76 of *P. recondita,* at 0.2135 mg of spores per plant. The inoculum was stored in a nitrogen freezer (−96 C) and then heat-shocked for 5 minutes at 43 C prior to use.

Because Monon and Knox carry the hypersensitive-type resistance from Chinese to some races of *P. recondita,* we used a single culture fully virulent to the Chinese resistance. The spray pattern was such as to distribute spores uniformly over the foliage. Plants were well watered before inoculation; water was sprayed on the leaves with an atomizer after inoculation and a plastic enclosure was placed over the plants for 16 hours. Plants of each cultivar were included in each inoculation to distribute randomly among cultivars variations due to inoculations within a given greenhouse experiment.

Latent period, pustule size, and pustule density were measured on each of the three uppermost leaves in the first experiment, January-April 1974, and on the two uppermost leaves in the following two experiments, September-December 1974, and January-April 1975. All plants in the first two experiments were inoculated in growth stages 47-56, as rated by the modified Feekes scale (17). In the third experiment, 12 plants of each cultivar were inoculated in each of seven different growth stages: 13, 22, 30-39, 47-56, 60-67, 68-72, and 73-77 (total of 84 plants for each cultivar).

Temperatures for the September-December experiment were 19-23 C. Nearly all days after inoculation were overcast with a low natural light intensity. Fluorescent lighting was supplied at 2×10^4 ergs $cm^{-1}sec^{-1}$ in a photoperiod of 16 hours light and 8 hours dark.

Temperatures for the January-April experiments were 24-27 C during the day on bright days and 19-23 C at night and on overcast days. Most days after inoculation were bright but again supplemental light was used to provide a 16-hour light period.

To reflect the average time required for appearance of pustules, we estimated the percentage of visible infection sites (flecks) that developed into pustules each day after inoculation. This was continued until all pustules had erupted. The resulting sum was the latent period. This method gave more consistent plant-to-plant measurements for latent period for F_1 plants and for the slow-rusting parent (9).

The average maximum pustule size on the center two-thirds of the leaf was estimated 18-19 days after inoculation by placing a scale (Fig. 1) next to the leaf. Infection sites at the tip and basal one-sixth of the leaves tended to erupt earlier and these pustules tended to be larger on some plants.

Pustule density (pustules per square centimeter of leaf area) was estimated for basal, mid, and tip portions of each leaf by placing a scale similar to that described by Peterson et al. (12) next to the leaf. The average of the three determinations was recorded.

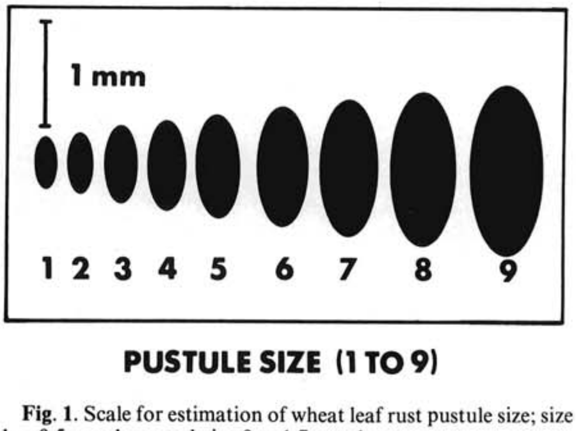

Fig. 1. Scale for estimation of wheat leaf rust pustule size; size 1 = 0.5 mm long and size 9 = 1.7 mm long.

Field experiment.—Plots that consisted of three rows 1 m long and 30 cm apart for each of the four cultivars were randomized six times throughout the slow-rusting breeding nursery at Lafayette. An aqueous (with a surfactant) spore suspension of *P. recondita,* race 76, was applied to foliage at 2000 hours on 5 May and again on 13 May 1975. Heading dates for Suwon 92, Suwon 85, Monon, and P6028 were 22, 19, 22, and 21 May, respectively.

Disease severity, using a modified Cobb scale (12) was determined on 4 June and 18 June. Pustule size on flag leaves was measured on 18 June as described above.

RESULTS

Latent period.—On fast-rusting Monon and Suwon 92 in the greenhouse, 50-80% of visible infection sites developed into pustules on the same day 7 or 8 days after inoculation and the remainder of the pustules appeared within only 1 or 2 more days. In contrast, pustules began to appear 9-10 days after inoculation for slow-rusting Suwon 85 and P6028 and, only 1-10% of all infection sites developed into pustules at that time.

Correlation values between latent periods for the flag leaf and those for the penultimate leaves ranged from 0.81 to 0.91. Means for latent period (Table 1) thus were averaged for the two leaves. Latent periods for Suwon 85 and P6028 were approximately 1.2 to 1.8 times as long as those for Monon and Suwon 92 (Table 1). Cooler temperatures and lower light intensity during the second greenhouse experiment (September-December 1974)

TABLE 1. Three stages of leaf rust development on slow- (Suwon 85 and P6028) and fast- (Suwon 92 and Monon) rusting winter wheat cultivars

Experiment, dates, and cultivars	Latent period (days)	Pustule size (1-9)[a]	Pustules (no./cm^2)
Exp. 1: January-April 1974			
Monon	8.12	5.90	18.5
Suwon 92	8.53	6.58	15.8
Suwon 85	12.77	3.70	14.0
P6028	11.57	3.25	17.0
LSD (0.05)	.52	.51	2.8
Exp. 2: September-December 1974			
Monon	8.63	5.32	22.5
Suwon 92	9.34	5.78	20.5
Suwon 85	15.46	2.44	16.2
P6028	13.79	2.87	19.0
LSD (0.05)	.55	.46	3.8
Exp. 3: January-April 1975			
Monon	8.26	5.61	17.8
Suwon 92	8.65	5.86	18.8
Suwon 85	11.76	3.28	12.5
P6028	10.39	3.54	17.0
LSD (0.05)	.84	.75	3.0

[a]Pustule size 1 = .5 mm long to size 9 = 1.7 mm long (see Fig. 1).

may have lengthened the latent period for Suwon 85 and P6028.

Pustule size.—Pustules on Suwon 85 and P6028 were only four-tenths to six-tenths the size of those on Suwon 92 and Monon (Table 1). Slight necrosis sometimes was associated with pustules on P6028. In the second greenhouse experiment, pustule size was reduced on all cultivars, but to a greater extent on Suwon 85 and P6028 than on Suwon 92 and Monon, presumably owing to the cooler temperatures and lower light intensity (Table 1). Pustules were largest on the flag leaves (Fig. 2), probably because these were the youngest leaves and thus did not begin to senesce as soon after inoculation as the other two leaves. Cultivars ranked similarly for pustule size on each of the three leaves; however, cultivar differences were greatest on the flag leaf. Pustule size for Suwon 92 and Monon on the second leaf below the flag leaf may have been limited due to leaf senescence.

Pustule density.—Suwon 85 had significantly fewer pustules per square centimeter of leaf area after uniform inoculation than the fast-rusting cultivars, Suwon 92 and Monon, or the other slow-rusting cultivar, P6028.

Plant growth stage.—Growth stage (GS) affects expression of the components of slow-rusting (Fig. 3). Latent period was longest on plants inoculated at growth stages (GS) between GS 39, flag leaf just emerged, and GS 60, flowering (Fig. 3-A). Pustule size was limited most on Suwon 85 and P6028 when plants were inoculated between flag leaf emergence (GS 39) and flowering (GS 60) (Fig. 3-B). Pustule size was limited least on the two slow-rusting cultivars when inoculated just after flowering (GS 68).

Pustule size on the fast-rusting cultivars was greatest when plants were inoculated between the time the flag leaf emerged and flowering. Senescence of the seedling leaves and senescence near maturity may limit pustule development on the fast-rusters. In this respect, data in Fig. 3-B support data in Fig. 2.

Although plant growth stage showed a significant effect on latent period and pustule size, particularly for Suwon 85 and P6028, the latent period clearly was longer and the pustule size definitely was limited for the slow-rusting cultivars at all plant growth stages.

Suwon 85 developed the fewest pustules per square centimeter of leaf area regardless of the growth stage at which plants were inoculated (Fig. 3-C). However, pustule density was greater on plants inoculated in the seedling stage compared to those inoculated in other stages.

Field experiment.—The two slow-rusting cultivars showed a relatively low severity of infection on 4 June

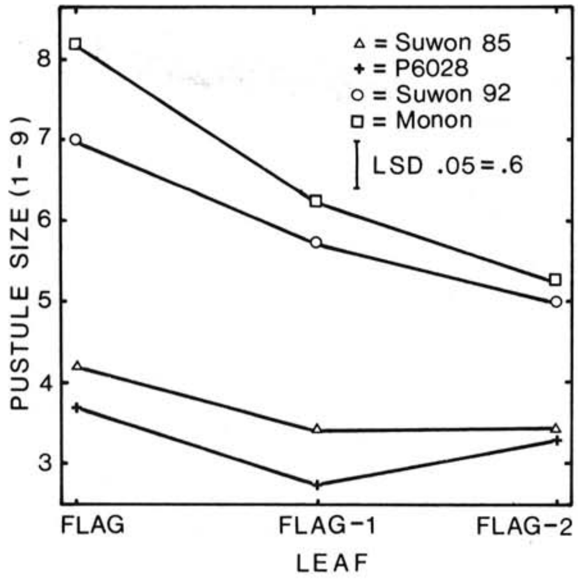

Fig. 2. Size of wheat leaf rust pustules at 18 days after inoculation of plants at the boot stage on each of three leaves for four greenhouse-grown winter wheat cultivars.

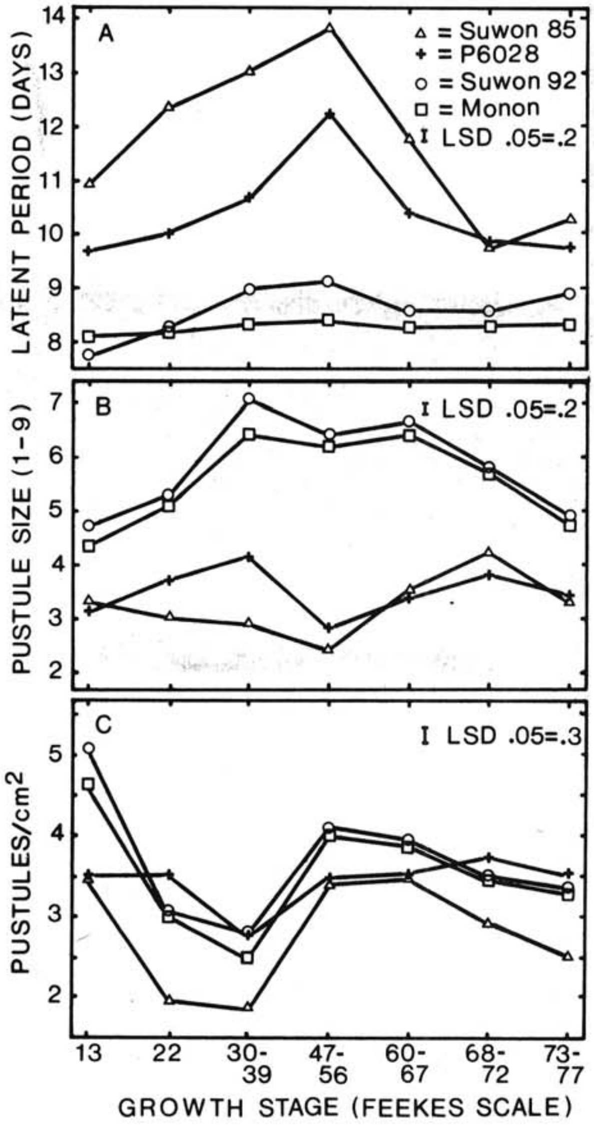

Fig. 3. Effects of stage of plant growth on three components of slow leaf-rusting for four greenhouse-grown winter wheat cultivars. Growth stages are recorded using a modification of the Feekes scale (see reference 17).

(Table 2), although it was not statistically less than Monon. Fourteen days later, however, severity of rust infection was less for Suwon 85 and P6028 than for Suwon 92 and Monon. Pustule size for Suwon 85 and P6028 also was limited compared to Suwon 92 and Monon. Our data suggest that pustules in the field experiment were smaller than those in the greenhouse experiments. However, in the greenhouse experiments, pustules were measured after most had reached maximum size. In the field experiment many pustules were not fully developed when measured.

TABLE 2. Leaf rust severity and pustule size on slow- and fast-rusting winter wheat cultivars in the field in 1975

Cultivar	Severity (%)[a] 4 June	Severity (%)[a] 18 June	Pustule size (1-9)[b]
Monon	9.0	71.7	3.0
Suwon 92	33.5	93.3	3.5
Suwon 85	1.8	50.9	1.3
P6028	1.7	27.5	1.3
LSD (0.05)	10.9	13.5	1.0

[a]Modified Cobb scale.
[b]Pustule size 1 = .5 mm long to size 9 = 1.7 mm long (see Fig. 1).

DISCUSSION

We identified three stages of pathogenesis (latent period, pustule size, and number of pustules per square centimeter of leaf area) which are altered by the slow leaf-rusting character in wheat. The effects of the slow-rusting character on these stages of pathogenesis may be considered as components of slow-rusting (longer latent period, restricted pustule size, fewer pustules per square centimeter of leaf area). Expression of these components was measurable by techniques adaptable for use on a large scale in the field, namely, the application of a suspension of spores onto the foliage as uniformly as possible. On the other hand, this technique probably is less useful for measuring small differences among cultivars for pustule density.

Basic to the potential value of slow-rusting as a form of resistance is the supposition that it is effective against all races of the pathogen. We have used a single culture virulent to the hypersensitive resistance in Suwon 85 and P6028 to demonstrate the existance of slow-rusting in these cultivars. Although our data do not prove that this resistance is general, the Knox resistance in P6028 has been exposed to many races of *P. recondita* over a period of several decades and on several million hectares. Testing to this extent provides evidence that this slow-rusting is a general resistance (2).

Knox resulted from a cross of a Vigo sib × Purdue 40110A1, a line with the Chinese resistance to *P. recondita*. Vigo, which is C. I. 12220, is a slow-ruster (3, 13). Monon derived the Chinese resistance from a Knox sib. The Chinese resistance has succumbed to new races (1); still, Knox continues to show a lower severity of leaf rust in the field than Monon. Monon apparently did not inherit slow-rusting resistance from Vigo via the Knox sib.

We are currently exposing P6028 and Suwon 85 to many races of *P. recondita* to determine if slow-rusting is indeed race nonspecific. Slow-rusting resistance in oats against *Puccinia graminis* f. sp. *avenae* and against *Puccinia coronata* f. sp. *avenae* and in wheat against *P. graminis* f. sp. *tritici* has been shown to be equally effective against several races (7, 14, 15).

The two slow-rusting cultivars, Suwon 85 and P6028, exhibited a long latent period and restricted pustule size, and Suwon 85 developed fewer pustules per square centimeter than the two fast-rusters. Restricted pustule size is a common component in other cases of slow-rusting in cereals and often is associated with a longer latent period and fewer pustules per square centimeter (4, 5, 6, 7, 8). Parlevliet and Van Ommeren (11) point out that selection for slow-rusting (they refer to slow-rusting as partial resistance) tends to select cultivars improved for several or even all the components. Studies with these types of cultivars would result in inflated correlations between components. Correlations between latent period and pustule size for F_2 populations (9) suggest linkage between or possible pleiotropic effects of genetic factors controlling these components.

Our data indicate that effectiveness of slow-rusting is influenced by environmental conditions. Also, on oats, the time required for development of pustules of *Puccinia graminis avenae* was less and pustule size was greater at 30-35 C than at 20-25 C (7). In the same report, the development of *P. coronata avenae* was relatively insensitive to change in temperature. However, in another study (5), pustule size of *P. coronata avenae* was larger at 21-26 C than at 10-15 C. This suggests that slow-rusting may be less effective against pathogenesis in some years at a location or at some locations than others.

Leaf senescence may contribute to the shorter latent period on the slow-rusters inoculated in seedling stages (before flag leaf emergence) and after flowering, compared to those inoculated in the boot stage. Seedling leaves begin to senesce sooner after they emerge than do the uppermost two leaves. The flag leaf does not begin to senesce until well after flowering. The reduced expression of slow-rusting at some plant growth stages is important in effectively identifying slow-rusting types in the breeding nursery. Although our data and those of other studies (4, 10) suggest that effectiveness of slow-rusting components is reduced in the seedling stages, rust development is still clearly impaired. Leaf rust infection in the seedling stages rarely occurs in Indiana. Our data suggest that slow-rusting resistance also is reduced after flowering. Vigo and other slow-rusting cultivars were reported to be more susceptible late in the season after senescence (3). In some years at Lafayette, leaf rust infection occurs late in the season, after heading. This was the case in 1974, and differences in leaf rust severity and pustule size were less distinct between fast- and slow-rusting cultivars than in 1975. Even though slow-rusting resistance is reduced after senescence begins, it may provide adequate protection to the crop before and during flowering to prevent significant yield loss from leaf rust.

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