Resistance

Two Components of Slow-Rusting in Asparagus Infected with *Puccinia asparagi*

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ABSTRACT

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Slow-rusting resistance in the field and latent period and number of uredinia per square centimeter of stem surface in the greenhouse were evaluated on several asparagus lines. In two years of observation in the field, rust development (as measured by the area under the disease progress curve) was less on lines 277E × 22-8, Jersey Centennial, Delmonte 361, 56 × 22-8, and UC 157 than on Mary Washington, Wash T2, and WSU-1. Lines with less rust in the field had longer latent periods and fewer uredinia per square centimeter of stem when inoculated uniformly with urediniospores of *Puccinia asparagi* in the greenhouse than lines that had more rust in the field. The area under the disease progress curve was negatively correlated with length of latent period (correlation coefficients ranged from −0.60 to −0.91) and positively correlated with number of uredinia per square centimeter of stem (correlation coefficients ranged from 0.71 to 0.92). Asparagus shoots of both slow- and fast-rusting cultivars became more resistant to rust, as indicated by a longer latent period and fewer uredinia per square centimeter of stem, as they matured.

Additional key words: epidemiology.

Puccinia asparagi DC. is an autoecious, macrocyclic rust that occurs sporadically in south central Washington where more than 12,000 ha of asparagus (*Asparagus officinalis* L.) are grown. Epidemics occur after spears are harvested in the spring and are associated with rain or frequent dews (8). When rust is severe, asparagus foliage ripens prematurely and carbohydrate storage in the crown is reduced, resulting in less yield the following spring (8).

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Efforts by several researchers have been directed towards identifying rust resistance in asparagus (2,6,8,19). However, the only type of resistance found in the genus *Asparagus* has been quantitative differences in the intensity of infection rather than qualitative differences (1,6,8,19). Even though uredinia on plants indicate susceptibility (1), some selections and the cultivar Jersey Centennial have recently been reported to have high levels of resistance as measured by reduced rust development (2).

Small grains infected with rust have also shown quantitative differences in rust development (7,9,18,20). This resistance has been known for many years (16) and is termed "slow-rusting" (18). Several components, including lower infection frequency, longer latent period, lower sporulation rate, and smaller pustules have

been found to be involved in the slow development of rust in small grains (5,7,11,15,18). Similar components of slow development of disease have been demonstrated in other host-parasite relationships (3,4,13,14,17).

In this study, I evaluated lines of asparagus for slow-rusting resistance in the field in Washington by using the area under the disease progress curve, and quantified latent period and infection frequency in these entries.

MATERIALS AND METHODS

Eight asparagus lines were established in the field from seedlings transplanted 12 April 1983 to a fine sandy loam soil at the Irrigated Agriculture Research and Extension Center (IAREC) near Prosser, WA, in three-row plots of 10–11 plants per row in a randomized complete block design with six replicates. The planting plan was a 2×24 plot arrangement. Transplants were spaced 26–30 cm apart in furrows 15–17 cm deep. Rows were spaced 1 m apart. Entries 277E×22-8, 56×22-8, and Jersey Centennial from Rutgers University, New Brunswick, NJ, were chosen because of reported rust resistance (2; J. H. Ellison, *personal communication*). Delmonte 361, Mary Washington, Wash T2, and WSU-1 from the Washington Asparagus Growers Association, Sunnyside, WA, were chosen because they are presently grown in Washington, and UC 157 from the University of California, Davis, was chosen because it rusted less severely than several cultivars grown in Washington in a replicated trial at IAREC in 1981 during a severe epidemic.

Sprinkler irrigation was used to irrigate plants and to encourage rust development. A border row of each plot was inoculated in May 1983 with approximately 5×10^5 urediniospores of *P. asparagi* per milliliter of oil (Soltrol® 170). The urediniospore suspension was sprayed with a 3-L hand pump garden sprayer until a thin film of oil was just visible on plants. Urediniospores were collected in bulk from asparagus plants in a field near Prosser, WA, in 1982 and increased on WSU-1 in the greenhouse.

Aecia developed naturally in plots in the spring of 1984. In May, the number of aecia per stem and the percentage of stems with aecia were determined in the center row of plots.

Rust severity was estimated as the proportion of surface area of the host covered with uredinia in the center row of each plot by using the modified Cobb's scale for cereal rust (12). Rust severity was estimated twice at a 14-day interval beginning at the end of August 1983 and six times at 10- to 14-day intervals over a 64-day period beginning in mid-June 1984. Rust development in 1984 originated from naturally occurring inoculum. The area under the disease progress curve (AUDPC) was calculated for each plot to indicate the slow-rusting character of the lines.

To compare latent period and infection frequency among asparagus lines, tests were conducted in the greenhouse. Asparagus seeds were germinated in petri dishes at 25 C and planted in a silt loam soil in 8-cm-diameter pots in the greenhouse. Plants were fertilized with a 16-16-16 (N-P-K) fertilizer (0.3 g per pot) at 3-wk intervals and natural light supplemented with fluorescent lamps was used to provide a photoperiod of 15 hr/day. Experiments in the greenhouse were done between January and March, 1983 and 1984, when temperature was relatively easy to control. Temperature ranged from 21 to 24 C during the day and 16 to 20 C at night.

Potted plants of seven or eight lines in randomized complete block designs in three experiments were inoculated as they rotated on a platform by spraying them as uniformly as possible with an atomized oil suspension of urediniospores. Urediniospores were from the same source as those used in the field study. The inoculum concentration was approximately 3×10^6 urediniospores per milliliter of oil in the first experiment and 1.6×10^6 urediniospores per milliliter of oil in the second and third experiments. Inoculum was applied so that a thin film of oil was not visible on plants after inoculation. Plants were placed in a mist chamber for 24 hr and then returned to the greenhouse.

In the first experiment, all plants were inoculated when cladophylls of the first shoot had just fully developed (6). There were ten replications with one plant per pot and three or six subsamples (pots) of each entry per replication. In the second and third experiments, three sets of replications were inoculated at different times so that plants were at a similar growth stage when inoculated. In the second experiment, the second shoot was removed and the first and third shoots to emerge were inoculated except in the case of line 56×22-8. Since this line developed more slowly than the other entries, the first and either the second or third shoots were inoculated, depending on growth. With all plants, the youngest shoot was at the 50–75% cladophyll stage of development (6) at the time of inoculation. There were 19 replications and one or two subsamples of each entry per replication. In the third experiment, the first, second and third shoots were inoculated when the third shoot had almost completely elongated with 0–10% of its cladophylls developed and the second shoot had reached the 100% caldophyll stage. There were ten replications.

Uredinia from initial points of infection (8) were counted on primary shoots of plants every day after inoculation for 10–11 days and then on alternate days up to 17 days after inoculation. Latent period was calculated by using regression analysis of probit percent of uredinia erupted on days after infection as described by Shaner (15). The number of uredinia per square centimeter of shoot surface was determined from the total number of uredinia on a primary shoot divided by the area of the primary shoot. Area was calculated from length and diameter measurements at 5 cm above the base of the shoot, using the formula for the area of the side of a cylinder ($2 \times 3.1416 \times$ radius $\times$ length). A mean value for latent period and uredinia per square centimeter was calculated and used in the analysis of variance when more than one sample per line was in a replication.

RESULTS

In 1983, severity of uredinia in plots was low due to few initial infections after inoculation in the border rows and dry weather during the summer. The highest severity observed in the center row of a plot was 20%. In 1984, rust was severe with the highest severity reaching 80%. Differences ($P = 0.01$) in severity of uredinial infection were found among the asparagus lines in 1983 and 1984 (Table 1). In both years, rust development (as determined by AUDPC) was less on lines 277E × 22-8, Jersey Centennial, Delmonte 361, 56×22-8, and UC 157 than on Mary Washington, Wash T2, and WSU-1 (Table 1).

Numerous aecia developed on asparagus in plots in 1984 (Table 1) and differences among lines in percentage of stems with aecia and aecia per stem were highly significant (Table 1). Line 277E×22-8 and Jersey Centennial, which were slow-rusting, had fewer aecia than Wash T2, and WSU-1, which were fast-rusting. The slow-rusting line, 56 × 22-8, did not differ significantly from the fast-

TABLE 1. The area under the disease progress curve (AUDPC) of asparagus lines and cultivars infected with *Puccinia asparagi* in the field in 1983 and 1984 and the percentage of asparagus stems with aecia and the mean number of aecia per stem in May 1984[x]

Cultivar or line	Uredinia-AUDPC[y]		Aecia-1984[z]p	
	1983	1984	Infected stems (%)	Aecia stem
277E × 22-8	0.0 a	29 a	36 a	2 a
Jersey Centennial	0.0 a	84 a	30 a	2 a
Delmonte 361	0.1 a	184 a	40 a	6 ab
56 × 22-8	0.0 a	319 ab	65 bc	5 ab
UC 157	1.0 a	429 b	66 bc	9 b
Mary Washington	6.0 ab	1,112 c	62 b	8 ab
Wash T2	10.0 bc	1,480 d	76 c	21 c
WSU 1	16.0 c	1,699 d	77 c	22 c

[x]Within a column, values with the same letter are not significantly different at $P = 0.05$ according to Fisher's Protected LSD.

[y]Values are means of five replicates in 1983 and six replicates in 1984. Time period over which AUDPC was calculated was 14 days in 1983 and 64 days in 1984.

[z]Values are means of six replicates.

rusting cultivars in percentage of stems infected with aecia and did not differ in number of aecia per stem from Mary Washington, but did differ in number of aecia per stem ($P = 0.05$) from Wash T2 and WSU-1 (Table 1).

In the greenhouse there were significant differences ($P = 0.01$) among the asparagus lines for both latent period and uredinia per square centimeter in the three experiments. When tested with single degree of freedom contrasts, the shoots shown in Table 2 of lines that rusted slowly in the field (277E × 22-8, Jersey Centennial, Delmonte 361, 56 × 22-8, and UC 157) had significantly longer latent periods and fewer uredinia per square centimeter of stem than did the fast-rusting lines. However, latent period and number of uredinia of some individual slow-rusting lines did not differ significantly from those of lines that rusted rapidly (Table 2). Latent period and number of uredinia of the slow-rusting lines for the first shoot in experiment II and latent period for the first and third shoots in experiment III (not shown in Table 2) were not significantly different ($P = 0.05$) from shoots of the fast-rusting cultivars, even though these shoots of the slow-rusting entries had larger values for latent period and smaller values for uredinia/per square centimeter than those of the fast-rusting lines.

Latent period and number of uredinia were influenced by age of the shoot. The youngest shoots (third shoots) had significantly shorter latent periods and more uredinia per square centimeter ($P = 0.01$) than the first shoots in the second experiment and the second and first shoots in the third experiment. Latent period and number of uredinia did not differ significantly ($P = 0.05$) between the second and first shoots in the third experiment. Mean values for latent period were 9.3 and 10.6 days for the third and first shoots, respectively, in experiment II, and 8.4, 9.7, and 9.9 days for the third, second, and first shoots, respectively, in experiment III. Means were 1.2 and 0.4 uredinia per square centimeter for the third and first shoots, respectively, in experiment II and 1.0, 0.3, and 0.4 for the third, second, and first shoots, respectively, for experiment III.

AUDPC in 1984 was negatively correlated with length of latent period and positively correlated with number of uredinia measured on shoots in the greenhouse. Latent period and number of uredinia were negatively correlated (Table 3).

DISCUSSION

Mary Washington, Wash T2, WSU-1, and Delmonte 361 are presently grown in south central Washington. Of these cultivars, only Delmonte 361 rusted slowly in this study. UC 157 has been moderately rusted in the field in Washington, but not as severely as Mary Washington, Wash T2, and WSU-1. However, UC 157 was grouped with the slow-rusting lines because its AUDPC values, although higher, were not significantly different from other lines that rusted slowly (Table 1). The ranking in AUDPC of the eight lines was similar both years. WSU-1 and Wash T2 were severely infected and Jersey Centennial had few uredinia in a field test in Minnesota (2) as in this study.

Some slow-rusting lines had fewer aecia than fast-rusting lines, whereas other slow-rusting lines did not differ in number of aecia from some fast-rusting lines. Differential responses in production of aecia and uredinia on asparagus cultivars have been noted previously (2,10). Production of aecia was high on WSU-1 and low on Jersey Centennial in this study and in Minnesota (2), but the production of aecia on Wash T2 was relatively low in Minnesota (2) and high in Washington. Telia in overwintering debris, including dead stems still attached to the crown, constituted the inoculum source for infection and subsequent development of pycnia and aecia. The fast-rusting cultivars produced more uredinia and telia the preceding year and therefore should have been exposed to higher numbers of basidiospores than the slow-rusting lines.

Extended latent period and low numbers of uredinia on stems contributed to the slow-rusting of asparagus. These components of slow-rusting have been reported in cultivars of small grains and other crops (7,11,17,20). However, latent period and number of uredinia did not differ significantly between some individual slow- and fast-rusting lines as was found in barley infected with *P. hordei* (7). A question that arises is why did some cultivars rust more slowly in the field than others and yet a significant difference in latent period or number of uredia was not detected among these cultivars in the greenhouse? There are several possible explanations. First—Even though some differences were not significant, usually latent periods were longer and uredinia were fewer among the slow-rusting than among the fast-rusting lines. A small effect of an individual component of disease resistance may accumulate over several cycles of infection in the field. One cycle of infection was observed in the greenhouse whereas many occurred in the field. Second—Small effects of two components of disease resistance may act together to significantly reduce rust in the field. Third—Components of disease resistance not studied (pustule size and sporulation rate) may be involved.

TABLE 2. Latent period and number of uredinia per square centimeter in three experiments when shoots of asparagus cultivars and lines that rusted slowly and rapidly were infected with *Puccinia asparagi*[v]

	Experiment I[w]		Experiment II[x]		Experiment III[y]			
	Latent period	Uredinia per cm^2	Latent period	Uredinia per cm^2	Latent period	Uredinia per cm^2		
Cultivar or line	First shoot	First shoot	Third shoot	Third shoot	Second shoot	First shoot	Second shoot	Third shoot
Slow-rusting								
277 × 22-8	9.8 a	4.7 a	...	...	...	...	...	...
Jersey Centennial	9.5 ab	5.4 ab	9.8 a	0.7 a	10.9 a	0.03 a	0.02 a	0.8 abc
56 × 22-8	9.5 ab	6.4 bc	9.8 a	0.5 a	10.4 a	0.10 a	0.20 ab	0.7 ab
Delmonte 361	9.1 bc	5.5 ab	9.8 a	0.7 a	9.8 ab	0.10 a	0.20 ab	0.6 a
44G × 22-8	...	...	9.4 ab	1.3 b	9.6 ab	0.02 a	0.10 a	0.6 a
UC 157	9.2 bc	6.7 bc	9.1 abc	0.7 a	10.0 ab	0.30 ab	0.10 a	0.8 abc
Fast-rusting								
Wash T2	...	...	8.9 bc	1.3 b	9.7 ab	0.5 ab	0.5 ab	1.0 bcd
Mary Washington	8.9 c	5.4 ab	8.8 bc	2.3 c	8.9 bc	0.9 b	0.7 ab	1.6 d
WSU 1	9.0 c	8.0 c	8.6 c	1.7 b	8.4 c	0.9 b	0.9 b	1.5 cd
Mean of slow-rusting[z]	9.4**	5.7*	9.6**	0.8**	10.1**	0.1**	0.1**	0.7**
Mean of fast-rusting	9.0	6.7	8.8	1.8	9.0	0.8	0.7	1.4

[v] Within a column, values with the same letter are not significantly different, $P = 0.05$, according to Fisher's Protected LSD.
[w] Plants were inoculated when 100% of their cladophylls had developed; values are means of 10 replications.
[x] Plants were inoculated when 50–75% of their cladophylls of the third shoot had developed. Values are means of 19 replications.
[y] Plants were inoculated when the third shoot had completed elongating and 0–10% of their cladophylls had developed. Values are means of 10 replications.
[z] Single-degree-of-freedom contrasts were used to test differences between slow-rusting and fast-rusting cultivars and lines. Asterisks (* and **) indicate statistical difference from the fast-rusting mean at $P = 0.05$ and $P = 0.01$, respectively.

TABLE 3. Correlation coefficients for the relationships between area under the disease progress curve (AUDPC) in 1984 and two components of slow-rusting when asparagus shoots were infected with *Puccinia asparagi* in three experiments

Experiment and shoot	AUDPC and latent period	AUDPC and uredinia/cm^2	Latent period and uredinia/cm^2
Experiment I	−0.73	0.71	−0.48
Experiment II			
Shoot 1	−0.90**[a]	0.90**	−0.94**
Shoot 3	−0.91**	0.77*	−0.81*
Experiment III			
Shoot 1	−0.60	0.88**	−0.50
Shoot 2	−0.82*	0.92**	−0.94**
Shoot 3	−0.69	0.79*	−0.71*

[a]Asterisks (* and **) indicate statistical significance at $P = 0.05$ and 0.01, respectively.

Length of latent period, number of uredinia, and severity of rust in the field were significantly correlated as found in previous studies on slow-rusting resistance (7,17). Both latent period and numbers of uredinia appeared to be good criteria to select for resistance in the greenhouse. Rapid progress should be made in breeding for rust resistance in asparagus by making selections both in the greenhouse based on latent period and numbers of uredinia and in the field based on the AUDPC.

Resistance to rust, as measured by the length of the latent period and numbers of uredinia, increased in both slow- and fast-rusting cultivars as the age of shoot increased. Ohm and Shaner (11) demonstrated that plant-growth stage had a significant effect on latent period and pustule size in wheat infected with *Puccinia recondita*. Wheat was more susceptible, as indicated by length of latent period and size of pustule, as seedlings and after anthesis than when in the boot stage of growth. As in this study, they found the latent period to be longer for the slow-rusting cultivars at all plant growth stages. With asparagus, differences among cultivars for latent period were most evident when shoots were between the 50 and 100% stage of cladophyll development (third shoot in experiment II and second shoot in experiment III). Differences in number of uredinia between slow- and fast-rusting lines were largest when shoots were between the 50 and 75% cladophyll stage (third shoot in experiment II). This agrees with the results of previous work in which the 50% cladophyll stage was considered the best developmental stage to evaluate asparagus lines for rust resistance in the greenhouse (6). Increased resistance in the field has also been noted as the age of shoot increased (6). This characteristic should be taken into account when evaluating asparagus selections for resistance.

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