Peanut Leaf Wettability and Susceptibility to Infection by *Puccinia arachidis*

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ABSTRACT

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The differential susceptibility to *Puccinia arachidis* observed among nonphysiologically resistant cultivars of *Arachis hypogaea* was related to leaf wettability. Regardless of leaf or plant age, a similar positive regression of leaf susceptibility on wettability was detected for all the nonphysiologically resistant cultivars that were studied. No other factors significantly influenced the susceptibility of these cultivars to *P. arachidis*. When physiologic resistance was not being investigated, this differential wettability provided a quick and discriminating technique for the preliminary screening of cultivars and lines within cultivars for resistance to *P. arachidis*.

Additional key words: leaflet size, stomatal density, surface wax, surfactant.

Few reports (13) of the relationship between the water repellency of leaves and their resistance to infection have been published . Although many authors (1,3,4,7–9,11,12) have implied that variations in leaf wettability may confer a differential susceptibility to disease, most investigations on the wettability of plants have assessed their ability to retain sprayed liquids.

The results of previous studies indicate a relationship between the wettability of peanut (*Arachis hypogaea* L.) leaves and their susceptibility to rust infection (caused by *Puccinia arachidis* Speg.) (5,6). During these investigations it was noticed that, while the abaxial surfaces of leaves became less susceptible as they matured, they also became less wettable, and that the virtually unsusceptible adaxial surfaces were always highly water repellent. Furthermore, there was a general trend towards greater wettability of the plants as they matured; leaves of older plants were more wettable than leaves of the same age on younger plants. Resistance to infection appeared to reside in this changing wettability of the leaves with age; the rate of change varied among the cultivars studied and affected uredospore retention and probably germination and appressorium formation.

These investigations were undertaken to determine the contribution of the wettability of the leaves of representative cultivars from each of the maturity/growth habit groups of peanut to their susceptibility to *P. arachidis* at various ages.

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MATERIALS AND METHODS

Two cultivars from each of the maturity/growth habit groups of peanut were studied: Starr and Tarapoto (early/erect) NC 4X and Georgia (medium/erect), NC 13 and F 393-6 (medium/semi-erect), and Florigiant and Virginia 61R (late/runner). These cultivars span the range of differential susceptibility to rust; Tarapoto (PI 259747) is physiologically resistant.

Twenty-five plants of each cultivar were grown. Seeds were sown in 20-cm diameter plastic pots placed in rows along greenhouse benches. Daily minimum and maximum temperatures were ~20 C and ~28 C, respectively. The pots were watered daily, each plant was given the same quantity of water, and care was taken not to wet the leaves. The dates of opening of the leaves on the main axis of each plant were recorded (peanut leaves open like a fan and the time of opening was assumed to be when the abaxial surfaces of the leaflets separated from one another since leaflet surfaces cannot be inoculated before that time). The leaves of these greenhouse-grown plants opened at 3- to 4-day intervals except those at the first two nodes, which opened almost synchronously 3–4 days after seedling emergence, and those at the third and fourth nodes, which opened within the following 7–8 days.

Uredospores of *P. arachidis* were collected from peanut plantings in the parish of St. Elizabeth, Jamaica, and increased on greenhouse-grown plants of cultivar Starr.

Two weeks after seedling emergence, wettability and susceptibility to rust were assessed among leaves 10.5, 7, 3.5, and 0 days of age from five plants of each cultivar on which the terminal leaf on the main axis had just opened. For each leaf, one of the terminal leaflets was used to determine its wettability and the other three leaflets were used to determine its susceptibility.

Because many leaf wettability estimates had to be made in a short time, a quick technique for estimating leaf wettability that was suitable for use in the greenhouse and field was developed (author, *unpublished*). The method relies on the retention of water by the surface when a leaf is held in a vertical position and water drained from around it at a constant rate. With peanut leaflets, a drainage rate of 2.5 cm/sec allowed good discrimination over a wide range of wettabilities of various leaves; the adaxial surface never retained water, regardless of the rate of drainage. It was not necessary to take repeated readings with the same leaflet since readings subsequent to the first either were the same or showed a slight rise in value. The relationship between mean contact angle measurements of the abaxial surface, θ, and milligrams of water retained per 10 cm^2 of abaxial surface, X, was approximately linear (the correlation between the two measurements was extremely high, $r = 0.997$, $P = 0.001$), and the conversion equation was:

$$\theta = 122.401 - 2.354 X$$

The three remaining leaflets of each leaf were placed on damp filter paper in a petri dish with their abaxial surfaces exposed. Susceptibility was determined by spraying the leaflets in the evening with a suspension of freshly harvested uredospores until a density of approximately 100 spores/cm^2 was attained on microscope slides interspersed among the dishes. No surfactant was used. The dishes were covered and placed in strong indirect light in a greenhouse. The filter papers were kept damp. On the 14th day after inoculation, susceptibility was assessed as the number of pustules that had developed per 10 cm^2 of abaxial leaf surface (the approximate size of an average leaflet). For leaves that had just opened at the time of inoculation, the leaflet areas at that time were used, not the areas at the time of pustule assessment because these leaflets were expanding rapidly. Although the leaves on opening had relatively more stomata per square centimeter, previous investigations of germ tube behavior indicated that stomatal density does not limit rust infection at this inoculum density. These procedures were repeated at 4, 6, 8 and 10 wk.

The most rapid changes in wettability occurred within a few days of leaflet opening. Other plants of each cultivar were grown and the wettability and susceptibility to rust of leaves at nodes not sampled on opening in the above investigation were determined until the plants were 6 wk old. Only every fourth leaf was removed to minimize the effect of leaf removal on each plant. Susceptibility was assessed as previously described.

The susceptibility of leaves from 10 replicate 6-wk-old plants of each cultivar also was determined when the wettability effect had been counteracted by a surfactant. Triton X-100 (Rohm and Haas, Philadelphia, PA 19105) at a concentration of 1.0 ml/L in the inoculating suspension allowed the surfaces of leaves with low wettability to retain a continuous film of liquid (this concentration of the surfactant was determined previously not to affect the germination of uredospores appreciably). Leaflets were placed in petri dishes; those from five of each set of 10 relicate leaves were arranged with their abaxial surfaces exposed and those from the other five with their adaxial surfaces exposed. Uredospore concentration in the suspension was adjusted to a density of approximately 100 spores per square centimeter. Pustule counts were made on the 14th day after inoculation.

RESULTS

The wettability and susceptibility to rust of the abaxial surfaces of the leaves are summarized for the 2-, 4-, and 6-wk assessments of

TABLE 1. Wettability of peanut leaves (X)[a] and their susceptibility to infection by *Puccinia arachidis* (Y)[b]

Plant age and nodal position[c]	Leaf age in days[d]	Cultivars															
		Starr		PI 259747		NC 4X		Georgia		NC 13		F 393-6		Florigiant		V 61R	
		X	Y	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y	X	Y
Two weeks:																	
6	0	25	103[e]	24	56	25	96	18	78	19	68	14	59	18	71	16	63
5	3.5	13	58	3	12	8	27	3	36	3	15	5	28	2	25	5	27
3,4	7	3	31	0	4	2	18	1	9	0	8	1	13	0	7	1	15
1,2	10.5	1	6	0	1	0	2	0	3	0	2	0	3	0	4	0	2
Four weeks:																	
10	0	32	151	29	74	29	107	25	115	27	118	25	116	26	101	24	99
9	3.5	21	92	13	32	17	79	11	61	13	67	11	66	15	63	15	65
8	7	19	73	11	18	14	63	8	37	9	41	6	35	10	51	10	36
7	10.5	13	56	9	17	7	31	1	18	4	26	2	20	5	35	5	21
6	14	7	35	5	10	2	12	0	9	1	15	1	18	0	16	2	12
5	17.5	4	26	1	4	0	6	0	12	0	7	1	10	0	8	0	9
3,4	21	1	12	0	0	0	1	0	2	0	2	0	5	0	4	0	11
1,2	24.5	1	7	0	0	0	3	0	4	0	1	0	3	0	0	0	3
Six weeks:																	
14	0	39	173	34	82	34	139	30	142	31	103	29	133	30	144	27	116
13	3.5	28	142	23	52	24	107	20	99	21	77	20	93	21	97	17	75
12	7	26	94	21	45	21	93	19	87	16	58	19	76	17	73	13	67
11	10.5	24	110	19	29	18	75	18	70	14	66	17	58	17	64	12	51
10	14	23	86	18	35	17	61	15	44	12	38	13	64	14	51	11	47
9	17.5	20	91	12	21	16	68	11	52	8	25	6	37	14	62	10	38
8	21	19	75	10	17	14	52	7	37	3	15	1	21	9	31	6	19
7	24.5	13	42	8	11	7	28	1	18	0	12	0	16	4	10	2	8
6	28	8	31	4	2	2	14	0	12	0	9	0	8	1	11	0	4
5	31.5	4	19	1	0	0	9	0	10	0	0	0	2	0	5	0	1
3,4	35	1	17	0	0	0	3	0	4	0	2	0	0	0	3	0	0
1,2	38.5	0	8	0	0	0	2	0	3	0	0	0	1	0	1	0	0

[a] Measured as the mean number of milligrams of water retained per 10 cm^2 of abaxial leaf surface (after drainage of water at the rate of 2.5 cm/sec from around vertically held leaflets).

[b] Assessed as the mean number of pustules per 10 cm^2 of abaxial leaf surface 2 wk after inoculation (when approximately 100 uredospores were deposited per square centimeter of abaxial leaf surface).

[c] Nodes were numbered sequentially from the base of the main axes of the plants. Leaves were sampled at the first or second and third or fourth nodes for leaf ages of 38.5 and 35 days, respectively.

[d] Leaf ages in days after opening.

[e] Each pair of means is for five replicate leaves.

the cultivars (Table 1). The most striking changes in these two factors occurred over the first 6 wk of the plants' growth; the new leaves on plants 8 and 10 wk old reacted similarly to leaves of the same age in the 6-wk-old sample for each cultivar. In each case, the leaf at the plant apex was the most wettable and susceptible and there was a rapid progressive decrease in these factors for leaves lower on the main axis. Similarly positioned leaves on plants of the same age, but of different cultivars, tended to differ in wettability and susceptibility and, for any one cultivar, leaves of the same age but on plants of different ages differed in wettability and susceptibility; both of these factors increased with the age of the plants up to 6 wk.

The relationship between the wettability of the leaves and their susceptibility to rust appeared to be linear. The two parameters were compared for plants 6 wk old because their leaves displayed almost the complete range of these two factors. Since the standard deviations of the mean pustule counts increased with the increasing means, the variance had to be stabilized before statistical analyses were performed. Because the data included counts with low means, the numbers of pustules per 10 cm^2 of abaxial surface for each leaf, y, were transformed to z according to the equation:

$$z = \frac{1}{2}(\sqrt{y} + \sqrt{y+1})$$

(the simpler equation $z = \sqrt{y + 0.5}$ gives the same value for counts greater than nine (author, *unpublished*). This transformation completely stabilized the variance. The standard deviations of mean wettability estimates were relatively small and showed no significant regression on wettability means. To maintain the apparent linearity of the relationship between the two parameters, however, wettability means were transformed to square roots. Analysis of covariance indicated that the regression lines for the cultivars of susceptibility on wettability did not differ significantly in their residual variances and slopes but did differ significantly (P = 0.01) in their elevations. When compared by the sequential Newman-Keuls method, the differences between the adjusted susceptibility means for the cultivars showed Tarapoto to be significantly more resistant to infection ($P = 0.05$) than the others. The homogeneity of the data for the nonphysiologically resistant cultivars having been established, the results for these were combined. Plots of pooled data for the regression of susceptibility on wettability (Fig. 1) showed a very highly significant correlation ($r = 0.973$, $P = 0.001$).

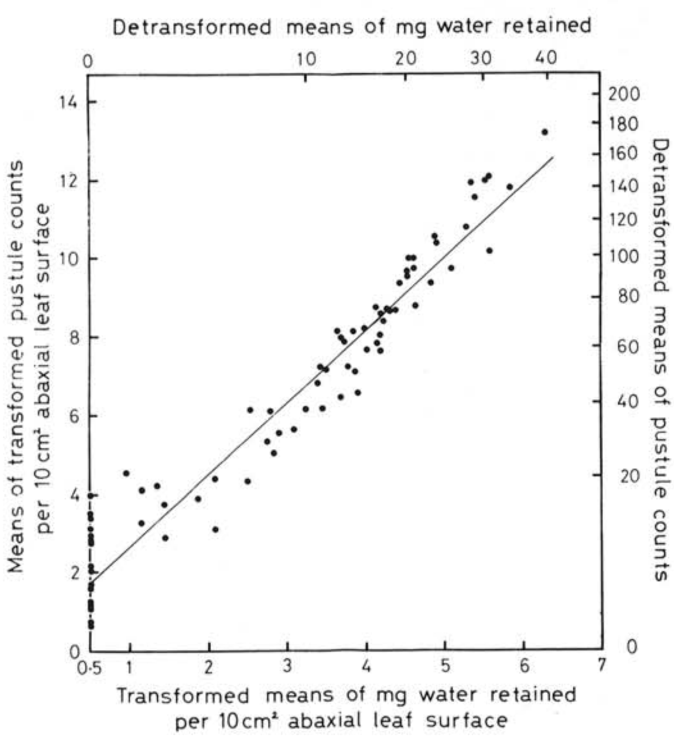

Fig. 1. Regression of the pooled data, transformed to stabilize the variance, for leaves of 6-wk-old plants of seven nonphysiologically resistant peanut cultivars of their susceptibility to rust infection (assessed as the mean number of pustules per 10 cm^2 of abaxial leaf surface developing 2 wk after inoculation with approximately 100 uredospores per square centimeter) on their wettability (measured as the mean mg water retained per 10 cm^2 abaxial leaf surface after the drainage of water at the rate of 2.5 cm/sec from around the leaflets when held vertically). Each determination is the mean for five replicate leaves.

To determine if the same relationship between wettability and susceptibility to rust also held true for plants of other ages, the regressions of susceptibility on wettability for the nonphysiologically resistant cultivars were compared for plants 2,4,8, and 10 wk of age. Differences were not significant in residual variances and slopes of the regression lines in any of these analyses and, although the elevations of the lines differed significantly ($P = 0.05$) in the 8-wk-old sample, the differences between the adjusted means for the cultivars were not significant when tested by the Newman-Keuls method. To test whether the relationship between these two parameters varied when the age of these plants was considered, the regressions of the pooled data of susceptibility on wettability were compared for plants 2, 4, 6, 8, and 10 wk old. Differences between the regression lines were not significant.

In general, the residual variances for the five plants of each cultivar at any sampling time were low. However, the residual variances for the 6-wk-old sample of F 393-6 and the 8-wk-old sample of Virginia 61R were higher than those for samples of other ages of those cultivars. Although these residual variances were relatively high, they were not high enough to preclude the legitimate use of the data in the analyses. Differences were not significant among the replicate plants in the 6-wk-old sample for F 393-6, but the wettability and susceptibility means of one of the replicate plants in the 8-wk-old sample for Virginia 61R were significantly lower ($P = 0.05$ in each instance) than those for the other four replicates; no significant differences were apparent among the replicates in their regressions of susceptibility on wettability.

The residual variances for the regressions of susceptibility on wettability for plants 2, 4, 6, 8, and 10 wk old of the physiologically resistant cultivar Tarapoto differed significantly ($P = 0.01$). The regression coefficients increased with plant age although the value of "a" in the regression equations fell; the overall effect was that leaves on older plants were more resistant than leaves with the same wettability on younger plants.

At opening, the leaves increased in wettability from the base of the plant upward. This phenomenon was particularly marked for leaves from nodes 1 to 10 (Fig. 2); from the leaf at node 10 onwards there was little further increase in wettability. The susceptibility to rust of the leaves on opening followed the same pattern. This increase in wettability of the leaves was accompanied by a corresponding increase in the opening areas of their leaflets and in each instance a close correlation was found between these two factors, $r => 0.95$ ($P = 0.001$), for each cultivar.

Multiple regressions for each cultivar of leaf wettability on the logarithm of leaf age, the logarithm of plant age and leaflet area showed each of these independent variables to be significant (P = 0.001 in each case) and yielded an R^2 of ~0.95 for each cultivar.

The susceptibility to rust of the abaxial surfaces of leaves when the effect of leaf wettability had been counteracted are summarized in Table 2. Analysis of variance showed no significant differences among the nonphysiologically resistant cultivars but there were significant differences among the leaves at different nodes (P = 0.01); leaves younger than 7 days and older than 28 days were significantly more susceptible ($P = 0.05$) than leaves of other ages. The data for each of these cultivars did not show this effect very clearly. The apparent slight decrease in susceptibility of the leaves of the physiologically resistant cultivar with age was not significant by Student's *t*-test. The adaxial surfaces of the leaves reacted similarly to the abaxial surfaces when the effect of wettability was counteracted; pustule counts appeared to be slightly lower, but this was not significant. There were no significant differences among nonphysiologically resistant cultivars, but there were significant differences among the leaves at different nodes ($P = 0.05$); the youngest and oldest leaves again being slightly more susceptible than the others.

DISCUSSION

A direct relationship, which was the same for all the non-physiologically resistant cultivars, always existed between the wettability of a leaf and its susceptibility to rust regardless of the age of the leaf or the plant on which it was borne. The resistance to rust in older leaves on older plants of the physiologically resistant cultivar Tarapoto was a little greater than would have been predicted from their wettability.

Both the age of a leaf and the age of the plant on which it was borne affected its wettability and susceptibility to rust. The leaf age effect was probably related to the state of maturity of the cuticle, whereas the plant age effect appeared to act mainly through leaflet size. Superficial wax seemed to play little role in the water repellency of the leaves; mean contact angles obtained before and after the washing of leaf surfaces with chloroform or benzene showed no significant differences.

The replicate plant among the 8-wk-old Virginia 61R that was significantly less wettable and less susceptible to rust than were the other replicates subsequently produced progeny less wettable and less susceptible to rust than were other plants of this cultivar. Peanut cultivars are not pure lines and slight differences often were noticed among replicate plants.

The results may explain the observations of Castellani (2) and McVey (10) that rust is not present on plants in the field until they are about 6 wk old. Because each uredial cycle takes approximately 2 wk and the leaves at the lower nodes are barely susceptible to infection when they open, primary infection usually will not develop before plants are about 4 wk old. The infected leaves tend to be masked by the preponderance of leaves free of infection at the base of the plants and of leaves that have not had time to develop pustules. Thus, secondary infection would be of little importance before 6 wk. Unless damaged or covered with a layer of dust, leaves on plants in the field tended to be less wettable and correspondingly less susceptible to rust than their counterparts at the same nodes on plants of the same age in the greenhouse. The leaflets tended to be smaller on field-grown plants than on those grown in a greenhouse, and the interval between leaf opening was usually longer in the field.

The finding that the susceptibility to rust of 6-wk-old plants of

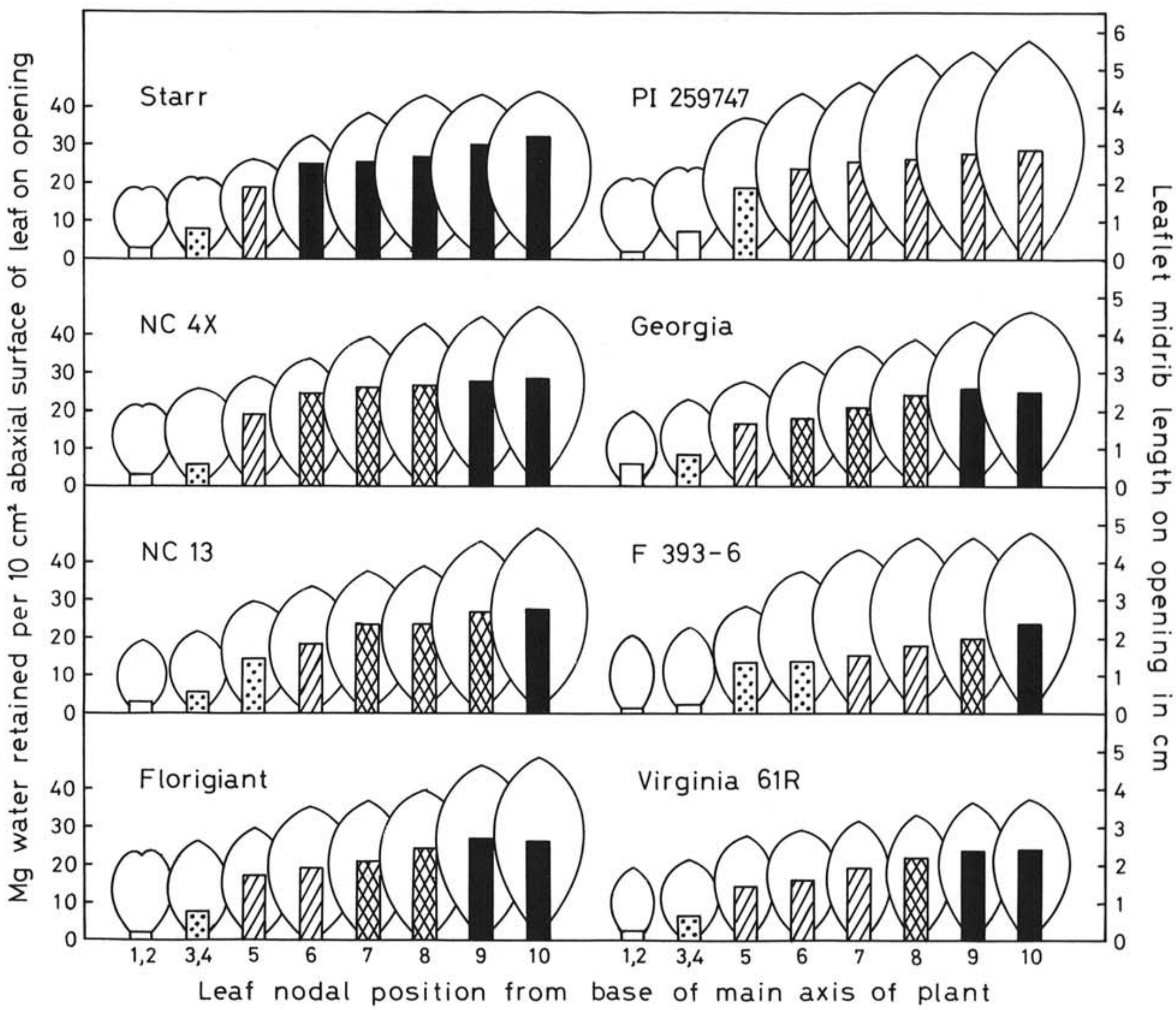

Fig. 2. Wettability, susceptibility to rust infection, and areas of leaflets of peanut leaves at the time of opening on the main axes of plants up to the age of 4 wk of eight cultivars. The height of each bar represents the mean milligram of water retained per 10 cm^2 of abaxial leaf surface for five replicate leaves (after the drainage of water at the rate of 2.5 cm/sec from around vertically held leaflets). Degree of shading of the bars indicates the number of pustules per 10 cm^2 of abaxial leaf surface for the five replicate leaves 2 wk after inoculation with approximately 100 uredospores per square centimeter: no shading = 0–25 pustules; stippling = 26–50 pustules; hatching = 51–75 pustules; cross-hatching = 76–100 pustules; solid shading = > 100 pustules. The leaflet template outlines indicate the relative areas of the leaves (approximate areas of leaflets are 0.75 × length × breadth). In each instance the leaves at the first or second and third or fourth nodes were sampled depending at which node a leaf was opening when the plant was 3.5 and 7 days old, respectively; leaves at the other nodes opened at approximate 3.5 day intervals.

TABLE 2. Susceptibility[a] to *Puccinia arachidis* of leaves of 6-wk-old peanut plants when the wettability of leaves had been increased by the inclusion of a surfactant in the inoculating suspension

Leaf nodal position[b]	Leaf age in days[c]	Cultivars							
		Starr	PI 259747	NC 4X	Georgia	NC 13	F 393-6	Florigiant	V 61R
14	0	138[d]	97	169	176	152	186	187	164
12	7	128	85	168	137	166	171	168	153
10	14	144	92	180	157	172	145	161	149
8	21	171	83	138	168	156	149	154	160
6	28	138	84	129	127	159	164	131	148
3,4	35	165	72	152	187	167	173	166	168
1,2	38.5	163	64	168	171	182	168	183	177

[a]Mean number of pustules per 10 cm^2 of abaxial leaf surface 2 wk after inoculation with approximately 100 uredospores per square centimeter.
[b]Nodes were numbered sequentially from the base of the main axes of plants. Leaves at the first or second and third or fourth nodes were sampled for leaf ages of 38.5 and 35 days, respectively.
[c]Leaf ages in days after opening.
[d]Each figure is the mean for five replicate leaves.

the nonphysiologically resistant cultivars was similar when the leaf wettability effect had been counteracted confirms the importance of wettability in governing the differential susceptibility of cultivars under normal conditions. There were no significant differences between leaf surfaces of these cultivars, but there were significant differences among the leaves at different nodes (the youngest and oldest leaves tended to be more susceptible). That the data for each cultivar did not always show this clearly was probably due to the crude inoculation technique used rather than to actual differences among the cultivars. One explanation for this effect is that stomatal density had an effect at the relatively high inoculum level that resulted when run-off of inoculum had been counteracted. Stomatal density was higher on the youngest leaves, because their leaflets were not fully expanded, and on the oldest leaves, because their leaflets were small in size. However, no stomatal effect in relation to susceptibility could be detected from studies of germ tube behavior on the leaves after inoculation with suspensions of uredospores with or without surfactant. Stomatal counts were always high, ranging from about 11,000 to 17,000/cm^2 on both leaf surfaces for each cultivar, and the germ tubes could hardly miss a stoma. Differences between leaf surfaces were not significant for the physiologically resistant cultivar, and although the younger leaves appeared to be more susceptible to rust, the apparent steady decrease in infection with leaf age was not significant. The fact that the surfactant overcame the effect of leaf surface and virtually overcame the effect of leaf age on susceptibility to rust probably explains McVey's findings (10) that plants in the greenhouse could be infected at any age and that the adaxial surfaces of leaves were susceptible to infection. Castellani (2) previously reported that pustules did not develop when adaxial surfaces were inoculated; McVey used a surfactant during inoculation, but Castellani did not.

This differential wettability provides a quick preliminary screening technique for selecting cultivars resistant to rust, when physiologic resistance is not being investigated, and lines less susceptible to infection within cultivars. The number of replicate plants and their leaves investigated depends on the material being screened. When many cultivars in the same maturity/growth habit group were compared, it usually was sufficient for preliminary screening purposes to investigate the wettability of the leaves at the second and fourth nodes from the apex of two replicate 6-wk-old plants for each cultivar n which the terminal leaf had just opened. If the cultivars belonged to different groups, the leaves at the second, fourth, and sixth nodes from the apex were investigated. However, the time required for the plants to mature had to be considered; a late-maturing cultivar that is less wettable than an early maturing one might not perform as well in the field since secondary infection has longer time in which to occur. Cultivars that appeared less wettable were further investigated through additional leaf wettability comparisons before large-scale field trials were initiated. If only a few cultivars were to be screened, it was best to compare the leaves at as many nodes as practically possible. For screening lines within cultivars, investigation of the leaf at the third node from the apex of plants of the same age on which the terminal leaf had just opened usually sufficed. The advantages of this method of screening were that it detected subtle differences among cultivars which did not become apparent except in large plot field trials and that it could detect differences among lines within cultivars.

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