EFFECT OF ABAXIAL LEAF PUBESCENCE ON RUST SPORE DEPOSITION, SPORE GERMINATION AND UREDINIA DENSITY

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Abaxial leaf pubescence has been associated with race non-specific resistance to Uromyces appendiculatus in Phaseolus vulgaris. Two resistance mechanisms for leaf pubescence have been proposed: one is a reduction in effective inoculum by physical trapping of spores by the trichomes and a second is a change in dew formation pattern resulting in germtubes formation without the leaf surface contact necessary for infection (5). Burage (3) demonstrated the formation of water droplets condensing first on the leaf trichomes and later forming a water layer that traps air between the water and the leaf epidermis. Since urediniospores of U. appendiculatus are highly unwettable, the second theory is based on the logic that dry urediniospores will float on a water film surface and germinate without contacting the epidermis. The objective of this study was to determine the effect of leaf pubescence on urediniospores deposition, and germination and resultant infection density when free moisture for spore germination is from dew or from fine mist provided by humidifiers.

Materials and Methods

Pubescent genotypes Pompadour Checa 83-30 (PC83-30) Jamaica Red (JR), Pompadour V(PV) and UAC 221 (UAC) and a glabrous genotype Pinto U.I. 114 (P114) were planted in the glasshouse at selected intervals to generate 4th trifoliolate leaves 10-30% expanded for simultaneous inoculation. Three experiments were conducted using a factorial experimental design and four replicates. Since the density of leaf trichomes is maximal in young leaves and decreases as the leaves expand, different trichome densities within each genotype were represented by different leaf expansion sizes. Leaves of the 4th trifoliolate were detached under water to maintain maximum turgidity and inoculated on the abaxial surface by a spore tower technique. The leaves were then incubated for 16 h at 19 $\pm$ 1°C and 100% relative humidity from dew formation in a dew chamber and from cool fine mist generated by humidifiers.

Leaf discs of 7 mm diam. were cut from each leaflet for microscopic observation and the rest of the leaves were maintained in glass containers for further disease development. The leaf discs were fixed and bleached. Fungal structures were stained on a filter paper bed with the abaxial surface up; no coverslip was used during microscopic observations so as to avoid displacement of loosely attached fungal structures. The number of spores deposited on the epidermis and trapped by the trichomes were counted under bright phase illumination. Infection was measured by the density of uredinia formed on the intact leaves.

Results and Discussion

Urediniospores were deposited in singlets and in clusters of varying size on the epidermis of the glabrous leaves (P114), but only singlets were observed on the epidermis of the pubescent leaves. The urediniospores that were deposited in clusters tended to be trapped by the trichomes and only some of the spores in singlets reached the epidermal surface. About 60-80% of the spores were trapped by the dense trichomes in the 10-20% expanded leaves (Table 1). Overall, the percentage of spores trapped was highly significant ($P = 0.001$) at all leaf sizes and caused a significant reduction of inoculum density on the leaf epidermis. Spores deposited in clusters and in singlets germinated equally well on both glabrous and pubescent leaf surfaces. Regardless of the form of moisture provided for spore germination, germlings from trapped spores tended to grow horizontal to the leaf epidermis without contacting it. Spores deposited at different distances from the epidermis had some germtubes twined on or appressed to the trichomes, some grew away from the epidermis following the trichomes while a few grew towards the epidermis. It was difficult to quantify appressoria formation or deduce whether germtube contact with trichomes provided enough stimuli for thigmodifferentiation.

There were significant differences between genotypes in uredinia density ($P = 0.01$), but the glabrous P114 did not always show the highest infection density (Table 1). Pubescent PC83-30 had the lowest infection density and UAC had the highest infection density in two experiments. Young trifoliolate leaves of P114, 10-20% expanded

and UAC had the highest infection density in two experiments. Young trifoliolate leaves of P114, 10-20% expanded had low infection densities similar to PC83-30, but larger leaves of P114 produced significantly higher infection densities than PC83-30. There was no correlation between infection density and the number of spores trapped, but there was a positive correlation (R = 0.45 to 0.74, P = 0.01) between uredinia density and the number of spores deposited on the epidermis and between the uredinia density and leaf size at the time of inoculation (R = 0.39 to 0.55, P = 0.01). Number of spores deposited on the epidermis also correlated positively with leaf size in all genotypes (R = 0.40 - 0.46, P = 0.01). Spore germination and uredinia density were not affected by the form of moisture provided during incubation. Leaves incubated in dew chambers that simulated natural dew formation developed infection density similar to the leaves provided with free water from humidifiers. During urediniospore deposition on the epidermis of pubescent leaves spores adhered to the leaf epidermis and germinated without floating to the water surface.

Recent studies on the adhesion of urediniospores of Uromyces viciae-fabae to its host showed a thin matrix around the spores that provided sufficient adhesion to bind the spores to the substratum (2). A similar process may also occur in U. appendiculatus. Even though pubescence provided significant spore trapping, the role of dense leaf trichomes appears to be in reducing the inoculum potential. Spores deposited in clusters have a higher inoculum potential than spores in singlets (1). This would have a significant effect in the field where environmental conditions are not always ideal for infection. Our results help explain why the effect of leaf pubescence on infection density has not been adequately demonstrated in glasshouse experiments, but is reported from field observations.

Table 1. Effect of leaf trichomes on deposition of Uromyces appendiculatus urediniospores on bean leaves of different sizes and on infection density.

Expt. No.	Host Genotype	Pub[1]	% leaf expansion	Spore density on trichomes	Spore density on epidermis	% spore trapping	Uredinia density
I	PC83-30	6	25	17 a[2]	26 a	49	6 b
	UAC221	5	25	22 a	24 a	49	12 a
	P114	1	25	0 b	72 c	0	10 a
II	PC83-30	6	10	24 abd	5 d	83	3 def
			25	43 a	32 b	57	8 de
	UAC221	5	10	29 ab	16 bcd	64	8 de
			25	17 bd	22 bcd	43	13 bcd
	P114	1	10	0 c	32 b	0	4 def
			25	0 c	83 a	0	23 a
	J. Red	5	10	29 ab	15 bcd	66	8 de
			25	35 ab	30 b	54	14 bcd
	P.V.	5	10	25 ab	19 bcd	70	8 de
			25	20 bd	24 bcd	43	11 cde
III	PC83-30	6	10	16 ab	10 ab	62	2 d
			20	12 ab	7 b	63	5 cd
			30	25 a	15 ab	62	5 cd
	UAC221	5	10	28 a	13 ab	68	9 bc
			20	35 a	24 ab	59	12 bc
			30	19 ab	44 a	30	18 a
	P114	1	10	0 c	19 ab	0	4 cd
			20	0 c	29 ab	0	7 bcd
			30	0 c	44 a	0	12 bc

[1]Pubescence rating on 0-9 scale (4); 1 = nearly glabrous; 9 = >1000 trichomes/cm^2.

[2]Numbers followed by same letter within a column in each experiment are not statistically different according to the New Duncan Multiple Range Statistical Analysis. Each experiment was analyzed separately.

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