Heat- and Cold-Induced Retention of Inoculum by Leaves

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ABSTRACT

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When rust-susceptible corn leaves were heated 5 sec at 55 C before being sprayed with either water or a suspension of uredospores of *Puccinia sorghi*, the retention of water or spore suspension on both leaf surfaces was increased about 9-fold and infection by *Puccinia* was increased about 5-fold over that on unheated leaves. If leaves were dipped in water instead of sprayed, the relative increase in retention due to the 55 C treatment was much greater, but the actual deposit was less from dipping than from spraying. With *Chenopodium quinoa* and *C. amaranticolor*, the increased retention of water as a result of heat treatment was greater than with corn, but with celery, safflower, cucumber, teasel, sunflower, tomato, tobacco, bean, willow, cowpea, and zinnia there was only a slight increase in retention due to heat, and with buckeye and cotton there was a significant decrease. When corn and *Chenopodium* leaves were dipped in water at 0 C the retention of water was greater than for water at 5-20 C, but less than that for water at 40-70 C. When corn leaves were heated at 1-10 sec at 55 C and then incubated overnight in a moist chamber, the internal water congestion was greater than for unheated leaves. When tobacco, bean, cowpea, cucumber, and cotton leaves were heated 1-10 sec at 55 C and then immersed in ice water, water congestion of intercellular spaces was slight but was greater than for heat alone, cold alone, or cold followed by heat.

In nature, and in experimental work, the success of inoculation depends on the deposition and retention of inoculum at an infection court, and usually on the retention of water with this inoculum. Many leaves such as cereals, onions, cabbages, and *Chenopodium amaranticolor* are difficult to wet. Two common methods of increasing retention of inoculum on leaves difficult to wet, are either rubbing the leaves between the fingers before applying the water suspension of inoculum (1), or atomizing the inoculum in a suspension of oil (3). Heating of leaves before inoculation, as reported here, will attain a similar objective.

Heating of leaves before inoculation increases infection with many viruses, bacteria, and fungi (6). With corn rust, heating leaves before inoculation greatly increased infection of both susceptible and resistant cultivars of corn. At the time of those experiments, no effect of heat on the retention of inoculum was noticed, but in subsequent trials much greater retention of inoculum was noticed when the leaves were heated before inoculation than when inoculum was applied to unheated leaves. This is an exploration of the effect of heat and cold before and during inoculation on the retention of inoculum, on subsequent infection, and on water congestion by leaves.

MATERIALS AND METHODS

To determine retention of water suspensions of inoculum, leaves or leaf pieces (80 to 1,500 mg green weight) were detached from greenhouse-grown potted plants, weighed quickly on a torsion balance, dipped in or sprayed with water or a water suspension of inoculum for 5 sec, and again weighed. To determine the effect of predisposition temperature on retention, the leaf was dipped in water at the desired temperature for 5 sec, then sprayed or dipped in water at about 20 C and weighed. The weight of water retained is expressed as a percent of the original untreated leaf. This can be readily converted to mg/cm^2 since the leaves of this study and presumably many other leaves, have a fairly constant ratio of green weight to area.

Based on three determinations for each species, the average milligrams of green weight per cm^2 of leaf was as follows: *Chenopodium amaranticolor* - 16.4, *Cucumis sativus* - 19.0, *Helianthus annuus* - 19.5, *Nicotiana tabacum* - 24.0, *Phaseolus vulgaris* - 20.4, *Salix* sp. - 15.3, *Vigna sinensis* - 22.0, and *Zea mays* - 14.7. The average value of 19 mg/cm^2 for the above would probably be applicable to many other species. To measure heat predisposition to rust, corn leaves were heated 0-21 sec at 55 C before inoculating them by spraying them for maximum deposit with a water suspension of uredospores of *P. sorghi*.

Retention of water vs. inoculum.—Since inoculum here, and likely in most trials with inoculum in the form of spore suspensions, consists of over 99% water, and since water is a more standard fluid than a spore suspension, the comparative retention of water and spore suspension was determined. In four determinations, the retention of water on sprayed unheated corn leaves averaged 24% of the green weight (3.5 mg/cm^2), and of a suspension of uredospores of *Puccinia sorghi* averaged 27% of the green weight. In four determinations, the retention of water on sprayed corn leaves which had been previously heated 5 sec at 55 C, averaged 242% and of a water suspension of uredospores averaged 211%. It is concluded that retention of inoculum can be closely simulated with water, and most tests of deposition on leaves were performed with water.

Heat predisposition to corn rust.—In two trials with a total of 11 replications, the average number of

TABLE 1. Effect of temperature and method of treatment on the adherence of water to leaves[a]

	Water retention as percent of original green weight			
Species	Leaves dipped in water at 0 C	Leaves dipped in water at 20 C	Leaves sprayed with water without prior treatment	Leaves sprayed with water after 5 sec at 55 C
Aesculus californica	114	70	366	214
Apium graveolens	148	117	207	237
Carthamus tinctorus	38	25	42	42
Chenopodium amaranticolor	35	0.2	4.8	104
Chenopodium quinoa	22	0.3	13	202
Cucumis sativus	122	142	248	179
Dipsacus fullorum	93	74	61	88
Gossypium hirsutum	88	72	248	86
Helianthus annuus	96	54	100	130
Lycopersicum esculentum	177	116	290	315
Nicotiana tabacum	47	22	164	84
Phaseolus vulgaris	50	85	155	122
Salix sp.	46	61	132	230
Vigna sinensis	38	59	114	117
Zea mays	14	1.5	19	172
Zinnia elegans	63	15	135	167

[a]Each value is the average of two or more determinations. For *Chenopodium* sp. and *Zea mays* each value is the average of four or more determinations.

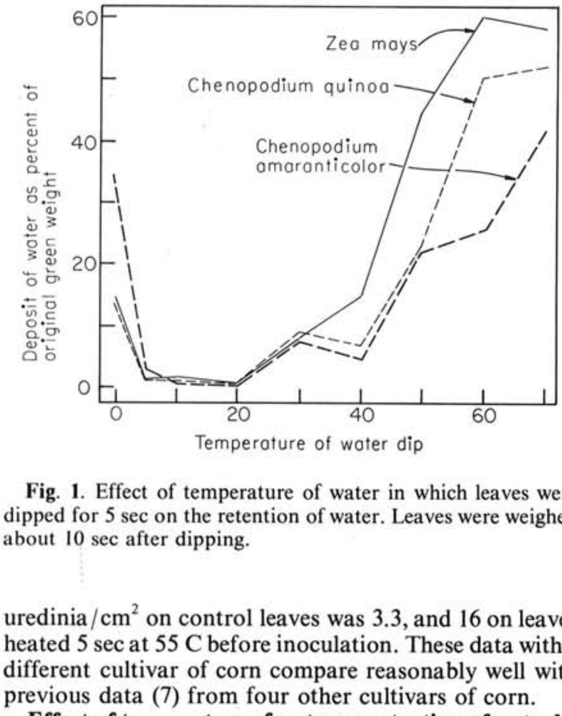

Fig. 1. Effect of temperature of water in which leaves were dipped for 5 sec on the retention of water. Leaves were weighed about 10 sec after dipping.

uredinia/cm^2 on control leaves was 3.3, and 16 on leaves heated 5 sec at 55 C before inoculation. These data with a different cultivar of corn compare reasonably well with previous data (7) from four other cultivars of corn.

Effect of temperature of water on retention of water by leaves.—Weighed leaves or leaf pieces were dipped for 5 sec in water at temperatures ranging from 0 to 100 C, and the deposit was determined after draining the leaves in a vertical position for 5 sec. With most species tested, the difference in retention due to temperature over this range of temperatures was no more than 2-fold, but with corn and *Chenopodium* the deposit at 0 C ranged from 14 to 35%, at 5 to 20 C it ranged from 0.2 to 1.5%, and at 70 C it ranged from 42 to 57% (Fig. 1 and Table 1).

Effect of heat before spraying or dipping on water retention.—Heat for various durations and temperatures before dipping leaves in water at 20 C resulted in great variation in retention of water. An increase in subsequent retention of water on corn resulted from as little as 2 sec at 45 C, but the dosage for maximum retention was about 80 sec at 45 C, 20 sec at 50 C, and 8 sec at 55 C. Eight sec at 55 C, followed by dipping in water at 20 C usually resulted in greater retention of water than 8 sec at 55 C only, or dipping in water at 20 C only. Five sec at 55 C was used as a standard predisposition treatment, and results with 15 species are given in Table 1. A large increase in retention due to heat was detected only with corn (Fig. 2) and *Chenopodium*. Only with secondary leaves of cotton and buckeye (four determinations of each species) was there an apparently significant reduction in retention of water as a result of prior heat.

Comparison of species.—The highest retention of water observed in a single determination was 437% (83 mg/cm^2) for spraying untreated buckeye (*Aesculus californica*) and the average of four determinations of this treatment was 366. The lowest retention of water was 0 for dipping untreated leaves of *Chenopodium amaranticolor* and *C. quinoa* in water at 20 C. For all methods of treatment the highest retention of water was with buckeye and tomato (210%), and the lowest was for *Chenopodium amaranticolor* and corn (30%).

Spraying vs. dipping.—The retention of water on unheated leaves was greater for spraying than for dipping for all hosts tested except teasel, and the average retention for all hosts was 57% for dipping and 141% for spraying (Table 1). The greatest relative increase of spraying over dipping was with *Chenopodium* (35-fold), but the greatest actual increase was with buckeye (296%).

Longevity of heat effect.—The effect of 55 C heat in increasing the retention of water lasted for at least 8 days and perhaps indefinitely. When corn leaves were heated 5 sec at 55 C and sprayed with water at 0 min, 13 min, 4 hr, 9 hr, 3 days, and 8 days after heating, the retention of water

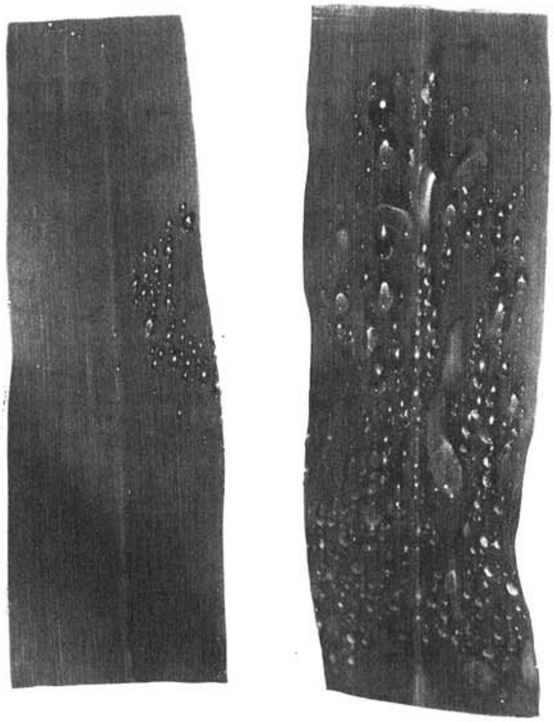

Fig. 2. Heat induced retention of water on corn. The left leaf piece was sprayed with water for maximum deposit by holding the leaf piece in a vertical position at right angle to the spray and about 10 cm from the atomizer. The right leaf was heated 5 sec at 55 C and then sprayed with water for maximum deposit. Both leaf pieces are adjacent pieces from the same leaf.

was 172, 165, 230, 232, 137, and 167%, respectively, whereas the retention on unheated leaves averaged 19%. At 4 days after heating, leaves had developed much anthocyanin (8), so it appears that anthocyanin had little if any effect on retention of water spray, though anthocyanin may reduce infection.

Age of leaves.—No clear effect of sequential position with age of corn leaves on retention of water applied as a dip treatment at 0 or 20 C or as a spray after heating at 5 sec at 55 C, was detected. But when unheated corn leaves were sprayed, the retention of water was greater on the older lower leaves. In two trials the average retention of water on unheated sprayed leaves was as follows: 1st leaf - 19%, 2nd leaf - 11%, 3rd leaf - 6.8%, and fourth leaf - 2.8%. No study of age of leaves was made with other species.

Heat and cold-induced water congestion of leaves.—Some bacteria and fungi cause infection primarily in water-congested leaves (2), and water congestion to aid transmission is commonly brought about by hydrostatic pressure, by hypodermic injection, by release of a vacuum, or by high impact pressure of a water spray (4). Heat-induced water congestion is no adequate substitution for the above in most cases, but that it does occur, is closely related to the subject of this report.

When young corn leaves were heated 1-10 sec at 55 C, before overnight incubation in a moist chamber, water congestion was commonly apparent the next morning only in the heated leaves. If these leaves were inoculated with bean rust uredospores (*Uromyces phaseoli*), but not corn rust uredospores, before incubation in a moist chamber, water congestion was increased. When cowpea, bean, tobacco, cotton, or cucumber leaves were heated 1-10 sec at 55 C and then immediately immersed in ice water, some water congestion occurred in a few seconds. If only the distal portion of the leaves was heated before placing the entire leaves in ice water, congestion was increased in the nonheated portions of the leaves, indicating a translocated effect of heat on water congestion. This effect of heat followed by cold was greatest with old bean, cowpea, or tobacco leaves. Water congestion due to heat and other treatments may be an important subject but it does not justify a detailed treatment here.

DISCUSSION

In previous studies (partially reviewed in reference 6), it was believed that heat-induced susceptibility to infection was primarily due to unknown biochemical changes in the host, induced by heat. This probably is still true, but this study shows that physical changes may be involved in some cases. Heat-induced susceptibility and heat-induced retention of inoculum are likely related to selective retention of inoculum by leaves (5), but the details are largely unknown. The application of these results to practical plant pathology is unclear, especially because in nature rusts are usually disseminated as dry spores whereas in experimental work and in this study, they usually were applied as a spore suspension, but temperature probably plays a role in the natural deposition of inoculum, or in the deposition of water necessary for infection. The surprising finding of this study is that retention of water on dipped corn leaves was greatest at temperatures either too low or too high for good plant growth of most plants or for infection with most plant pathogens.

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