Leaf Wettability of Wheat in Relation to Infection by *Puccinia recondita* f. sp. *tritici*

G. D. Statler and J. T. Nordgaard

Professor and former graduate student, Department of Plant Pathology, North Dakota State University, Fargo 58105. Current address of junior author: Agvise Inc., Benson, MN 56215.
Published with the approval of the Director of the North Dakota Agricultural Experiment Station, as Journal Series Paper 1040.
Accepted for publication 14 December 1979.

ABSTRACT

STATLER, G. D., and J. T. NORDGAARD. 1980. Leaf wettability of wheat in relation to infection by *Puccinia recondita* f. sp. *tritici*. Phytopathology 70:641-643.

All wheat cultivars tested had significantly more uredia when Tween-20 was added to the incubation chamber water mist, or when the plant leaves were rubbed prior to inoculation. Hard red spring wheat cultivar Thatcher had significantly more pustules than did the durum cultivars Botno and Rolette. There were more uredia at the longest incubation time (24 hr) for all treatments and cultivars. There was a significant correlation between number of uredia and incubation times between 4 and 24 hrs. Rubbing the leaves or the addition of Tween-20 to the water droplet resulted in significant reductions in water droplet contact angles. The angles were not significantly different among cultivars. Leaves from all cultivars that were studied retained significantly more water when leaves were rubbed or dipped in water-dye plus Tween-20 than when undamaged leaves were dipped in water-dye. Rubbing the leaves or the use of Tween-20 in the water mist improved wettability, increased infection, and reduced variability of infection when water mist was used.

Additional key words: leaf wettability, droplet contact angle, water retention.

The relationship of moisture in the incubation period of *Puccinia recondita* Rob. ex. Desm. f. sp. *tritici* has been known for some time (4). The more free moisture (to the point of complete wetness) that is present on the leaf surface for an incubation period, the more penetrations and infections will occur (1,11). This is why rust is usually more prevalent in years with high rainfall (5). Moisture factors have been used in equations to predict rust development (3,5).

Wettability experiments have been conducted to determine the adhesion of water and pesticide solutions and mixtures to leaf surfaces. The contact angle between a water droplet and the leaf surface has been used as a measure of wettability (7,9,10). Contact angle gives an inverse measurement of the adhesion between a solid and a liquid (6,8). Complete wetting results when the contact angle approaches zero ($\cos \theta \cong 1$) (6). The roughness and waxiness of the leaf surface govern the droplet contact angle. Glaucous leaves with contact angles greater than 120 degrees have many deposits of wax rodlets or platelets (7,9). Contact angles less than 110 degrees occur on leaves that have little wax and a flat wax topography.

Spray retention by plants was affected by the pesticide in the liquid, size and impacting velocity of the droplet, leaf surface properties, presence of a surfactant, and leaf angle (8). The above factors and air/liquid surface tension may influence the amount of water which adheres to leaves by altering the advancing and receding contact angles (8). Weathering throughout the season may cause plants to become highly water retentive (2).

We have observed that uniform infection by *P. recondita tritici* can best be obtained on durum (*Triticum turgidum* L. var. *durum*) cultivars by rubbing the leaves prior to or after misting with water or by the addition of Tween-20 (a surfactant) to the water mist. Water mist alone is sufficient for hard red spring wheat (*Triticium aestivium* L.) cultivars to become uniformly infected. Therefore, this experiment was conducted to investigate the effect of Tween-20 in mist or rubbing leaves on water retention of wheat and infection by *P. recondita tritici*.

MATERIALS AND METHODS

Two cultivars of *T. aestivum*, 'Thatcher' and 'Waldron', and two cultivars of *T. turgidum*, 'Botno' and 'Rolette', were used in this study. Botno and Rolette are moderately susceptible (infection type 3) to *P. recondita tritici* culture 73-35 in the greenhouse. Thatcher is susceptible and Waldron is resistant to culture 73-35.

Culture 73-35 was purified by two successive isolations from single pustules. The purity of 73-35 was tested on isogenic wheat lines. Uredospores were increased on Little Club wheat and stored in glass vials in liquid nitrogen.

Plants were grown in an autoclaved mixture of peat, sand, and clay soil. Plants were placed on a greenhouse bench at 22 ± 3 C with a 16-hr photoperiod until inoculation.

Primary leaves of 10-day-old seedlings were inoculated by spraying with a mixture of 5 mg of uredospores in 1 ml Soltrol 170 (Phillips Petroleum Company, Bartlesville, OK 74004) prior to misting with water or 1% Tween-20 (Sargent-Welch Scientific Co., Skokie, IL 60076) in water. Plants were misted after inoculation. A hand-held mist sprayer with the same size nozzle was used in all experiments. Inoculations and incubation were performed in moist chambers at 18.5 ± 2 C with a 24-hr photoperiod (1,400 lux). Inoculated plants were held at approximately 100% relative humidity. After designated incubation periods, the plants were returned to the greenhouse bench at 22 ± 3 C for the duration of the experiment.

Three treatments were used after inoculation to elucidate the action of the epicuticular wax layer in the adhesion of free moisture to wheat leaves: water misted onto an undisturbed or undamaged leaf surface; water misted onto leaves which had been rubbed lightly to damage the epicuticular wax layer; and water containing 1% Tween-20 was misted onto undamaged leaves (Table 1). Plants were removed from the incubation chamber at 3,4,5,6,8,12, and 24-hr intervals, placed on a greenhouse bench, and dried with a fan. The uredia were counted on 10 leaf sections, each 5 cm long, taken 1 cm from the apical end. The entire experiment was repeated four times. Data were analyzed with a factorial design and Duncan's multiple range test.

The contact angle of water droplets, determined by Fogg's method (7), was taken to measure wettability by water of the external surface of the leaf. A Bausch and Lomb microprojector with its optical axis horizontal was used to project the profile image of a water drop resting on a horizontal leaf surface. A portion of a leaf from Botno, Rolette, Thatcher, or Waldron, was mounted with the adaxial surface up on the horizontal platform. Five treatments were used, of which three utilized a 0.5% crystal violet in distilled water (water-dye) and two a 0.5% crystal violet plus 1% surfactant

0031-949X/80/07064103/$03.00/0

Tween-20 in distilled water (Table 2). In each treatment a droplet about 3 mm in diameter was placed on the leaf surface with a glass pipet and its projection outline was traced. The angles were computed by using the equation of Ebeling (6). Ten measurements were taken for each treatment and the data were analyzed according to Duncan's multiple range test.

The amount of water retained by wheat leaves was determined with a modified method described by Amsden and Lewins (2). This was accomplished by measuring the amount of 0.5% crystal violet retained after leaves were either: dipped in water-dye; dry rubbed and dipped in water-dye; wet rubbed and dipped in water-dye; dipped in water-dye + Tween-20; or wet rubbed and dipped in water-dye plus Tween-20. The leaves were then dipped in 10 ml of 95% alcohol and the optical density determined with a Bausch & Lomb Spectronic 20 set at 420 μm. The amount of water retained per unit leaf area was determined from a calculated dilution curve.

RESULTS

All three wheat cultivars studied (Thatcher, Botno, and Rolette) developed significantly more uredia when Tween-20 was added to the water mist, or when plants were rubbed prior to inoculation (Table 1). Because two- or three-way interactions were not significant, $P = 0.05$, only the treatment means for cultivars, incubation treatments, and time of incubation are shown in Table 1. The coefficient of variability was significantly greater for the water mist treatment than for rubbing or the addition of Tween-20 to the water mist. The hard red spring wheat cultivar Thatcher developed significantly more pustules than did durum cultivars Rolette and Botno. Waldron was resistant to culture 73-35.

There were fewer uredia at the shorter incubation periods and none at 3 hr (Table 1). There were significantly more pustules at 8, 12, and 24 hr of incubation than at 4, 5, or 6 hr. The correlation coefficient between incubation time and number of uredia was 0.85 and was statistically significant, $P = 0.01$.

Data from the water droplet contact angle experiment demonstrated that the advancing ($> 90°$) or receding ($< 90°$) angles were not statistically different among cultivars except for the water-dye droplet on a dry-rubbed leaf (Table 2). Dry rubbing the leaves reduced the droplet contact angle on the leaves of durum wheats Botno and Rolette more than on those of the hard red spring wheats Thatcher or Waldron. Rubbing the leaves or the addition of Tween-20 to the water droplet resulted in significant reductions in contact angles (Table 2, Fig. 1). Rubbing with a wet finger prior to applying the water-dye plus Tween-20 droplet produced the lowest contact angles.

Leaves from all cultivars studied retained significantly more water when leaves were rubbed or dipped in water-dye plus Tween-20 than when undamaged leaves were dipped in water-dye (Table 3). The treatment in which leaves were rubbed then dipped in water-dye plus Tween-20 retained the greatest amount of liquid.

Tween-20 increased water retention by leaves of all cultivars, especially after the epidermal wax layer had been mechanically damaged by rubbing. The most water was retained in the water-dye plus Tween-20 treatment. The water-dye plus Tween-20 adhered to all areas of the leaf resulting in a liquid film on the leaf surface. An undamaged leaf retained the least water-dye which was usually a droplet at the tip, the edge, or midrib of the leaf.

TABLE 1. Average number of *Puccinia recondita* f. sp. *tritici* uredia for three cultivars, three incubation treatments, and seven incubation times

	Uredia produced (avg. no.)
Cultivar	
Botno	4.46 a[z]
Rolette	6.04 a
Thatcher	9.16 b
Incubation treatment	
Water	3.08 a
Water plus Tween-20	7.99 b
Rub and Water	8.59 b
Incubation time (hr)	
3	0.00 a
4	0.84 a
5	1.93 a
6	3.87 a
8	9.63 b
12	13.88 c
24	15.72 c

[z]Values with the same letter are not significantly different according to Duncan's multiple-range test, $P = 0.05$.

TABLE 2. Water droplet contact angles[a] on leaves of two durum and two hard red spring wheat cultivars

Type and Cultivar	Epidermis treatment[b]				
	1	2	3	4	5
Durum					
Botno	143 ab	119 d	92 e	132 bc	63 f
Rolette	143 ab	100 e	95 e	134 bc	59 f
Hard red					
Thatcher	145 a	128 cd	91 e	129 cd	64 f
Waldron	146 a	133 bc	90 e	125 cd	62 f

[a]Mean value of 10 samples. Values with the same letter are not significantly different by Duncan's multiple-range test, $P = 0.05$.

[b]Treatments: 1, water droplet on undamaged leaf surface; 2, water droplet on leaf surface that had been lightly rubbed with a dry finger; 3, water droplet on leaf surface lightly rubbed with a wet finger; 4, water plus 1% Tween-20 droplet on an undamaged leaf surface; and 5, water plus 1% Tween-20 droplet on a leaf surface rubbed with a wet finger.

DISCUSSION

There was a general trend for fewer uredia at shorter incubation times for all treatments and cultivars. This observation corresponds to the work of Politowski and Browning (11). The

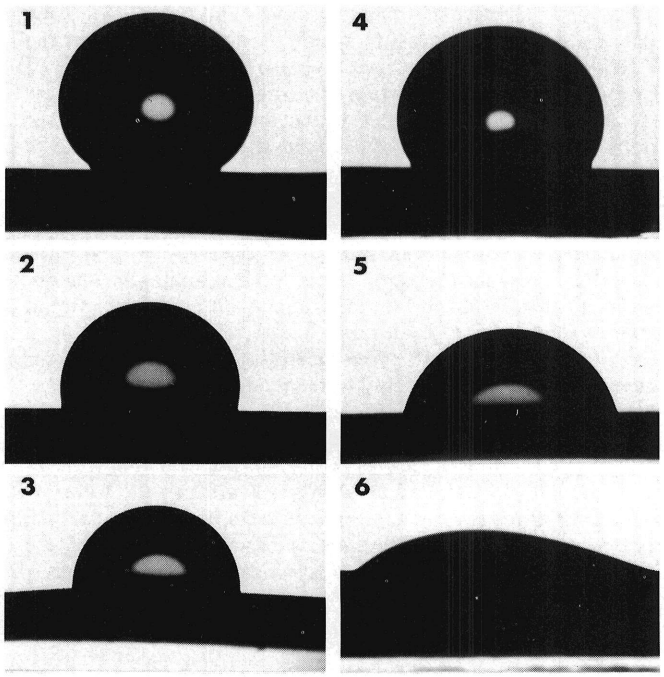

Fig. 1. Advancing and receding contact angles on a leaf of durum wheat cultivar Rolette. **1,** Water-dye droplet on an undamaged leaf, $\theta_a = 139.18°$. **2,** Water-dye droplet on a dry-rubbed leaf, $\theta_a = 107.28°$. **3,** Water-dye droplet on a wet rubbed leaf, $\theta_a = 91.38°$. **4,** Water-dye/Tween-20 droplet on an undamaged leaf surface, $\theta_a = 131.05°$. **5,** Water-dye/Tween-20 droplet on a wet rubbed leaf, $\theta_r = 67.81°$. **6,** Water droplet on a leaf that is covered with a water film, $\theta_r = 27.1°$.

correlation coefficient between number of uredia and incubation time was 0.85, indicating that the longer the incubation time, the more infection, at least between 4 and 24 hr and under our conditions.

Significantly more uredia developed in plant leaves that had been rubbed or misted with water and Tween-20 than in those misted with water only for all cultivars and all incubation times except at 4, 5, or 6 hr (Table 1). The increased numbers of uredia that developed on leaves rubbed prior to inoculation or misted with Tween-20 solution coupled with the fact that more water was retained by wheat leaves after the leaves were rubbed or when Tween-20 was added indicated that wettability factors are very important in the infection process. Data from water droplet contact angle and water retention studies indicated that rubbing or the addition of Tween-20 to the water mist increased leaf wettability and thus was responsible for the increased number of pustules that developed in leaves that received those treatments. Presumably the Tween-20 solution had a lower surface tension which allowed more of it to adhere to the plant surfaces.

Results of prior research on pesticide spray retention suggested an inverse relationship between leaf surface roughness and spray retention (6,7,8,9). The data from our water droplet contact angle and leaf-dipping experiments supported this inverse relationship. Mechanically rubbing the leaves probably flattened and (or) removed parts of the wax layer and left a smoother surface, thereby allowing water to adhere to the leaf. Work of Netting and Wettstein-Knowles (10) indicated that wax morphology was more important in determining contact angles on vegetative wheat leaves than were other structural modifications of the leaf. According to Hall et al (9), water droplet contact angle values lower than 110° indicate that water droplets are in contact with more of the leaf surface than those above 110°. Thus, rubbing, the use of a surfactant, or combinations of these treatments caused droplets to be in closer contact with the leaf surface.

In previous experiments we had difficulty obtaining uniform infection on leaves of durum wheats when they were misted with water after inoculation. Rubbing prior to inoculation or the use of Tween-20 in the water mist improved the amount of infection. Therefore, we hypothesized that some leaf factor such as leaf wettability may be involved. The facts that significantly more pustules developed on plant leaves that had been rubbed prior to the water mist or when Tween-20 was added to the water mist, coupled with the development of significantly more pustules on leaves of the hard red spring wheat Thatcher with all treatments supported differences in leaf wettability. However, data on water droplet contact angles (Table 2) and water retention (Table 3) did not indicate differences between hard red spring and durum cultivars in most cases. But these data did support the idea that the addition of Tween-20 to the water mist or rubbing made the leaf more wettable and increased water retention.

This study also demonstrated that longer incubation periods result in more infection, perhaps due to increased probability of stomatal contact. We also have demonstrated that rubbing the leaves and the use of Tween-20 in the water mist improve wettability and infection and reduce the variability that results from use of water mist only.

TABLE 3. Milliliters of water ($\times 10^{-4}$) retained (per square centimeter) by leaves of two durum and two hard red wheats after dipping in a water-dye solution[a]

Type and Cultivar	Epidermis treatments[b]				
	1	2	3	4	5
Durum					
Botno	5.0 a	11.0 b	31.5 e	16.0 c	35.0 f
Rolette	5.0 a	10.0 b	31.0 e	17.5 c	35.0 f
Hard red					
Thatcher	5.0 a	11.0 b	27.5 de	18.0 cd	37.5 f
Waldron	6.0 a	10.0 b	29.0 e	24.0 de	40.0 f

[a] Mean value from 10 samples. The values with the same letter are not significantly different by Duncan's multiple-range test, $P = 0.05$.

[b] Treatments: 1, undamaged leaf dipped in water-dye; 2, leaf surface rubbed lightly between dry thumb and forefinger and dipped in water-dye; 3, leaf surface rubbed lightly between wet thumb and forefinger and dipped in water-dye; 4, undamaged leaf dipped in water-dye/Tween-20; and 5, leaf surface rubbed lightly between wet thumb and forefinger and then dipped in water-dye/Tween-20.

LITERATURE CITED

1. ALLEN, P. J. 1955. The role of a self-inhibitor in the germination of rust uredospores. Phytopathology 55:259-266.
2. AMSDEN, R. C., and C. P. LEWINS. 1966. Assessment of wettability of leaves by dipping in crystal violet. World Rev. Pest Control 5:187-194.
3. BURLEIGH, J. R., M. G. EVERSMEYER, and A. P. ROELFS. 1972. Development of linear equations for predicting wheat leaf rust. Phytopathology 62:947-953.
4. CHESTER, K. S. 1946. The nature and prevention of the cereal rusts as exemplified in the leaf rust of wheat. Chronica Botanica Co. Waltham, MA. 269 pp.
5. DIRKS, V. A., and R. W. ROMIG. 1970. Linear models applied to variation in numbers of cereal rust urediospores. Phytopathology 60:246-251.
6. EBELING, W. 1939. The role of surface tension and contact angles in the performance of spray liquids. Hilgardia 12:665-698.
7. FOGG. G. E. 1948. Adhesion of water to the external surface of leaves. Faraday Soc. of London Discussions 3:162-166.
8. FURMIDGE, C. H. L. 1962. Studies at phase interfaces: 1. The sliding of liquid drops on solid surfaces and a theory for spray retention. J. Coll. Sci. 17:309-324.
9. HALL, D. M., A. I. MATUS, J. A. LAMBERTON, and H. N. BARBER. 1964. Infra-specific variation in wax on leaf surfaces. Aust. J. Biol. Sci. 18:323-332.
10. NETTING, A. G., and P.WETTSTEIN-KNOWLES. 1973. The physico-chemical basis of leaf wettability in wheat. Planta 114:289-309.
11. POLITOWSKI, K., and J. A. BROWNING. 1975. Effect of temperature, light, and dew duration on relative numbers of infection structures of *Puccinia coronata avenae*. Phytopathology 65:1400-1404.