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TRANSPIRATION CHANGES IN OAT PLANTS INFECTED WITH CROWN RUST[1]

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Water, which is vital in the metabolism of plants, is lost in considerable amounts in normal plant growth. It is lost in two ways: as vapor in the process of transpiration, or as liquid by guttation. Transpiration occurs as evaporation of water from the moist cell walls into the intercellular spaces and then through natural openings such as stomata and lenticels into the outside atmosphere. Some examples of factors that influence the rate of transpiration are: amount of leaf area, leaf structure, orientation of leaves, water content of leaves, temperature, relative humidity, light and wind (5). Abnormal increases in transpiration are usually detrimental to plants resulting in decreased growth.

Investigations of several types of rust diseases have shown that transpiration usually increases with infection and this increase is believed correlated with the rupture of the host epidermis which occurs when the fungus sporulates (1, 2, 6, 9).

The objective of this study was to test the rupture hypothesis using oat plants infected with the crown rust fungus **Puccinia coronata**, Cda. var. **avenae** Fraser and Led. (7). The approach was to use a fully susceptible variety of oats which when infected would produce sporulating pustules that rupture the epidermis, and also a resistant variety that would produce only flecks with no sporulation and epidermal rupture.

MATERIALS AND METHODS

Plants of the susceptible oat variety Lee and the resistant line CI 7998 were grown in half-strength Hoagland's solution in either sealed glass or plastic containers. Glass containers were 100 ml beakers painted black and covered with black plastic covers containing 5 evenly spaced holes 10mm in diameter. The plastic covers were sealed to the top of the beakers with modeling clay. Plastic containers were one-half pint polyethylene refrigerator jars painted black with 10 holes cut in each of the tops. Plants selected for uniformity were inserted into the holes and held in place with polyurethane foam plugs. In each experiment comparable control containers without plants were maintained to correct for water loss through the polyurethane plugs. The solution in all the containers was changed every third day. No apparent difference in growth response was noted between plants in the glass and plastic containers.

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The experiments were carried out in a growth chamber at 60°F. Alternating periods of 14 hours light and 10 hours darkness were maintained using "Gro-Lux" VHO flourescent lamps which produced 450-500 foot-candles at plant height.

Plants were inoculated with uredospores of crown rust race 216. Spores were mixed 1:10 with talc and dusted onto the primary leaves of the plant which were then lightly sprayed with distilled water containing 0.5% "Tween 20." The plants were placed in a saturated atmosphere in the dark for 16 hours then transferred to the growth chamber.

Water loss was determined by differential weight loss of plants and containers weighed to the nearest 0.01 gm. daily at 8:00 a.m. and 9:00 p.m. Measurements were made for 9 days which permitted full sporulation of the fungus. At the end of the experiment the plants were divided into three parts: primary leaves, second leaves, and roots then dried for 36 hours at 100°C and weighed to the nearest mg.

RESULTS

The symptom pattern of crown rust infection was observed in the susceptible Lee oats as follows: on the fourth day following inoculation light flecking appeared which developed into heavy flecking with a few sporulating pustules on the fifth and heavy sporulation and rupture on the sixth and seventh days. On the resistant CI 7998 very faint flecking appeared on the fourth day and increased in size and intensity through the fifth and sixth with the whole leaf becoming chlorotic then necrotic through the seventh and eighth days. In no instance were sporulating pustules that ruptured the epidermis produced on the resistant CI 7998.

The water loss data from four separate experiments using 75 inoculated and non-inoculated plants each of the susceptible Lee were pooled and are shown in graphic form in Figure 1. The pooled data were also subjected to analysis of variance and are presented in summary in Table 1.

The graph shows that infected plants on the second to fourth day after inoculation lost less water than uninoculated plants. This reduction was apparent during the day, but not during the night. Five days following inoculation, the infected plants began to show a greater water loss than the uninoculated controls. This pattern increased in magnitude and persisted until the ninth or final day. The increased water loss was apparently associated with the rupture of the epidermis, since pustules began to appear on the fifth day and increased in size and number from that day onward. Analysis of the data shows the day x treatment interaction (Table 1) to be highly significant which indicates that the change from the fifth day onward was a true relationship.

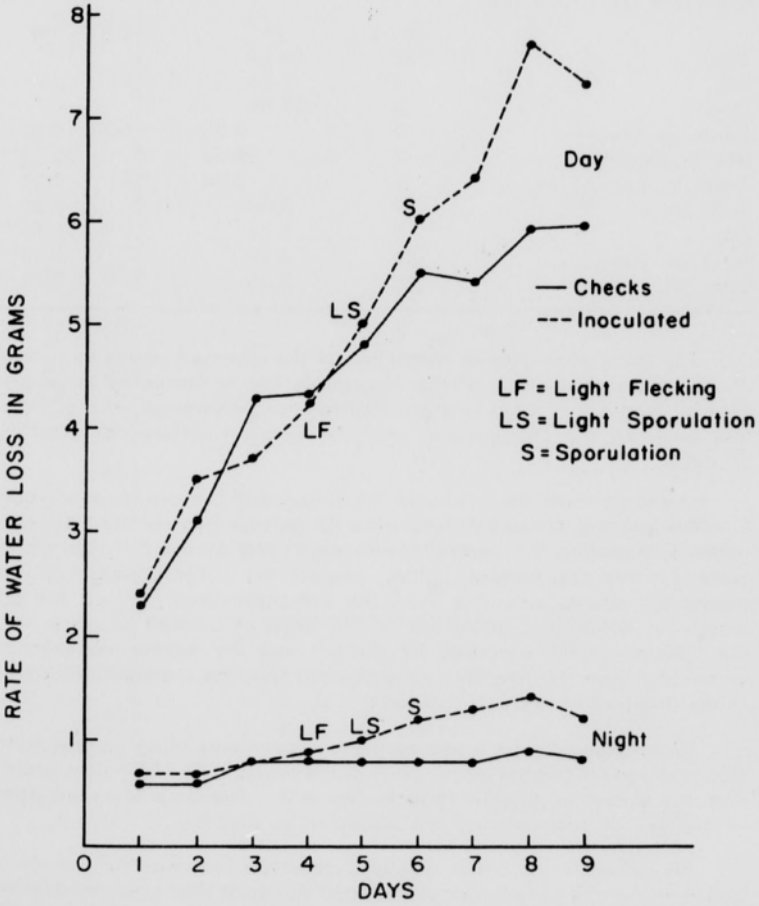

Figure 1. The effect of crown rust race 216 and time on the day and night water loss of plants of the susceptible variety LEE. Graph constructed from averages of four experiments.

Table 1. This effect of crown rust race 216 on the water loss of the susceptible variety Lee. Data presented from four experiments in analysis of variance summary table.

ANALYSIS OF VARIANCE

	df	ss	ms
Total	143	54.03	
Rep.	3	2.76	
Treat.	3	35.89	
Inocu. vs. Checks	1	0.33	0.33
Day vs. Night	1	35.52	35.52**
Treat. vs. Time of Day	1	0.04	0.04
Error (A)	9	3.00	0.33
Days	8	5.74	0.72**
Days vs. Treat.	24	4.39	0.18**
Error (B)	96	2.25	0.02

The daily difference in water loss of the diseased plants as percent of non-diseased controls of the susceptible Lee is presented in graphic form in Figure 2. There is a great difference percentage wise in water loss between the two groups, with the greatest differential occurring at night.

Dry weights of the tissues of the susceptible Lee are given in Table 2. The infected primary leaves were 71 percent heavier than the non-infected, however, the second leaves and roots of the diseased plants were 21 and 35 percent lighter, respectively. Total weight of the parasitized plants was less than the non-parasitized. When the dry weight of tissue was calculated on the basis of amount of water loss, the infected plants produced 27 percent less dry matter per gram of water loss than the healthy. It is obvious that the metabolic efficiency of the diseased plants was reduced.

Water loss data from two separate experiments using 35 inoculated and and non-inoculated plants each of the resistant CI 7889 were pooled and are shown in graphic form in Figure 3. The data were subjected to analysis of variance and are presented in summary in Table 3.

No difference in water loss was observed between the inoculated and non-inoculated resistant plants until the sixth day after inoculation. Following this time the diseased plants lost less water during the day than the healthy through to the final day. Examination of the days x treatment interaction (Table 3) shows this value to be highly significant which indicates that the reduction in water loss represents an actual relationship. No marked difference in nighttime water loss was observed between the two groups of plants.

Consideration of the dry weight data (Table 3) shows that the fungus did damage the resistant oat plants although the weight reduc-

tion was not nearly as great as in the susceptible Lee plants. Furthermore, the parasitized CI 7998 showed greater efficiency in converting water loss to production of dry matter than did Lee.

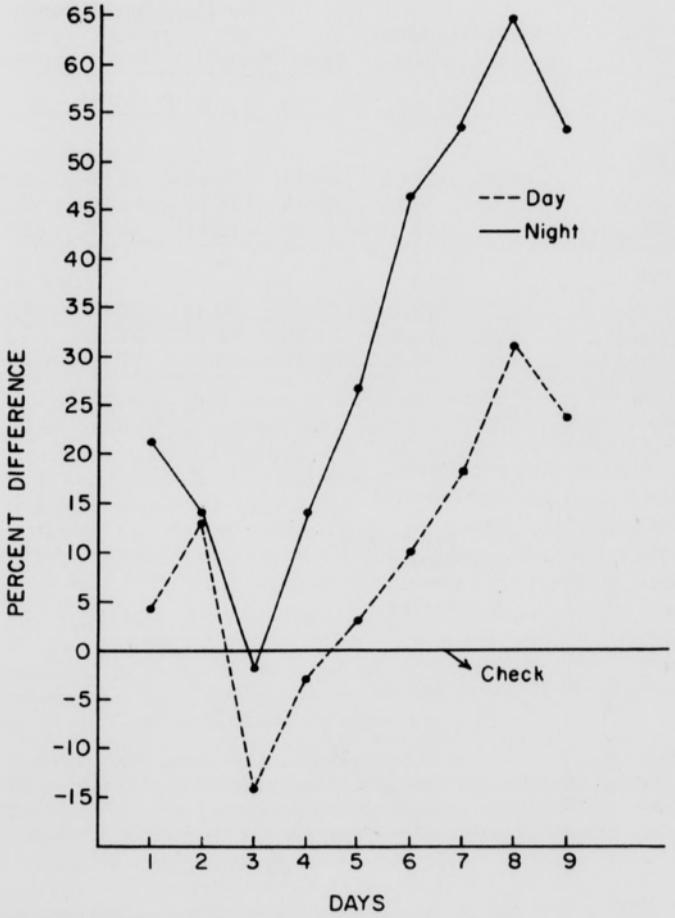

Figure 2. Percent difference in daily rate of water loss between inoculated and non-inoculated plants of the susceptible LEE. Graph constructed from averages of four experiments.

Table 2. Dry weight of plant parts of the inoculated and non-inoculated varieties used in the water loss experiment.

	Primary Leaves	Secondary Leaves	Roots	Total dry weight	Total water loss	One Gram water loss produces dry weight
	in mg	in mg	in mg	in mg	in Grams	in mg
Lee						
Checks	14.21	89.02	46.81	150.04	48.55	3.09
Inoculated	24.33	70.20	30.49	125.11	55.51	2.25
% Difference	+71.22	—21.04	—34.86	—16.61	14.33	—27.18
CI 7998						
Checks	8.29	56.94	15.25	80.48	30.33	2.65
Inoculated	6.47	53.94	11.78	72.19	28.13	2.57
% Difference	—21.95	—5.27	—22.75	—10.30	—7.25	—3.02

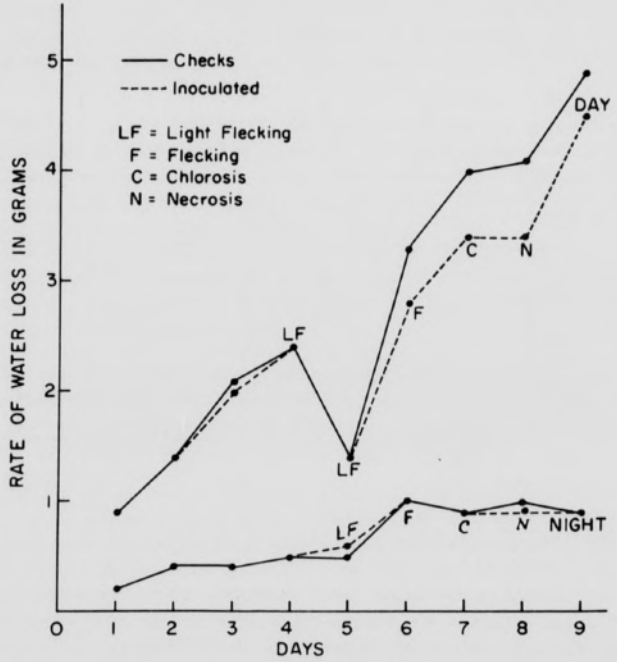

Figure 3. The effect of crown rust race 216 and time on the day and night water loss of plants of the resistant line CI7998. Graph constructed from averages of two experiments.

Table 3. The effect of crown rust race 216 on the water loss of the resistant line CI 7998. Data presented from two experiments in analysis of variance summary table.

ANALYSIS OF VARIANCE

	df	ss	ms
Total	71	31.53	
Rep.	1	0.01	
Treat.	3	17.21	
Inocu. vs. Checks	1	0.06	0.06
Day vs. Night	1	17.07	17.07**
Treat. vs. Time of Day	1	0.08	0.08
Error (A)	3	0.06	0.02
Days	8	9.56	1.19**
Day vs. Treat.	24	4.58	0.19**
Error (B)	32	0.11	0.003

DISCUSSION

The data show that there is increased water loss in susceptible crown rust infected oat plants. This increase appears to be correlated with sporulation of the fungus and the consequent rupture of the epidermis since it begins and increases during this period. This conclusion is further supported by the fact that this increase was observed during the night when the major avenues of transpiration, the stomata, were closed. These results agree with the observations of other workers (1, 2, 6, 9, 10).

The nighttime water loss in the susceptible Lee was proportionately greater than the daytime loss which corresponds to the findings of Johnston and Miller with leaf rust of wheat (3, 4). This effect is undoubtedly due, for the most part, to the lack of stomatal transpiration during the night and its confounding effect during the day.

A reduction in water loss such as occurred in the inoculated susceptible Lee on the second through fourth day was also reported by Gerwitz and Durbin to occur in bean plants affected with rust (2). This reduction is apparently associated with stomatal transpiration since it did not occur during the night periods when the stomata were closed. It is possible that this reduction in water loss is due to the plugging of the intercellular spaces and stomata with fungal tissue, such as described by Rothman (8), so that the normal movement of water vapor to the exterior of the leaf is blocked.

The reduction in water loss that occurred in infected resistant CI 7998 plants from the time of flecking onward corresponds to the reduction in the susceptible Lee during the flecking stage. It would appear that there is a common transpirational reaction to rust infection by both susceptible and resistant plant types prior to and during the flecking stage, but this changes markedly upon sporulation of the fungus and

rupture of the susceptible host epidermis. Further evidence that rupture is important in increased water loss is indicated by the fact there was no increase in nighttime loss in inoculated plants of CI 7998 in which rupture did not occur (Figure 3).

The tremendous increase in the weight of the infected primary leaves of the susceptible Lee contrast sharply with the marked decrease in the corresponding resistant CI 7998 (Table 3). Part of the increase in Lee was undoubtedly due to the weight of the fungal tissue, but the exact amount could not be calculated. When the decrease in weight of the non-infected **second leaves and roots of Lee is considered,** it would seem that there was a preferential translocation of metabolites from these organs to the infected primary leaves. This preferential translocation apparently did not occur in comparable degree in the resistant CI 7998.

It is clear from the data (Table 3) that the rust fungus adversely affects growth in both types of oat plants, although the effect is much less in the resistant CI 7998. The results also show that the pathogen affects the whole plant and not just the infected tissue in which it resides.

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