PERONOSPORA TABACINA IN TOBACCO: TRANSPIRATION, GROWTH, AND RELATED ENERGY CONSIDERATIONS

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Summary

An investigation of the influence of *Peronospora tabacina* Adam on the transpiration and growth rates of the leaves of *Nicotiana tabacum* L. in three environments is described.

In the presporulation stage of infection the following results were obtained:

(1) The main contribution to the enhanced total daily water loss from diseased plants occurred at night when the ratio of diseased to healthy plant transpiration approached 2·0.

(2) Leaf growth was almost zero in the diseased plant from the third day after inoculation while growth in healthy plants continued at approximately 10% per day.

In the postsporulation stage of infection, transpiration from the diseased tissue decreased to less than that from healthy tissue, the exact ratio being controlled by the environment.

Temperature and temperature gradient measurements over healthy and diseased leaves at night showed that:

(1) Temperature of presporulation diseased tissue was above that of healthy tissue by 0·1–0·2°C.

(2) The transport of sensible heat from the air to the leaf surface was less for diseased than healthy tissue by an almost constant amount.

(3) Energy balance considerations adequately accounted for the measured night transpiration from healthy material but only half the requirement for the diseased tissue.

The implication of the observed transpiration rates and of energy balance considerations are discussed.

I. INTRODUCTION

Blodgett (1901) observed that plants of *Rubus* sp. infected by *Gymnoconia interstitialis* (Schl.) Logh. transpired nearly twice as much as disease-free plants. Reed and Cooley (1912) working with *Gymnosporangium juniperi-virginianae* Schm. claimed that infection lowered the transpiration rate from apple leaves to one-quarter that of comparable healthy leaves.

A more complete analysis of the effect of rust infection on foliage transpiration was reported in a series of papers by Johnston and Miller (1934, 1940). They found that the overall transpiration of a susceptible wheat plant heavily inoculated with *Puccinia triticina* Eriks. at anthesis was increased by 17%, and that the diurnal rhythm of transpiration was seriously disturbed.

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Other studies concerning the influence of disease on transpiration include the effect of smut (*Ustilago tritici* Pers. Rostr.) (Kourssanov 1928) and the powdery mildews *Erysiphe graminis* f. sp. *hordei* (Graf-Marin 1934) and *E. polygoni* D.C. (Yarwood 1936) on their appropriate hosts, are in general agreement with the results of Johnston and Miller (1940).

The studies on leaf-infecting fungi referred to above have been confined to a demonstration of changes in transpiration rates in tissues in which sporulating lesions occurred. We report here an analysis of the effect of blue mould (*Peronospora tabacina* Adam) on the transpiration and growth rates of the tobacco leaf during the incubation phase of infection and following induction of sporulation. Preliminary measurements of air temperature gradients above an infected *P. tabacina* leaf surface are discussed in relation to the phenomenon of increased nocturnal transpiration of diseased leaf tissue.

II. Materials and Methods

Three parallel series of experiments were conducted to measure the effect of *P. tabacina* on the transpiration and growth rate of the tobacco leaf under three different controlled environmental regimes. The glass-house conditions during the course of the experiments described below were:

Regime I: day temperature 20°C, day R. H. *c.* 48%; night temperature 15°C, night R. H. 50–70%.

Regime II: day temperature 25°C, day R. H. *c.* 35%; night temperature 18°C, night R. H. 35–50%.

Regime III: day temperature 31°C, day R. H. 40–42%; night temperature 21°C, night R. H. 42–50%.

The change from night to day temperatures and vice versa occurred at 0830 hr and 1630 hr respectively. Experiments were made under natural light conditions in July in Canberra (lat. 35° 20′).

Tobacco plants (*Nicotiana tabacum* L. cv. Virginia Gold) of similar age and size (mean stem length 15 cm) grown under uniform conditions in 6-in. clay pots were used as the host material. The apical buds of the plants were removed and the leaf number reduced to three medium grown leaves. Five plant replicates and two pot blanks were used in each experiment.

Initially, the soil water in the pots was adjusted by watering to saturation and then allowing them to stand until excess water had drained away (Bliss, Kramer, and Wolf 1957). This level of soil moisture was maintained by daily addition of water, equivalent to the measured daily water loss. Water loss from surfaces other than that of the plants was prevented by enclosing each pot in a double polythene bag which was firmly tied around the base of the plant stem. Inoculation with a dense spore suspension of *P. tabacina* was done on a rotary turntable to ensure uniform infection of all leaves (Cruickshank 1958) and transpiration was measured gravimetrically.

Individual leaf areas were calculated as 60% of length by breadth measurements on the 3rd, 5th, and 7th days after inoculation. Leaf area as used in the

calculation of leaf growth rate is the sum of the individual areas of three leaves on each plant. Stem area was considered negligible in relation to leaf area and was ignored.

Transpiration is expressed as grams per square centimetre of leaf area (one surface only). Weighings were made at 0830 and 1630 hr from the 3rd–8th day after inoculation. On the 8th night uniform sporulation over the inoculated leaves was induced by returning the plants to a humidity cabinet (R.H. 97%, temp. 20°C)

TABLE 1

CHANGE IN TRANSPIRATION RATIOS DURING THE INCUBATION PHASE OF INFECTION (PRESPORULATION)

Ratios of healthy to diseased transpiration given

Day	Time after Inoculation (hr)	Transpiration Period	Regime		
			I	II	III
4	72–88	Night	0·875	0·619***	0·718***
	88–96	Day	0·900	0·842	0·863*
5	96–112	Night	0·655**	0·529***	0·558***
	112–120	Day	0·912	0·984	0·914
6	120–136	Night	0·742*	0·514***	0·509***
	136–144	Day	1·025	1·202	1·026
7	144–160	Night	0·630**	0·530**	0·479***
	160–168	Day	1·134	1·212	0·943
8	168–184	Night	0·475***	0·457**	0·376***
	184–192	Day	1·562*	1·244	1·018
Significance of trend in transpiration ratios		Night	*		***
		Day	**	**	*

* $P<0·05$. ** $P<0·01$. *** $P<0·001$.

from 1630 hr to 0830 hr. Transpiration from the sporulating leaf tissue was determined over a further 24 hr after the plants had been returned to their respective environments and conditioned to them for 12 hr. For statistical analysis leaf area and transpiration data were subjected to logarithmic transformation. Results are presented in Tables 1 and 2 and Figures 1, 2, and 3.

Stomatal number per unit area of leaf was determined after fixing and clearing the leaf tissue with lactophenol (Berry 1959).

Temperature gradients above healthy and infected leaves exposed side by side at night in environmental regime III were measured by fine wire thermocouples. Figure 4 shows the apparatus clamped on the healthy leaf. Each leaf was held

between two "Perspex" frames. The top frame carried an inner smaller frame which supported the thermocouple wires of 48 S.W.G. (*c.* 0·004 cm dia.) copper and copper–nickel. The couples in each frame were connected differentially and the absolute temperature of one couple in each frame was measured with respect to a standard junction maintained in melting ice. The vertical separation between adjacent couples was 0·1 cm and the lowest was 0·1 cm from the bottom of the inner frame which was adjustable in height within the main frame. The couples were not placed one above the other in the vertical plane but were arranged in ascending order over a horizontal distance of 2·5 cm. This was an aid to construction and prevented the screening of any one by its neighbours. Leads were connected to one side of each upper main frame and terminated in a switchboard. An eye-reading reflecting

TABLE 2

MEAN TRANSPIRATION RATES OF DISEASED AND HEALTHY TOBACCO LEAF IN IMMEDIATE POSTSPORULATION PERIOD

Transpiration rates given as g cm^{-2} 24 hr^{-1}

Regime	Diseased Tissue	Healthy Tissue
I	0·105	0·111
II	0·084***	0·144***
III	0·123**	0·179**

** $P<0·01$. *** $P<0·001$.

galvanometer was used to indicate the temperatures and temperature differences at and between the various positions in turn. Alternate runs for the two leaves were made about every 30 min over a period of several hours. Temperature differences could be read to 0·01°C and the absolute temperature to 0·1°C as the circuit resistances and galvanometer sensitivity were adjusted to give these resolutions.

III. RESULTS

(*a*) *Effect of Infection on Transpiration*

It was clear from inspection of the data that the 24-hourly transpiration from non-sporulating diseased tissue under each of the three environmental regimes was greater than that from healthy tissue growing under the same conditions. The transpiration response curves over 5 days of the incubation phase (72–182 hr after inoculation) of diseased and healthy tobacco leaves separated into night (1630–0830 hr) and day transpiration (0830–1630 hr) are illustrated in Figures 1 and 2. The ratio of the transpiration rate of healthy to diseased leaves over the same period of the incubation phase and the significance of trend in these ratios are presented in Table 1. No significant water loss occurred from the pot blanks.

The absolute magnitudes of the day transpiration were greater in all cases than those for the nights and for both day and night the transpiration levels increased with increasing temperature from regimes I to III. The transpiration rates were higher for the diseased than the healthy plants at night and the difference increased during the presporulation period. On the other hand, the transpiration

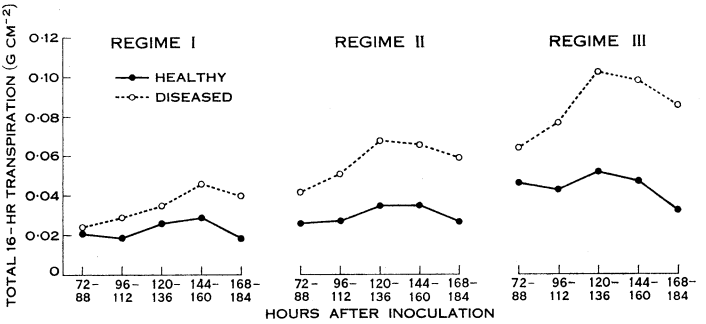

Fig. 1.—Night (1630–0830 hr) transpiration comparison between healthy and diseased plants (presporulation phase) in three environments.

rates during the day for the healthy and diseased plants were not very different but there was a definite trend towards a reduction in transpiration of the diseased relative to the healthy plants during the presporulation period. In Table 1 we give the ratios of healthy to diseased transpiration rates together with the statistical significances of the departure of the ratios from the expected value of unity.

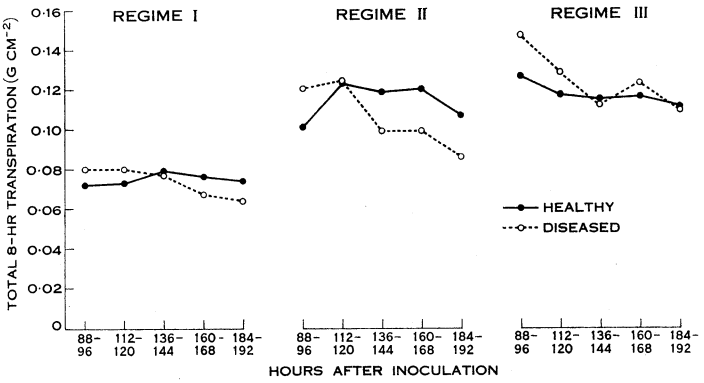

Fig. 2.—Day (0830–1630 hr) transpiration comparison between healthy and diseased plants (presporulation phase) in three environments.

Transpiration from diseased leaves over the 24-hr period before induction of sporulation was greater than that from healthy leaves. The mean total transpirations in the immediate postsporulation period are presented in Table 2. It is apparent from these results that transpiration of tobacco leaf tissue after *P. tabacina* had been induced to sporulate was not significantly different in regime I and was significantly less in regimes II and III than that of healthy tissue.

(*b*) *Effect of Infection on Leaf Growth*

To get a clear picture of the effect of *P. tabacina* on growth, young plants with rapidly expanding leaves were selected. Growth response curves of diseased and healthy leaves from the 3rd to the 7th day after inoculation under the three environmental regimes are illustrated in Figure 3. As for transpiration, earlier measurements were not possible as an initial 48-hr incubation period at high humidity and a 24-hr period for equilibration of the plants to their changed environment were required before measurements could be taken.

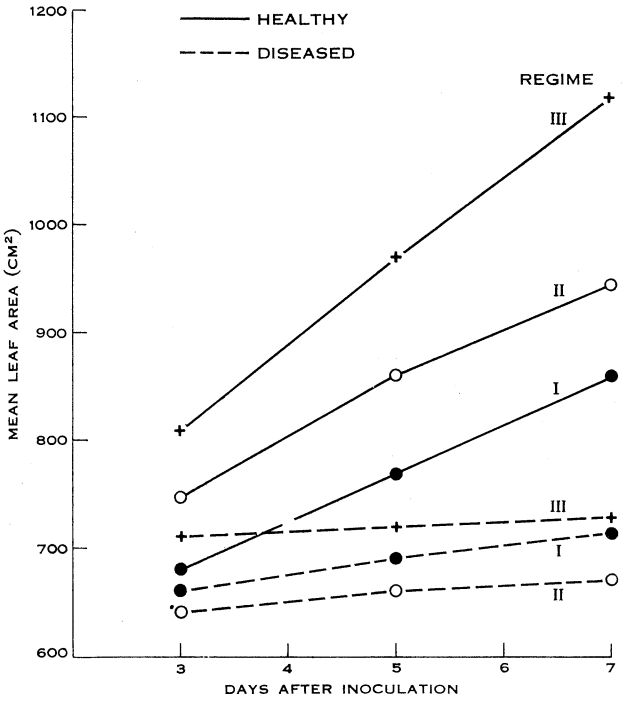

Fig. 3.—Leaf growth in healthy and diseased plants (pre-sporulation phase) in three environments.

In all instances the growth over the incubation period and over the same time interval for the healthy plants increased linearly with time. In terms of relative growth the diseased *v.* healthy slope comparisons within the same environments were significantly different ($P<0{\cdot}001$). Comparisons between healthy tissue subject to environmental regimes I and III showed a significant difference ($P<0{\cdot}05$) as was expected. For diseased plants the relative growth under environmental regime I differed significantly ($P<0{\cdot}01$) from those in the other two; regimes II and III were not significantly different in growths.

(*c*) *Possible Difference in Leaf Characteristics associated with Increased Transpiration: Stomatal Number per Unit Area of Leaf*

Mean counts of stomata per unit area (mm^{-2}) of leaf surface in both upper and lower epidermis (diseased 40·8, 177·0; healthy 39·3, 180·3 respectively) over

a series of 10 leaf samples show that in spite of the large difference in leaf areas, there was no significant difference between the diseased and healthy tissue (cf. Ziegenspeck 1944).

(*d*) *Temperature Gradient Measurements above Non-sporulating Leaves*

Figure 5 shows the result for two 20-min periods separated by about 2 hr. Each curve gives the mean for five separate measurements during the time intervals concerned. In both instances the temperatures at 0·8 cm from the leaf surfaces were the same to within the accuracy of measurement (0·1°C). In drawing the curves of Figure 5 it is assumed that these temperatures were identical although the subsequent argument would not be affected if they were not. In both these periods and in all others investigated but not illustrated the temperatures of the air immediately above the surfaces were higher over the diseased than over the healthy leaves. In the period 2030–2050 hr there was no definite temperature gradient above the diseased tissue but a marked gradient above the healthy tissue.

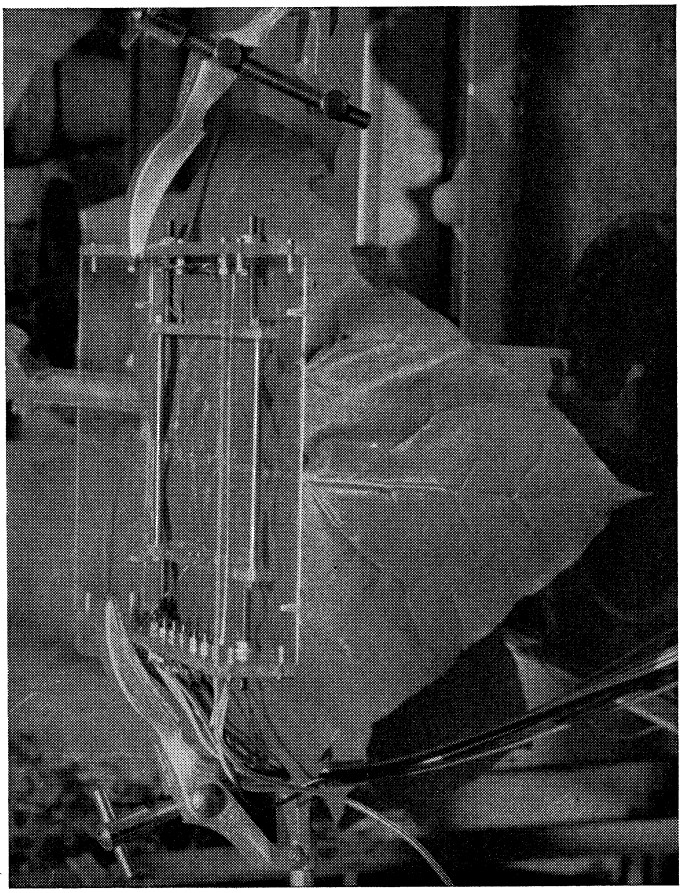

Fig. 4.—Method of clamping thermocouple apparatus against tobacco leaf.

From 2220–2240 hr there were gradients above both, that above the healthy tissue being larger than that above the diseased. In all cases where the gradient existed there was a transfer of sensible heat from the air towards the leaf surface (i.e. down the temperature gradient).

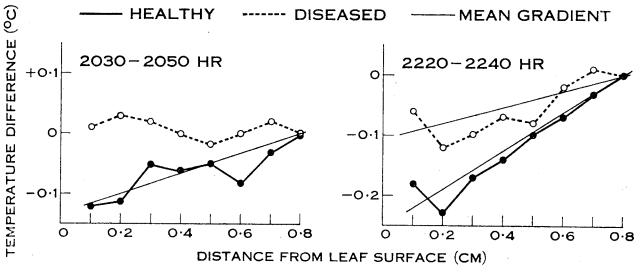

Fig. 5.—Examples of temperature profiles above healthy and diseased leaves in environmental regime III at 120–136 hr following inoculation.

(*e*) *Energy Considerations*

It is possible, by assuming that the heat transfer was due to molecular processes, to calculate the quantities being supplied from the air. The assumption that the processes are molecular rather than turbulent may not be valid but some support for this approach is given by the work of Vehrencamp (1953). He found that in the open at night in very calm and stable conditions close to a smooth natural surface half the actual heat transfer could be accounted for by assuming molecular

TABLE 3

SENSIBLE HEAT TRANSFER (CAL CM^{-2} SEC^{-1}) TO LEAF SURFACE

Period	Diseased Tissue	Healthy Tissue	Difference
2030–2050 hr	0	$1{\cdot}05\times10^{-5}$	$1{\cdot}05\times10^{-5}$
2220–2240 hr	$0{\cdot}81\times10^{-5}$	$1{\cdot}92\times10^{-5}$	$1{\cdot}11\times10^{-5}$

transfer. In a closed glass-house in which the layers of air immediately above the leaf are stable and undisturbed it is not unrealistic to use the concept of molecular diffusion to indicate the likely magnitude of this transfer. Later, more evidence which suggests that molecular rather than turbulent processes were operative will be given. Taking the thermal conductivity of air as $6{\cdot}14\times10^{-5}$ cal cm^{-1} sec^{-1} deg^{-1} and drawing mean temperature gradients through the curves of Figure 5 the sensible heat transfer in the two periods has been calculated and is given in Table 3. We should note that these values represent the minimum transfer that could have taken place as the presence of turbulent processes would have enhanced the values. Further, gradients above one of the two leaf surfaces have been considered so to

arrive at likely figures for the total transfer the values given in Table 3 should be doubled. Thus it appears that the presence of disease in the tissue reduces the amount of sensible heat transfer from the air by about 2×10^{-5} cal cm^{-2} sec^{-1}.

Although leaf surface temperatures were not measured the curves of Figure 5 suggest that the diseased leaf was *c.* 0·1–0·2°C higher than the healthy leaf. This implies that the outgoing radiation from one surface was about $1\cdot5\times10^{-5}$ cal cm^{-2} sec^{-1} greater for the diseased than the healthy leaf. Considering both surfaces the excess radiative heat leaving the diseased leaf was about 3×10^{-5} cal cm^{-2} sec^{-1}.

As the general environment of both leaves was the same an extra energy supply of about 5×10^{-5} cal cm^{-2} sec^{-1} must have been available to the diseased leaf on these accounts. To this must be added the energy required to maintain the enhanced transpiration rate. The temperature observations reported were made in the period 120–136 hr after inoculation when, according to Figure 1, the diseased and healthy leaves transpired *c.* 0·1 and 0·05 g water per cm^2 leaf surface respectively in the 16-hr night. This represents an average energy consumption of *c.* 10×10^{-4} and 5×10^{-4} cal cm^{-2} sec^{-1} respectively. The consideration above shows that only a very small fraction of these energy requirements was satisfied by extraction of heat from the air.

However, since the glass-house was heated the temperature of the inside of the roof was near to the general ambient temperature. Assuming this surface to be at 20°C and the leaf surface temperature to be at 16·5°C we may calculate the net gain of radiative energy to the upper surfaces of the leaves. The temperature observations indicate that the leaf surfaces were in the region of 16·5°C but there was some fluctuation throughout the predominantly clear night. Considering the surfaces in question to radiate as black bodies the net gain to the leaves amounted to about $4\cdot5\times10^{-4}$ cal cm^{-2} sec^{-1}. There would have been little or no net transfer of radiant energy to the undersurfaces of the leaves. Thus the energy requirement necessary to sustain the observed transpiration rate from the healthy leaves was provided in the main by radiative exchange. There was a small contribution of heat directly from the air and presumably also from the respiratory process.

For the diseased leaf we can offer no such satisfactory explanation. The energy required for about half the observed rate of transpiration is provided by radiative exchange but it is difficult to envisage any exchange process external to the tissue itself which could supply the deficiency. One is therefore forced to consider the energy released in the respiratory process as a possible source.* The dry weight of the plants was in the region of 5 g and each milligram of diseased tissue would be required to liberate 70 mm^3 CO_2/hr/mg dry weight in order that the extra energy required could be relased. In arriving at this value it is assumed that the reaction

$$C_6H_{12}O_6+6O_2\rightarrow6CO_2+6H_2O+673 \text{ kcal}$$

was operative, that the plant tissue consisted of hexose alone, and no energy was consumed in growth and synthesis. A respiration and corresponding release of

* It has been suggested to us that if the water transpired originated from the soil, then there would have been a transport of heat to the leaves. Calculation shows that even if this water arrived in the leaf at its day temperature (31°C) then, in falling to 16°C, the quantity of heat made available for the diseased leaf would be *c.* $3\cdot0\times10^{-5}$ cal cm^{-2} sec^{-1} or about 3% of the total requirement.

energy at this rate was quite impossible as a weight of hexose in excess of the dry weight of the plant would have been consumed in the one night. However, the rate of growth of the healthy plant was about 10% and that of the diseased plant less than 1% per day at this time. A 10% increase in leaf area corresponded to a dry weight increase of 0·5 g per day. The energy liberated by 0·5 g hexose in oxidation in accordance with the above reaction is about 1·9 kcal. This energy spread throughout the night over the plant leaf area represents $3{\cdot}3\times10^{-5}$ cal cm^2 sec^{-1}, or less than 10% of the extra requirement.

Support for the assumed molecular nature of the heat transport is obtained from a consideration of the water vapour transfer away from the leaf surface. Making the assumption that the air in immediate contact with the leaf surface is saturated (it may contain somewhat less water than this) and that at a distance of 2 cm from the surface the water vapour content has fallen to the ambient level (about 45% R.H.), the amount of vapour transferred may be calculated. Taking the diffusivity for water vapour as 0·25 cm^2 sec^{-1}, the amount of water leaving each surface of the leaf (top or bottom) is $1{\cdot}03\times10^{-6}$ g cm^2 sec^{-1} and the energy used in evaporating this was $6{\cdot}2\times10^{-4}$ cal cm^{-2} sec^{-1}. For the two surfaces this amounts to $1{\cdot}2\times10^{-3}$ cal cm^{-2} sec^{-1}. This very approximately is the energy used in the diseased leaf as calculated from the 16-hr water loss.

IV. Discussion

The downy mildews caused by leaf-infecting members of the Peronosporaceae constitute a large group of economically important diseases which as a whole are very sensitive to the water relations of their hosts and their environments (Yarwood 1956). Sporulation of *P. tabacina*, the causal organism of downy mildew of tobacco (blue mould) is closely dependent on the diffusion pressure deficit of infected leaf tissues and the relative humidity of the ambient air in the immediate vicinity of the leaf surface (Cruickshank 1958).

Although the overall daily transpiration of infected tobacco leaf is greater than that of healthy leaf tissues it seems clear from the foregoing data that the increase in the transpiration of infected leaves prior to sporulation is very largely due to the higher rate of transpiration of infected plants during the night. The higher overall rate of transpiration of diseased plants could be expected to disturb the water economy of the host plant which under some field situations could influence the growth and quality of the tobacco leaf. Under near optimal relative humidity conditions for the sporulation of *P. tabacina* (Cruickshank 1958), the higher transpiration rate of diseased tissues during the night could produce conditions in the leaf sublamina layer favourable for sporulation. The former situation would be directly unfavourable for the growth of the tobacco plant. The latter condition would influence the reproduction of *P. tabacina* and thus play an important role in the epidemiology of blue mould of tobacco.

Previous workers studying foliage infections have confined their experiments to tissues exhibiting normal disease symptoms, viz. sporulating lesions. Studies of *P. tabacina*-infected leaf, during the 24 hr after sporulation had been induced, showed

that transpiration was either equal to or less than that of healthy plants grown under similar conditions (Table 2). This result is in agreement with that of Reed and Cooley (1912) but is in direct opposition to most of the literature in this field. It is suggested that this difference is due to the physically different nature of tobacco leaf tissue in comparison with that of cereals studied by most other workers, and to its rapid physiological collapse after sporulation. This is supported at least in part by Schramm and Wolf (1954) who, in a study of the transpiration of tobacco infected with black shank (*Phytophthora parasitica* var. *nicotiana* (Breda de Haan-Tucker)), state that water loss decreases as the disease progresses.

Reduction in plant growth due to disease has been reported by several authors (Graf-Marin 1934; Johnston and Miller 1934). The most outstanding feature of the growth data presented in Figure 3 is the almost complete inhibition of leaf expansion and the rapidity with which it occurred after infection. This effect on growth probably explains the distortion of young partially infected leaves which are sometimes observed in the field. It could also be responsible for the characteristic downward curving of unilaterally infected leaf petioles, and for the stunting of tobacco plants which have become systemically infected at an early stage of growth.

Several authors have speculated on the reasons for the higher rate of transpiration from diseased tissues. A change in the distribution and number of stomata was claimed by Dodge (1923). Graf-Marin (1934) attributed the increase mainly to an increase in the opening of stomata in diseased leaves. Johnston and Miller (1940) considered the higher rate of transpiration of diseased plants at night was due partially to transpiration through ruptures in the cuticle caused by sporulating uredia, in the case of the leaf rust (*P. triticina*) which they studied, and partially to the transpiration of the fungus itself. Stomatal number, cuticle rupture, and direct transpiration from external fungal conidiophores or other reproductive structures were not demonstrated in the present studies to be important contributing factors to greater transpiration. It must be concluded that in the present instance nocturnal transpiration prior to sporulation is primarily cuticular.

In a recent comprehensive review Raschke (1960) considers the factors which influence the energy balance and transpiration rates of individual healthy leaves. However, we believe the present paper to be the first attempt to investigate the different energy requirements of healthy and diseased leaves in identical environments. In the previous section we showed that the energy requirements necessary to sustain the observed transpiration rates from healthy tissue were satisfied. We were unable to suggest how the extra energy required for the enhanced transpiration from diseased tissue was obtained from external sources. We can only conclude that some other energy source is available associated with the presence of the fungus in the leaf tissue. According to Allen (1954) diseased tissue shows an increased respiration which he attributes to the utilization of energy-rich phosphate which permits a more rapid aerobic breakdown of carbohydrate. We see here that this explanation is not tenable as complete breakdown of all available carbohydrate does not give the required energy level necessary to maintain the enhanced transpiration rate. We are not able at the present stage to account, on an energy basis, for the high transpiration observed.

One characteristic of the curves of Figure 5 for the period 2220–2240 hr requires comment. It will be noted that the minimum temperature measured occurred not at the 1-mm level but at the 2-mm level and that the temperature distribution is reminiscent of that found by Lake (1956) above bare soil on very calm nights in the open. Normally it is considered that the only important source of radiative heat loss is the surface itself, in our case the leaf surface. However, it has been suggested (Ramanathan and Ramdas 1935; Rider and Robinson 1951) that under very stable conditions the air layer in contact with the surface may be in radiative rather than conductive equilibrium with its surroundings, the eddy conductivity approaching the molecular value. The unusual density distribution is remarkable and its maintenance for long periods not easily explained. However, for our present purpose it is sufficient to note that this type of temperature distribution in very stable conditions is not unknown and its existence may indicate the onset of molecular diffusion as we have assumed in our calculation above.

These simple and preliminary measurements of microenvironment reported here at least serve to show that the presence of disease in the leaf exercises some control. We hope to undertake a further more comprehensive investigation along these lines in the hope of providing a satisfactory energy balance at least for individual leaves.

V. Acknowledgments

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VI. References

Allen, P. J. (1954).—Physiological aspects of fungus disease in plants. *Annu. Rev. Pl. Physiol.* **5**: 225–48.

Berry, S. Z. (1959).—Resistance of onions to downy mildew. *Phytopathology* **49**: 486–96.

Bliss, L. C., Kramer, P. J., and Wolf, F. A. (1957).—Drought resistance in tobacco. *Tob. Sci.* **1**: 120–3.

Blodgett, F. H. (1901).—Transpiration of rust-infected *Rubus*. *Torreya* **1**: 34–5.

Cruickshank, I. A. M. (1958).—Environment and sporulation in phytopathogenic fungi. I. Moisture in relation to the production and discharge of conidia of *Peronospora tabacina* Adam. *Aust. J. Biol. Sci.* **11**: 162–70.

Dodge, B. O. (1923).—Effect of the orange-rusts of *Rubus* on the development and distribution of stomata. *J. Agric. Res.* **25**: 495–500.

Graf-Marin, A. (1934).—Studies on powdery mildew of cereals. *Mem. Cornell Agric. Exp. Sta.* **157**: 1–48.

Johnston, C. O., and Miller, E. C. (1934).—Relation of leaf-rust infection to yield, growth, and water economy of two varieties of wheat. *J. Agric. Res.* **49**: 955–81.

Johnston, C. O., and Miller, E. C. (1940).—Modification of diurnal transpiration in wheat by infections of *Puccinia triticina*. *J. Agric. Res.* **61**: 427–44.

Koursanov, A. L. (1928).—De l'influence de l'*Ustilago tritici* sur les fonctions physiologiques du froment. *Rev. Bot.* **40**: 343–71.

Lake, J. V. (1956).—The temperature profile above bare soil on calm nights. *Quart. J. R. Met. Soc.* **82**: 187–97.

RAMANATHAN, K. R., and RAMDAS, L. A. (1935).—Derivation of Ångstrom's formula for atmospheric radiation and some general considerations regarding nocturnal cooling of air-layers near the ground. *Proc. Indian Acad. Sci.* A**1**: 822–9.

RASCHKE, K. (1960).—Heat transfer between the plant and the environment. *Annu. Rev. Pl. Physiol.* **11**: 111–26.

REED, H. S., and COOLEY, J. S. (1912).—The effect of *Gymnosporangium* on the transpiration of apple leaves. Rep. Va. Agric. Exp. Sta. for 1911–12. pp. 82–90.

RIDER, N. E., and ROBINSON, G. D. (1951).—Study of the transfer of heat and water vapour above the short grass surface. *Quart. J. R. Met. Soc.* **77**: 375–401.

SCHRAMM, R. T., and WOLF, F. T. (1954).—The transpiration of black-shank infected tobacco. *J. Elisha Mitchell Sci. Soc.* **70**: 255–61.

VEHRENCAMP, J. E. (1953).—Experimental investigation of heat transfer at an air-earth interface. *Trans. Amer. Geophys. Un.* **34**: 22–30.

YARWOOD, C. E. (1936).—The diurnal cycle of the powdery mildew *Erysiphe polygoni*. *J. Agric. Res.* **52**: 645–7.

YARWOOD, C. E. (1956).—Humidity requirements of foliage pathogens. *Plant Dis. Reptr.* **40**: 318.

ZIEGENSPECK, H. (1944).—Vergleichende Untersuchungen der Entwicklung der Spaltöffnungen von Monokotyledonen und Dikotyledonen im Lichte der Polariskopie und Dichronskopie. *Protoplasma* **38**: 197–224.