Patterns in the Growth, Oxygen Uptake, and Nitrogen Content of Single Colonies of Wheat Stem Rust on Wheat Leaves

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ABSTRACT

Patterns of hyphal growth were determined for individual colonies of *Puccinia graminis* f. sp. *tritici* on wheat leaves 5 to 19 days after inoculation and related to the rates of O_2 uptake and total N content of 1.4-mm discs cut from positions within and adjacent to the colonies. Rates of colony elongation for partially incompatible host-parasite combinations (infection types 2 and 2-) were 18 to 55% of those for a compatible combination (infection type 4). The growth rate for each infection type was nearly constant from the time of first visible fleck until host leaves became senescent, even though a ring of chlorotic host tissue developed around colonies of incompatible combinations.

Rates of O_2 uptake in the sporulating centers of the colonies of infection type 4 were 10 to 15 times the rates of healthy tissues, whereas the N content increased threefold. Tissues invaded by nonsporulating hyphae at the border of type 4 colonies had rates of O_2 uptake two to four times those of controls without detectable change in N content. Uncolonized tissues located just beyond hyphal tips at the apical or basal ends of type 4 colonies had no alteration in respiratory activity. In contrast, the rate of O_2 uptake in noninvaded barley tissues adjacent to colonies of powdery mildew was 120% of the rate in healthy tissue.

With infection types 2 and 2-, the rates of O_2 uptake at colony centers were increased two- to four-fold, but only to rates one-fourth to one-half those of infection type 4 for equal amounts of fungus development. However, O_2 uptake increased slightly in the noninvaded tissues adjacent to colonies of infection type 2- when peripheral chlorotic rings were produced. Thus, an increase in respiration in hyphal-free tissues near advancing rust colonies could be detected only in the case of an incompatible host-parasite combination. Phytopathology 60:92-99.

Samples for measuring respiratory activities of rusted tissue usually contain many infection centers, each with fungal cells of several types, and cells of the host in varying degree of response to the parasite (10). Tissues with high numbers of infections per unit leaf area are often used under the assumption that crowding of pustules will reduce such heterogeneity. In such tissue, the contribution of the host or parasite to the total respiratory activity of rusted tissue has been a matter of conjecture, based largely on comparisons with powdery mildew diseases. Furthermore, results with host-parasite combinations of differing compatibilities in samples with several infection centers have been obscured by differences in rate of fungus development.

In an attempt to avoid such sampling problems, the microsampling methods used by Bushnell & Allen (7) for powdery mildew were applied here to single colonies of wheat stem rust. Specific objectives were twofold: to learn if respiratory alterations in the host might be detected in the noninvaded tissues bordering rust colonies, thereby providing a direct measure of changes in the host's respiration; and to compare the respiratory activity of host-parasite combinations of different infection types using small samples relative to the size of individual colonies. Colony elongation was used to measure fungus development; nitrogen content to indicate total accumulation of fungal and host materials. Samplings from the periphery of powdery mildew colonies on barley leaves were included for comparison with similar samplings from rusted wheat leaves.

MATERIALS AND METHODS.—Five host-parasite combinations (Table 1) were studied, including two partially incompatible combinations (infection types 2 and 2-) in which hyphae developed much less than in fully compatible combinations, but grew enough to spread throughout the 1.4-mm discs used for respirometry. The hosts included a near-isogenic pair of wheat lines (supplied by W. Q. Loegering) which differ in susceptibility to a culture of race 29 of *P. graminis* f. sp. *tritici*. The two lines are near-isogenic with respect to the Sr 9 locus. They were derived from a third-generation selfed heterozygous plant in the program from which Loegering & Harmon (18) later obtained (in the 11th selfed generation) near-isogenic lines ISr9a-Ra and ISr9a-Sa.

Plants were grown at 22-26 C in a controlled environment growth room described elsewhere (5), in the peat-sand U.C. soil mix C of Matkin & Chandler (19) with their fertilizer 1 and L-7 nutrient solution supplemented with 0.5 g NH_4NO_3/liter. Fritted trace elements (F.T.E., E. I. Dupont De Nemours & Co., Inc.) were added to the soil at a rate of ½ lb per yd^3.

Primary leaves were inoculated lightly on the abaxial side 9 days after seeding. Rust uredospores were dusted onto a glass slide (in a settling tower) which was then pressed against tissues 4 to 6.5 cm from the leaf tips. Mildew conidia were applied directly to a similar zone through a stencil in a settling tower. Plants were returned to the plant growth room immediately after

TABLE 1. The host-parasite combinations used in the present study

Disease	Infection type	Host: Species	Host: Variety or other designations	Host: Line or C.I. No.	Parasite: Species	Parasite: Race	Parasite: Culture
Stem rust of wheat	4	*Triticum aestivum* ssp. *vulgare* (Vill., Host) MacKey	RE XIII,S	I6-13-1/ 3-1-10S[a]	*Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E.Henn.	29	17-53B
Stem rust of wheat	2	*Triticum aestivum* ssp. *vulgare* (Vill., Host) MacKey	RE XIII,R	I6-13-1/ 3-1-4R[a]	*Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E.Henn.	29	17-53B
Stem rust of wheat	2-	*Triticum aestivum* ssp. *vulgare* (Vill., Host) MacKey	Marquis	C.I. 3641	*Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E.Henn.	38	60-S21-36
Powdery mildew of barley	4	*Hordeum vulgare* L.	Hanna	C.I. 906	*Erysiphe graminis* D.C. ex Mérat f. sp. *hordei* Em. Marchal	9	59.4
Powdery mildew of barley	4	*Hordeum vulgare* L.	Atlas	C.I. 4118	*Erysiphe graminis* D.C. ex Mérat f. sp. *hordei* Em. Marchal	3	CR 3

[a] One of two near-isogenic lines differing in susceptibility to race 29. See text.

inoculation in the case of mildew, and after an overnight dew period in the case of rust.

Two to 5 days after inoculation, the tip of each primary leaf was clipped to a support rack about 10 cm below the fluorescent light tubes of the growth room. This held the inoculated parts of the leaf 20-45 degrees from the vertical, about 15 cm beneath the tubes at a light intensity of 1,100 to 1,300 ft-c (12 hr/day). Samples from within or near infection sites were taken from positions at least 1.5 cm from the nearest neighboring colony, and from leaves with no more than three rust or mildew infections. Healthy leaves were usually sampled from a zone 4.4 to 5.6 cm from the leaf tip, comparable to the inoculated region of diseased leaves.

The tips of hyphae which bordered fungal colonies were located by microscopic examination of living leaves. Mildew hyphae were visible in dry mounts, whereas rust hyphae were examined in wet mounts (0.1% Tween 20 [polyoxyethylene sorbitan monolaurate]) under a cover glass. The rust hyphae just beneath the epidermal tissue could be seen at about ×400 with most microscopes if the leaf tissues were green. The hyphal tips on the edge of the diamond-shaped colony were traced until the hyphae at the tip of the colony were located (Fig. 1). Such tips were within 0.1 mm of the front-running hyphae within mesophyll as judged from leaves cleared with chloral hydrate (courtesy of K. Leath). Since no visible injury developed after inspection of fresh wet mounts, leaves were used for respirometry immediately after colonies were measured, or were kept attached to potted plants for repeated measures of colony development. The

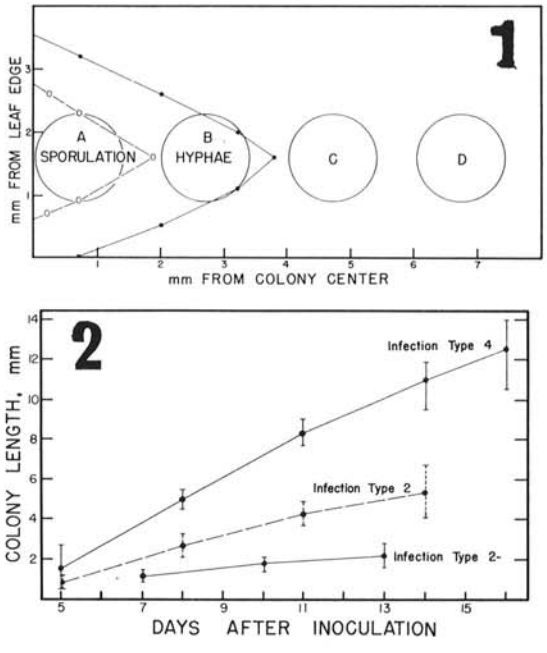

Fig. 1-2. **1)** The standard pattern for cutting 1.4-mm discs from wheat leaves for microrespirometry. Shown with the hyphal and sporulation zones of a 10 day wheat stem rust pustule of infection type 4. **2)** Growth of wheat stem rust colonies on wheat leaves. From repeated measurements of 8 to 10 colonies for each infection type. Vertical lines indicate range. Infection type 4, RE XIII,S—Race 29; infection type 2, RE XIII,R—Race 29; infection type 2-, Marquis—Race 38.

starchlike materials at infection sites were stained with IKI as described elsewhere (5).

Discs of tissue 1.4 mm in diam were cut for respirometry in the pattern of Fig. 1 with a circular knife made from a No. 15 syringe needle. Actual cuts were no more than 0.1 mm from those indicated. Four discs were always from positions in a line parallel with the midrib of the leaf, on the longitudinal axis of the rust or mildew colony, but did not include midrib tissue. The end-most disc of each set was from a position bordering either one tip of the colony (with all discs from noninvaded tissue) or the center of the colony as in Fig. 1. In both cases, the disc from the position closest to the center was designated the "A" disc; successive discs away from the center were designated "B", "C", and "D", respectively. Whether the samples were from the basal or apical end of the colony is indicated for each experimental series.

Oxygen uptake of individual 1.4 mm discs was measured with Gregg microrespirometers as described earlier (7), but with 2-hr periods of measurement. The volume of empty respirometers was 18.2 to 19.5 μliter. Respirometer drift was eliminated by thermostating the respirometers with a one-liter stirred water bath within a 50-liter, electrically thermostated water tank, and by placing mercury within the sheaths of each respirometer to accelerate heat exchange with the water bath. Five respirometers were run simultaneously, one serving as the thermobarometer. Differences among the five in blank runs usually did not exceed the equivalent of 0.004 μliter O_2/hr/mm^2. This error did not exceed 8% of the rate of O_2 uptake by samples of healthy tissues in this study, and a lesser percentage of the rates of diseased tissues.

Total N content was determined for selected 1.4 mm discs after they were removed from microrespirometers. Each disc was digested following the methods of Grunbaum et al. (13) in a sealed tube (4 × 50 mm outside dimensions) which contained 250-300 μliter of gas space and 8.3 μliter of 50% H_2SO_4. The ammonia in the digest was determined by back titration after diffusion distillation in 40-mm Conway diffusion cells (9).

The $s_{\bar{x}}Q$ values of Snedecor (28) at the 5% level were used to indicate the least differences for significance among the means for discs from a given fixed position relative to hyphal tips in successive samplings. Differences as small as 10% of the rate of O_2 uptake and 15% of the total N content of healthy tissues were significant by such analysis. Where successive samplings could not be used as replicates (successive samplings from a fixed position relative to the center of rapidly growing colonies), minimum differences for significance between any two members of a set of four discs were based on results from healthy leaves as described below.

RESULTS.—*Fungus and symptom development.—Puccinia graminis* f. sp. *tritici* of each infection type grew at uniform rates from the time of first visible fleck until the host leaves became senescent 15 to 19 days after inoculation (Fig. 2). The hyphal colonies of infection type 4 elongated 1.1 mm/day; those of infection types 2 and 2- grew 0.6 and 0.2 mm/day, respectively. Colonies of *Erysiphe graminis* f. sp. *hordei* of infection type 4 grew uniformly at rates of 0.7 to 0.9 mm/day, somewhat less than the growth rate of the stem rust fungus or a compatible host. The growth of individual rust on mildew colonies was much like that expressed by the averages of Fig. 2 in that each grew without major changes in growth rate during the period of measurement. The rust colonies of infection type 4 were bordered by unbranched runner hyphae 0.4 to 0.6 mm long which extended apically and basally parallel to the central axis of the colony. In contrast, the rust hyphae of lower infection types were highly branched near colony borders.

The chlorotic rings characteristic for infection type 2 were produced late in disease development; at 8 days after inoculation with infection type 2- and at 14 days with infection type 2. The distance from the outer to the inner circumference of the ring was 0.3 to 0.6 mm, with the outer boundary never more than 0.1 mm beyond hyphal tips at the colony edge. The onset of this chlorosis apparently did not change rates of colony development, for a slow growth rate relative to that of infection type 4 was evident before chlorosis appeared, and continued at an uninterrupted rate afterwards. No yellowing was visible in the colonized tissues of infection type 4, although the presence of hyphae and the loosening of epidermis at sporulation may have masked some chlorosis. The normal yellowing of the wheat leaf tissues, which started at leaf tips 24 to 28 days after seeding and proceeded basally at 1 to 2 cm/day, was delayed 1 to 2 days by rust pustules of infection type 4.

Patterns of staining with IKI varied among the several host-parasite combinations examined. With rust of infection type 4, a heavily stained region usually extended from the colony center to the junction between the runner hyphae and the more highly branched hyphae nearer the colony center. The contributions of fungus and host to the total stain were not determined. Tissues extending 1 to 1.5 mm beyond the tips of the rust hyphae sometimes stained with IKI, but only in diffuse patterns near the end of daily light periods. In contrast, the tissues bordering colonies of powdery mildew stained consistently in a well-defined pattern extending about 0.3 mm beyond hyphal tips [see Fig. 5 in Bushnell (6) for a more complete description]. Tissues near the chlorotic rings of rust infections of types 2 and 2- likewise stained consistently, usually in a band extending 0.2 to 0.7 mm beyond the outer boundary of the ring (which was near hyphal tips).

O_2 Uptake and N content of tissues from noninfected leaves.—Patterns in the O_2 uptake of healthy leaf tissue are illustrated by the data for RE XIII, S wheat (Fig. 3). Variation among daily samplings was high, largely from leaf to leaf variation. Differences among the members of each set of four discs was smaller, usually less than 0.01 μliter O_2/hr/mm^2. The samples at 26 and 28 days after seeding with large differences between members were taken from senescing leaves in which chlorosis had progressed downward into the sampling zone 5 cm from leaf tips. Such visibly senescent

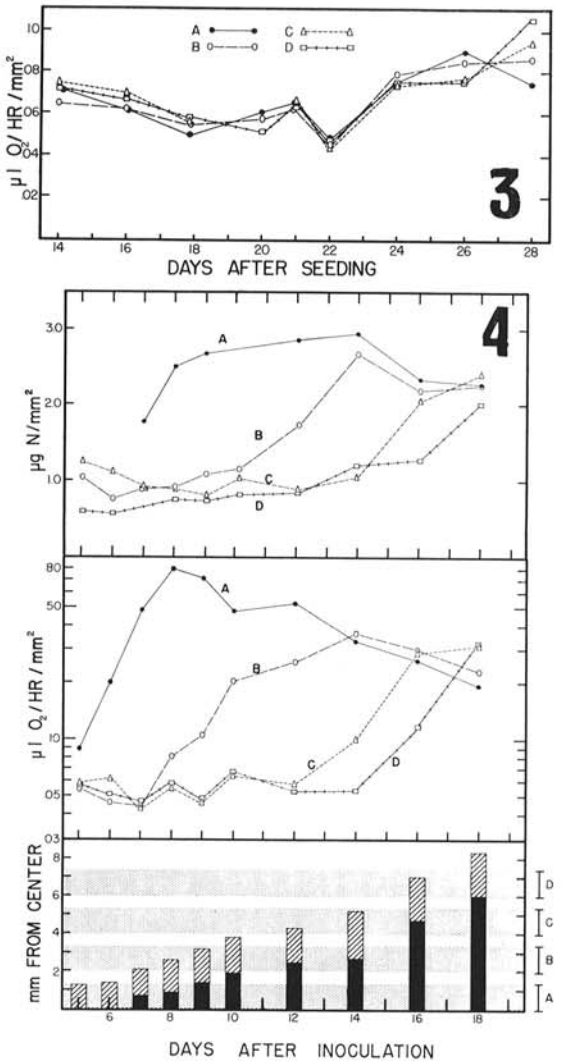

Fig. 3-4. **3)** Oxygen uptake of individual 1.4-mm discs from healthy wheat leaves (RE XIII,S). A set of four discs was cut from a single leaf at each sampling time in the pattern of Fig. 1. Disc A was from the most apical position of each set. **4)** Oxygen uptake and nitrogen content of discs from wheat stem rust pustules of infection type 4 (RE XIII,S—Race 29) on wheat leaves. Zone of sporulation is indicated by solid bars; hyphal zone by shaded bars. Disc position is indicated at right of graph. Each set of 4 discs was cut from the basal side of the pustule with disc A in most apical position bordering the colony center as shown in Fig. 1.

tissues were avoided in subsequent samplings of diseased tissues, although samplings 15 to 19 days after inoculation (24 to 28 days after seeding) may have included tissues with O_2 uptake rates enhanced as a consequence of aging.

The average rates of O_2 uptake for several noninfected host varieties are listed in Table 2 with the average total N content for RE XIII, S wheat, and Hanna barley. The rate of O_2 uptake for Atlas barley as measured here with 1.4-mm discs was 150% of the rate obtained earlier with 1.1-mm squares (7), probably because the nutritional and lighting regimes were improved in the present study. As in the earlier study, the rates of small excised samples (the 1.4-mm discs) were about 1.5 times the rate of leaf segments 1 to 2 cm long. The maximum range among the discs within each set of four was always less than 0.02 μliter/hr per mm^2, and 0.4 μg N/mm^2, the values used as the minimum differences for significance between any two discs within individual sets of four in subsequent experiments with diseased leaves.

O_2 Uptake and N content of tissues invaded by rust hyphae.—Discs cut from rust pustules of infection type 4 had extremely high rates of O_2 uptake coupled with large amounts of total N (Fig. 4). The rates of O_2 uptake for tissues near pustule centers (the A discs) 8 to 10 days after inoculation were 10 to 15 times the rate of healthy tissues. Total N content was increased about three times. In spite of such intense activity near the pustule center, the increase in respiration spread laterally in pace with the growth of the rust hyphae. Tissue not yet invaded by hyphae (such as the B disc on the 7th day or the C discs on the 10th and 12th days) were virtually the same in rate of O_2 uptake as tissues cut from noninfected leaves. As hyphae spread into each disc position, the rates of O_2 uptake increased two- to threefold, but without any significant change in total N content. Finally, as the zone of sporulation enlarged to include a given position (Fig. 4), the rate of O_2 uptake increased further; and the total N content increased two- to threefold.

Colonies of infection type 2 had relatively small changes in respiratory activity and N content (Fig. 5, Table 3). As hyphae spread to include all the A discs (9 to 16 days after inoculation), rates of O_2 uptake were about four times those of healthy tissue. Total N increased only slightly 13 to 16 days after inoculation. The zone of sporulation never exceeded 1 mm in length and, therefore, spread through only one-third of the 1.4-mm disc nearest the colony center.

High and low infection types can be compared using colonies of approximately equal size. The colonies of infection type 2, sampled 11 to 15 days after inoculation (Fig. 5), were about the same as colonies of infection type 4 on the 7th and 8th day after inoculation (Fig. 4) in size of hyphal and sporulating zones.

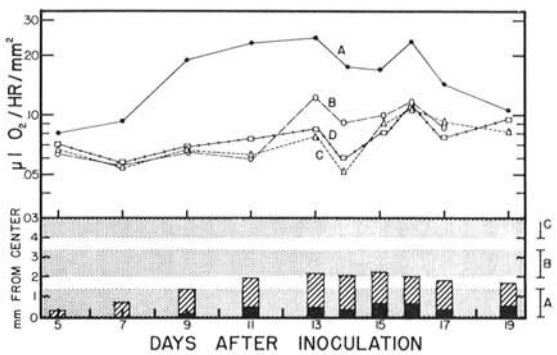

Fig. 5. Oxygen uptake of discs from wheat stem rust pustules of infection type 2 (RE XIII,R—Race 29) on wheat leaves. Conventions as for Fig. 4.

TABLE 2. O_2 Uptake and N content of 1.4-mm discs from healthy wheat and barley leaves

Var.	No. samplings[a]	Age, days after seeding	Oxygen uptake, μliter/hr/mm²		Total nitrogen, μg/mm²	
			Avg[b]	Range[c]	Avg[b]	Range[c]
RE XIII,S	8	14-26	0.064 ± 0.004	0.016	0.95 ± 0.05	0.36
RE XIII,S	6	14-24	0.071 ± 0.002	0.011		
Marquis	5	15-23	0.068 ± 0.011	0.013	1.00 ± 0.06	0.32
Hanna	5	18-25	0.053 ± 0.003	0.004		
Atlas	4	15-21				

[a] Each sample was a set of four 1.4-mm discs cut from positions as shown in Fig. 1.

[b] $\bar{X} \pm t_{.05}\, s_{\bar{x}}$ following Snedecor (28).

[c] The maximum range in entire series between individual discs within sets of four.

TABLE 3. Nitrogen content of 1.4-mm discs from wheat stem rust pustules of infection type 2 (RE XIII,R-Race 29). Oxygen uptake, disc position, and pustule development shown in Fig. 5

Disc position	Total N content, μg N/mm²	
	5-11 Days after inoculation	13-16 Days after inoculation
A	1.13	1.41
B	1.06	1.19
C	1.15	1.13
D	1.18	1.13
$s_{\bar{x}}Q$		0.13

On this basis, the rate of O_2 uptake for infection type 4 was higher than for infection type 2.

O_2 Uptake and N content of noninvaded tissues near rust and mildew colonies.—Respiratory activity at the borders of rust colonies was measured by sampling in the standard pattern of Fig. 1 with the A discs from a position beyond hyphal tips at one end of the fungal colony. None of the samples included rust hyphae, but the A discs included tissue originally within 0.1 mm of hyphal tips. No alteration in rate of O_2 uptake was detected at the borders of rust colonies of infection type 4, on either the apical or basal ends (Table 4). The A discs were not significantly different in rate from samples from positions more distant from the colony, and were comparable to samples from non-infected leaves (Table 2). However, the A discs had 121% of the N content of discs farther from the colony in a series of samplings from the basal side of the colony (Table 4).

The absence of a detectable change in the O_2 uptake at the borders of wheat stem rust colonies of infection type 4 prompted a reexamination of tissue bordering powdery mildew colonies on barley leaves. The data of Bushnell & Allen (7) for 1.1-mm squares of tissue indicate a doubling in the respiratory activity of tissues 2 mm beyond mildew hyphae on the 12th day after inoculation. With 1.4-mm discs in the current study, the rate of O_2 uptake was only 1.2 times that of controls (Table 5) as measured 7-14 days after inoculation. The tests included Atlas—race 3, as used by Bushnell & Allen (7), and Hanna—race 9, which likewise gave infection type 4. Total N content was not altered (Table 5) (measured only in the case of Hanna—race 9). The relatively small increase in O_2 uptake suggests that respiratory activity may not have spread beyond the zones which stained with IKI, about 0.3 mm beyond hyphal tips. (Samples of the host with mildew hyphae removed from positions within the boundaries of the colonies had rates of O_2 uptake increased 1.8 times.) Nevertheless, the enhanced activity was more than that encountered at the border of rust colonies on wheat when host and parasite were compatible. Appropriate host-parasite combinations (barley rust or wheat mildew) were not tested to learn

TABLE 4. Oxygen uptake and N content of 1.4-mm discs from noninvaded wheat leaf tissues adjacent to wheat stem rust colonies of infection type 4 (RE XIII,S—Race 29)

Disc position[a]	Sampling from apical end of colony[b]	Sampling from basal end of colony[c]	
	μl O_2/hr/mm²	μl O_2/hr/mm²	μg N/mm²
A	0.064	0.059	1.04
B	0.064	0.056	0.89
C	0.061	0.059	0.83
D	0.064	0.063	0.85
$s_{\bar{x}}Q$			0.14

[a] Sampling pattern as in Fig. 1, but with disc A from noninvaded tissue bordering hyphal tips, and other discs from positions extending away from the colony.

[b] Seven samplings 5-12 days after inoculation. Colonies 1.3 to 9.1 mm long.

[c] Six or 7 samplings 10 to 18 days after inoculation. Colonies 7.6 to 13.6 mm long.

TABLE 5. Oxygen uptake and N content of 1.4-mm discs from noninvaded barley leaf tissues adjacent to powdery mildew colonies of infection type 4

Disc position[a]	Atlas—Race 3[b]	Hanna—Race 9[c]	
	μl O_2/hr/mm²	μl O_2/hr/mm²	μg N/mm²
A	0.058	0.076	0.97
B	0.048	0.066	1.00
C	0.047	0.063	0.96
D	0.050	0.061	0.96
$s_{\bar{x}}Q$	0.004	0.007	

[a] Sampling patterns as in Fig. 1, but on the apical end of colonies with disc "A" from noninvaded tissue bordering hyphal tips and other discs from positions extending away from the colony.

[b] Four samplings, 7 to 13 days after inoculation. Colonies 3.7 to 8.5 mm long.

[c] Six samplings, 9 to 14 days after inoculation. Colonies 5.9 to 9.5 mm long.

if the peripheral increase was a property of the barley host, the mildew parasite, or both.

Respiratory activity apparently increased at the borders of rust pustules of infection type 2 (RE XIII, R—Race 29) as the border tissue began to become chlorotic 13 and 14 days after inoculation (Fig. 5). Such activity was more clearly indicated with Marquis—race 38, a combination which produced a chlorotic ring earlier in disease development. Discs free of hyphae, but including small portions of the chlorotic ring tissue, did have higher rates of O_2 uptake than tissues farther from the colonies (Table 6). Unfortunately, the small amount of chlorotic tissue beyond hyphae (a band only 0.1 mm wide) precluded an accurate measure of respiratory rates of the chlorotic tissue.

Discussion.—The respiratory activity at the centers of individual rust pustules of infection type 4 (host and parasite fully compatible) was more intense than previously reported for either rusted or mildewed tissues. Using 2.8- and 5-mm discs cut from heavily rusted wheat leaves, Samborski & Shaw (23) found maximum rates per unit leaf area about one-third those obtained here. On a dry wt basis, their rates were 2 to 3 times the rates of healthy tissue, similar to the increases reported by others for heavily rusted segments of wheat leaves (3, 11, 14). The high measured rates of O_2 uptake in the present study probably relate to the use of small (1.4-mm) tissue discs. The discs of high activity contained no interpustular tissue, and were tightly packed with sporophytic hyphae. The low numbers of infections per leaf may have further influenced activity, since the development of rust pustules increases when the density of infections is reduced (23, 31). However, powdery mildew colonies at low numbers per leaf had rates of O_2 uptake only one-third those obtained here with rust (7), probably because mildew hyphae are more loosely packed than are rust hyphae at the centers of sporulating colonies.

The total N content of 1.4-mm disc from pustule centers did not increase in pace with rates of O_2 uptake, but did increase fourfold when host and parasite were compatible. Likewise, the N content doubled in 2.8-mm discs from pustules of *P. graminis* on wheat (27). Larger sample pieces from heavily infected leaves have generally yielded small differences in N content between healthy and rusted tissues (8, 12, 26), possibly because the pieces contained interpustular tissues. Variables such as infection density, nutrition of the host plant, and the proportion of the leaf or plant that is rusted, also influence the degree of N increase at sites of infection. The present results show, however, that the large increase in N was a late event in the course of pustule development. The increase in N occurred only after an increase in O_2 uptake had been established, much like the delayed increase in N-acetyl glucosamine indicated by Bassett's data for wheat stem rust cited by Allen (1). Thus, the accumulation of N probably was associated with massive development of the fungus. The large increase in total N did not occur with a partially incompatible host-parasite combination in which the fungus developed in relatively small amounts (Table 3). Others have likewise found only small alterations in total N with low infection types (12, 27).

Table 6. Oxygen uptake of 1.4-mm discs from positions within and adjacent to wheat stem rust colonies of infection type 2- (Marquis—Race 38)

Disc position	Oxygen uptake, µl/hr/mm²	
	Disc A from near pustule center[a]	Disc A from noninvaded tissue bordering the pustule[b]
A	0.129	0.081
B	0.073	0.069
C	0.073	0.066
D	0.070	0.065
$s_{\bar{x}}Q$	0.025	0.008

[a] Sampling pattern as in Fig. 1 on the apical side of colony center. Five samplings, 7 to 15 days after inoculation. Colonies 1.4 to 2.6 mm long.

[b] Sampling pattern as in Fig. 1 on the apical end of colonies with disc A from noninvaded tissue bordering hyphal tips and other discs from positions extending away from the colony. Four samplings, 8 to 14 days after inoculation. Colonies 1.4 to 2.6 mm long. Chlorotic ring first visible 8 days after inoculation.

The evidence from excised 1.4-mm discs indicates that the respiratory activity of host tissues bordering colonies of the wheat stem rust fungus was not increased when host and parasite were compatible. The sampling and measuring techniques did detect small increases at the borders of well-developed powdery mildew colonies on compatible hosts, and of rust colonies on incompatible hosts. Only respiratory changes less than 10% of control rates would have gone undetected, except for increases specifically masked by excision-induced respiration. Since the respiration of mildewed host tissues was not increased by cutting or brushing treatments, it was concluded that micro samples gave reliable estimates of activity before excision (7). This may be true also for the present study, but the effects of wounding on rusted tissues were not directly assessed.

The absence of an increase in respiration in tissues peripheral to rust colonies has no bearing on whether an increase develops in the host cells close to the colony center. The peripheral noninvaded tissues were separated from zones of abundant hyphae by about 0.5 mm, the length of the unbranched runner hyphae. Cytological evidence of synthetic activity (4, 15) and enzyme activity (30) suggests that increased oxidative activity does occur in the host cells in close contact with rust hyphae.

Rust fungi are known to alter compatible host tissues in advance of growing hyphae regardless of the present evidence that respiratory increase is absent. Tetrazolium reduction by some oxidative enzymes increased while that of others decreased in tissues extending 0.5 mm beyond the border of bean rust pustules (30). Increases extending 0.2 to 2.0 mm ahead of hyphae have been found in RNA (15), in N content (Table 4), and apparently in NAD (22). A decrease in the incorporation of cytidine into host nuclei extended about 0.1 mm ahead of hyphae (21). Alterations in IKI staining have been described many times in tissues peripheral to rust

colonies (as was found here with stem rust of wheat), although the altered zone is not always as clearly delineated as with powdery mildew of barley. Like powdery mildew, rust produces green islands 1 to 2 mm in advance of hyphal tips in detached leaves (Bushnell, *unpublished data*). The changes in advance of rust hyphae partly resemble alterations induced by cytokinins, a parallel described more completely elsewhere (6, 15).

Sempio & Barbieri (24) found a respiratory increase in the noninfected portions of partly rusted bean leaves and in noninfected leaves of partly rusted plants. Increases equaling 30 to 40% of control respiratory rates occurred with both compatible and incompatible host-parasite combinations. They found similar increases in noninfected portions of several nonhost species inoculated with the bean rust fungus (25). Such systemic changes in respiration were not indicated by the present study with wheat stem rust or elsewhere, although changes in photosynthesis have been found in tissue remote from infection sites (17), and growth of roots and shoots is frequently reduced by foliar rust infection. The systemic respiratory alterations of Sempio & Barbieri (25) may relate more directly to altered plant development rather than to the respiratory changes in host cells within infected parts of the plant.

The respiratory activity of 1.4 mm discs from partially incompatible host-parasite combinations was less than that of a compatible combination, whether colonies of equal age or of equal size were compared. Similar conclusions have been drawn from measurements with heavily infected leaves (3, 14). On the other hand, the host tissue bordering rust colonies of an incompatible host-parasite combination had slightly enhanced rates of O_2 uptake (in contrast to a compatible combination) late in disease development. However, the concept of a "hypersensitive" burst of high respiratory activity may be more valid for the infection types in which the fungus stops growing early in the course of infection instead of ramifying within mesophyll tissues as in all the cases cited above. Such activity has been indicated for some powdery mildew diseases (16, 20). Sempio & Barbieri (24) likewise found a very rapid respiratory response with bean varieties "immune" and "highly resistant" to the bean rust fungus.

The available data on growth suggest that rust colonies of a given host-parasite combination enlarge at a nearly constant rate from the time of first visible fleck until leaf senescence, competition with nearby colonies, or external factors limit fungus development. Thus, Yarwood (31) found that the diameter of mycelial areas of a bean rust fungus on a compatible host increased at a uniform rate, which, in turn, was about one-fourth the rate obtained here for elongation of stem rust colonies. Qualitative descriptions of growth involving incompatible host-parasite combinations (infection types 0; and 2) suggest that rust hyphae continue to grow slowly for many days after symptoms are first visible (2, 29) as with infection types 2 and 2- in the present study. Thus, the expression of incompatibility may result from continued interaction between the rust fungus and its host instead of any single event or short period of interaction.

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