Respiratory Changes During Germination of Urediospores of Puccinia graminis f. sp. tritici

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ABSTRACT

Urediospores of *Puccinia graminis* f. sp. *tritici* germinated rapidly when agitated in a buffer containing nonyl alcohol and Tween 20 (polyoxyethylene sorbitan monolaurate). Synchronous and nearly complete germination occurred when urediospores were vapor-phase hydrated prior to use. This method permitted measurements of endogenous respiration of urediospores with an oxygen electrode during the germination process. The results were compared with those obtained using conventional manometric techniques. Initially the manometric measurements of respiratory rates were higher than the polarographic measurements, but as germination began the situation was reversed. The differences observed in the two methods of measurements were attributed to problems of wetting the spores, the drying out of germ tubes, and a metabolic restriction in Warburg respirometer flasks caused by the lack of carbon dioxide.

The respiration of germinating urediospores, when measured with an oxygen electrode, was characterized by four distinct phases. Phase 1 was characterized by the development of respiratory activity upon contact of urediospores with liquid, preceding germination. Phase 2 was initiated by a decline in respiratory rate, during which germination was initiated. Phase 3 was marked by a second and greater increase in respiratory rate which paralleled the rate of germination and germ tube elongation. Phase 4 showed a gradual decline in respiratory rate which commenced after germination was completed and the germ tubes had grown more than half of their maximal length. In contrast to germinating spores, nongerminating ones incubated under conditions of self-inhibition did not show phase 3.

The addition of the partially purified self-inhibitor of urediospore germination inhibited both respiration and germination, with the latter being more sensitive.

The respiratory quotient of vapor-phase hydrated urediospores declined from an initial high value of 0.88 to lower values from 0.33 to 0.59. The respiratory pattern observed was suggestive of a qualitative change in urediospore metabolism during the progress of germination. Phytopathology 60:1357 1364.

Additional key words: respiration and germination of urediospores, wheat stem rust, polarographic and manometric measurement of spore respiration.

The germination of most fungus spores is accompanied by marked increases in respiratory rate (17). One exception is the urediospores of *Puccinia graminis* f. sp. *tritici,* whose respiratory rate has been reported to remain unchanged (4, 15). In these studies, respiratory rates were measured after germination of urediospores had progressed for several hr; moreover, the percentage germination was low. For example, Shu et al. (15) used spores after 72 hr of germination, whereas Caltrider et al. (4) used spores that had been germinated for 6-24 hr. Therefore, any transitory changes in respiration preceding germ tube emergence and during germ tube growth may have been missed.

The uncertainty concerning respiratory changes during germination has persisted because urediospores have not germinated satisfactorily during continuous incubation in respirometer flasks. Germination of high densities of urediospores is inhibited because of the release of self-inhibitory compounds (1), so manometric determinations have been made either of urediospores whose germination was self-inhibited (8, 19) or, as noted above, of spores which had already completed germination on large volumes of water. Another difficulty in manometric measurements is the liberation of a sizable volume of a gas, perhaps air, when the spores are wetted (19).

Recently, Bush (3) used an oxygen electrode for measurements of respiratory rates of urediospores. Oxygen consumption when measured polarographically was 4-7 times greater than when measured manometrically, but both rates declined with time. Bush (3) gave no data on the degree of synchrony and the timing of germination, but it appears that measurements were made under conditions of self-inhibition. Furthermore, the germination conditions in his experiments were not constant throughout the course of respiratory measurements. Thus, the question of respiratory changes during germination of urediospores has remained unresolved.

Consequently we decided that the respiratory characteristics of urediospores during germination warranted a re-examination, so we attempted to overcome the problem of germinating urediospores during measurements of respiration. As a result, a method of obtaining synchronous, rapid, and almost complete germination of urediospores in suspension under constant environmental conditions has been developed. This paper reports the respiratory changes during germination of urediospores as determined with an oxygen electrode in comparison with those obtained employing standard Warburg techniques.

MATERIALS AND METHODS.—*Production and germi-*

nation of urediospores.—Urediospores of *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn., race 56, were grown on *Triticum aestivum* L. var. Baart in a controlled environment chamber under a photoperiod of 16 hr at 22 C and 8 hr dark at 20 C. Spores used in the present experiments were mostly collected the same day as used and in no case were more than 4 days old. Two kinds of spores were used in the study: (i) nonhydrated spores, which were not given any treatment and had a moisture content of 11-16% as determined by drying them at 60 C for 48 hr; and (ii) hydrated spores, whose wt was increased by vapor-phase hydration and had a moisture content of 50 to 60%. This was accomplished by placing weighed aliquots of spores in clumps on aluminum weighing dishes which were kept over distilled water in a desiccator for 16 to 24 hr at 20 C under diffused light.

Rapid and synchronous germination of urediospores was obtained in a medium (BNT) of the following composition: calcium phosphate-potassium phosphate buffer, pH 7.0 (10), containing 1×10^{-4} M nonyl alcohol and 0.01% Tween 20 (polyoxyethylene sorbitan monolaurate). Urediospores were suspended by oscillating 10 to 40 mg of spores in 10 ml BNT with a Mickle vibratory tissue homogenizer (H. Mickle, Hampton, Middlesex, England) for 2 to 5 min or by vigorously shaking them in medium by hand in a closed Erlenmeyer flask for 1 to 2 min. Optimal germination was obtained by agitating a suspension of 30 to 120 mg of urediospores in 30 ml BNT in a 125-ml Erlenmeyer flask on a reciprocal shaker (180 strokes/min) at 20 C in diffused light. When hydrated urediospores were used at a concn of 4 mg (original wt)/ml, excellent germination and synchrony were obtained, the emergence of germ tubes being visible after 40 to 60 min in almost all spores. This method was simpler and gave better results than that described by Schipper et al. (14). In all experiments, the actual percentage germination was determined using the first appearance of a germ tube as the criterion, and results were based on counts of at least 100 spores.

Measurement of oxygen uptake.—Polarographic measurements of oxygen uptake were made with a Clark-type oxygen electrode. It consisted of a platinum cathode with a silver reference anode, both of which were covered by a Teflon membrane. The electrode was used in conjunction with an amplifier and a chart recorder, and a polarizing voltage of 0.64 v was applied across the electrodes. The sample chamber was constructed of Plexiglas to accommodate the oxygen electrode, which fitted into the sample chamber from above. Another hole, provided with a detachable Plexiglas stopper with a capillary bore, allowed addition of materials into the sample chamber. The volume required to fill the chamber was 2.4 ml, and the contents were stirred magnetically. The output from the amplifier was 10 μv. The instrument was calibrated to 100% oxygen saturation with the electrode in air-saturated incubation medium and to zero oxygen tension in the presence of an anaerobic suspension of yeast cells. The full scale deflection from the calibrated oxygen electrode corresponded to 48 chart units.

A continuous recording of the rates of oxygen uptake of urediospores incubated in the sample chamber was not possible because the spores were difficult to wet. Therefore, the procedure followed was to germinate urediospores in suspension on a reciprocal shaker as described. Then 1 ml of spore suspension was added to the sample chamber at regular intervals. After each transfer of spores, tracings of oxygen uptake were followed for 5 min, or more, and then zeroing was accomplished by adding a drop of a concd suspension of yeast cells to the chamber (Fig. 1). This also served to check the zero calibration before the start of the next determination.

The rate of oxygen uptake was calculated from the slope, corrected to linearity, on the basis of the solubility of oxygen (240 μM) in the buffered medium (5). Thus, if 48 chart units equal 240 μM oxygen, and if the pen deflection of 4 mg urediospores in 1-ml incubation medium in 5 min is equal to 18 chart units (Fig. 1), then the rate of O_2 uptake in μM/mg per min $= \frac{240 \times 18}{48 \times 4 \times 5}$, or 4.5. To express rates in μliters O_2/mg per hr, the values for the concn of air dissolved in water and the percentage oxygen in dissolved air, as functions of temp, were taken from the literature (9), and calculations were made as follows: μliters O_2/mg per hr $= \frac{X \cdot Air_{H_2O} \cdot O_2' \cdot V \cdot 60}{100 \cdot O_2}$ where X = μM O_2/mg per min, Air_{H_2O} = concn of air dissolved in water in μliters/ml, O_2' = percentage oxygen in air dissolved in water, V = volume of sample chamber in ml, and O_2 = concn of oxygen dissolved in water in μM.

Manometric measurements were made with a Warburg constant volume respirometer with air as the gas phase at 22 C (18). The main compartment contained 2.5 ml liquid, and 10 mg spores were placed in the side arm in all experiments. Unless indicated otherwise, the center well contained 0.2 ml of 10% KOH with a folded filter paper. Germination of urediospores in Warburg flasks in the presence of nonyl alcohol was at least 60%. Carbon dioxide was determined by the

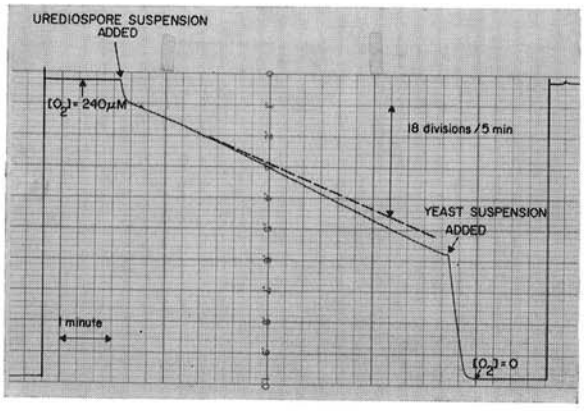


Fig. 1. An oxygen electrode trace of germinating *Puccinia graminis* f. sp. *tritici* urediospores after 240-min incubation. See text for details.

direct method of Warburg, and correction was not made for its retention in the incubation medium.

Contamination during respiratory measurements was monitored under the light microscope, and very few bacteria were found. Moreover, observation of the results indicated that the significant changes occurred soon enough after incubation commenced to eliminate the possibility that contaminants exerted a significant effect.

The terms Q_{O_2} and Q_{CO_2} in the text refer to μliters of oxygen taken up and carbon dioxide given off, respectively, per mg fresh wt of spores per hr.

Isolation and partial purification of self-inhibitor of urediospore germination.—The self-inhibitor of the germination of urediospores of *Puccinia graminis* f. sp. *tritici* was prepared according to the method of P. J. Allen & R. Strange (*unpublished data*). Ten g of urediospores (6 to 12 months old and stored at 4 C) were homogenized in 200 ml calcium phosphate-potassium phosphate buffer (10), pH 7.0, for 5 to 10 min in a Waring Blendor. The spores were filtered off, and the clear filtrate was extracted 2 times with ether, using one-third of a volume of ether for each extraction. The ether was removed with a flash evaporator, and the residue was redissolved in one-tenth the volume of the original buffer. This solution was filtered and re-extracted with an equal volume of ether. The ether-extract was evaporated and the residue was dissolved in a known volume of ether and stored at −20 C. For use, an aliquot of the ethereal solution of the self-inhibitor was pipetted into a germination vessel, the ether was evaporated off, and the inhibitor dissolved in BNT. The amount of self-inhibitor required to counteract the stimulatory effect of nonyl alcohol upon urediospore germination, i.e., for 100% inhibition, was approximately equal to that present in 200 mg urediospores.

RESULTS.—*Oxygen uptake.*—1) *Polarographic measurements.*—The respiratory rate of nonhydrated urediospores (4 mg/ml) incubated in BNT increased in the first 30 min after contact with liquid (Fig. 2-A). The rate then abruptly began to decrease before germination commenced. By 75 min, the oxygen consumption further declined, at which time the emergence of germ tubes was visible in 30% of the urediospores. A rapid increase in the rate of oxygen consumption began at about 90 min, paralleling an increase in the percentage germination. The increased rate continued through 150 min, then leveled off. Over 70% of the urediospores germinated by this time, and the germ tubes were elongating rapidly. After 240 min, the rate of oxygen consumption gradually began to slow down, and after 450 min it was decreased almost one-third. Thus, a secondary and very pronounced increase in oxygen uptake paralleled the rise in urediospore germination, and followed the initial increase and decline in respiration. The maximal rates of oxygen uptake occurred within 4 hr after incubation had started, when contamination by other microorganisms was negligible.

When nonhydrated urediospores were suspended at a concn of 4 mg/ml in buffer containing Tween 20 but

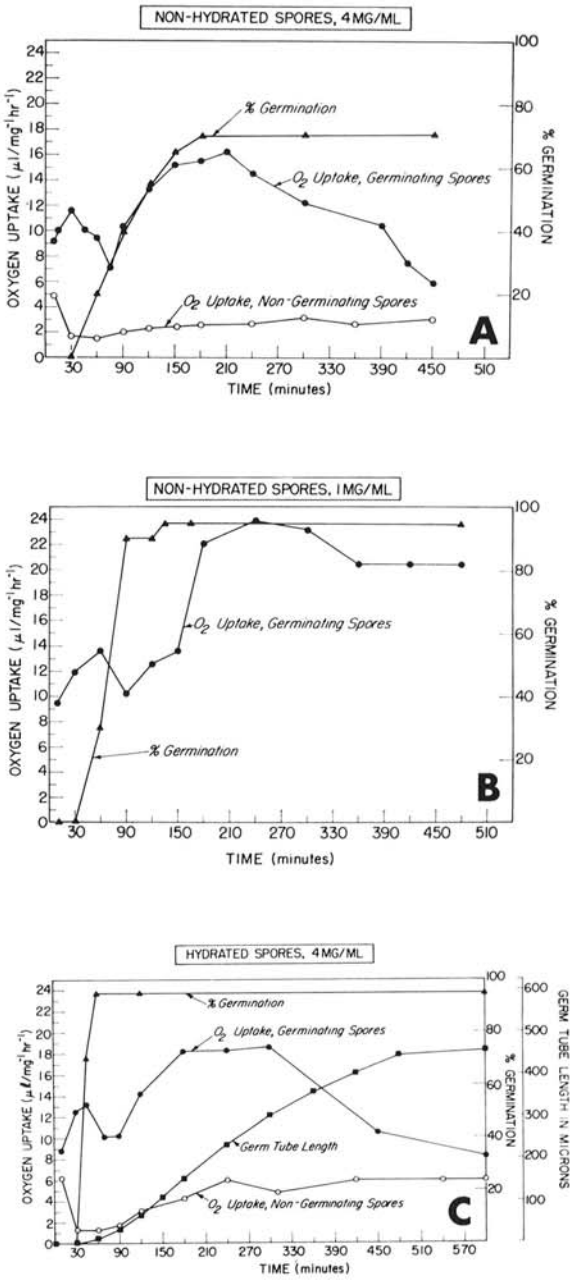


Fig. 2. Oxygen electrode measurement of oxygen uptake by *Puccinia graminis* f. sp. *tritici* urediospores. Germinating spores were incubated in presence of nonyl alcohol; nongerminating spores were incubated in absence of nonyl alcohol.

without nonyl alcohol, the spores did not germinate because of self-inhibition. The rate of oxygen uptake under these conditions declined from the time measurements were begun (Fig. 2-A). It may be noted that polarographic measurements of respiratory rates within a few min after the urediospores were suspended in liquid were not possible because of the time involved in preparing spore suspensions. After 30 to 60 min,

the Q_{O_2} declined to a min of 1.5 and increased, but to a very small extent, during further incubation. The low rates of respiration of such spores was not due to their death under conditions of self-inhibition for, even after prolonged incubation under these conditions, they germinated within 30-45 min when nonyl alcohol was added. The Q_{O_2} of nongerminating urediospores was one-sixth that of germinating urediospores.

To determine whether crowding of urediospores affects the rate of oxygen consumption, urediospores were also used at a concentration of 1 mg/ml. As shown in Fig. 2-B, the respiratory rates achieved were higher than at 4 mg/ml (Fig. 2-A), and the decline in rate after 6 hr of incubation was much less. Moreover, the synchrony and percentage of germination were also improved. Comparison of the data in Fig. 2-A and B shows that the relative magnitudes of increase in respiratory rate and germination are almost identical. For example, at the end of 210 min of incubation, when a plateau is reached in respiratory rate and germination at each concn of urediospores, both rates are about 30% greater at the lower spore concn than at the higher.

Germinating hydrated spores at a concn of 4 mg (original wt)/ml of BNT showed a similar pattern of respiratory activity (Fig. 2-C) to nonhydrated spores, although there was an over-all increase in the rate of oxygen uptake. It should be noted that hydration resulted in the almost complete germination of urediospores, and improved the synchrony of germination even over that when only 1 mg/ml urediospores was used. The rate of germ tube elongation under these conditions was exponential in the first 120 min of incubation and, thereafter, was more or less constant until 300 min. At the end of 480 min, germ tube growth was nearly completed. For the most part, increases in respiratory rate and germ tube growth appear parallel, although the former declines sooner.

Like nonhydrated spores, hydrated ones did not germinate in the absence of nonyl alcohol. But the rate of oxygen consumption was twice that of the same concn of nonhydrated spores incubated under these conditions. On the other hand, hydrated spores which germinated in the presence of nonyl alcohol respired 3 times faster than those incubated in its absence.

2) *Manometric determinations.*—Upon contact with liquid, nonhydrated urediospores liberated an alkali-insoluble gas as indicated by the rise in manometer level which persisted for up to 5 min. In order to test whether the evolution of gas is a metabolic process, nonviable urediospores were studied and found to liberate gas upon wetting, as shown in Table 1. These observations confirm the findings of Williams & Allen (19). By contrast, hydrated living urediospores evolved no gas at all.

Following the liberation of gas, nonhydrated urediospores showed an immediate increase in gas uptake (Fig. 3-A). The respiratory rate increased to a max after 20 min of contact with BNT. It then declined sharply but with transient increases. At 90-105 min, the Q_{O_2} was only one-third that at 20 min. Gas uptake

TABLE 1. Gas evolution by viable and nonviable urediospores of *Puccinia graminis* f. sp. *tritici* during contact with a liquid medium for 5 min[a]

Spore condition and treatment	Gas evolution μliters[b]	No. determinations
Viable spores		
Hydrated[c]	−2.4 to 3.2	3
Hydrated + self-inhibitor	−3.7	2
Nonhydrated	2.9 to 8.8	3
Nonhydrated + self-inhibitor	5.6	2
Wetted and dried[d]	13.2	1
Nonviable spores		
Stored 7 months at 4 C	11.7	1
Killed at 60 C, hydrated	7.7	1
Killed at 60 C, nonhydrated	15.4	1

[a] 10 mg spores/2.5 ml buffer + nonyl alcohol + Tween 20 (polyoxyethylene sorbitan monolaurate) (BNT).

[b] Total mm deflection × flask constant for oxygen. Measurements in Warburg apparatus.

[c] Vapor-phase hydration for 16 to 20 hr prior to placement in liquid medium.

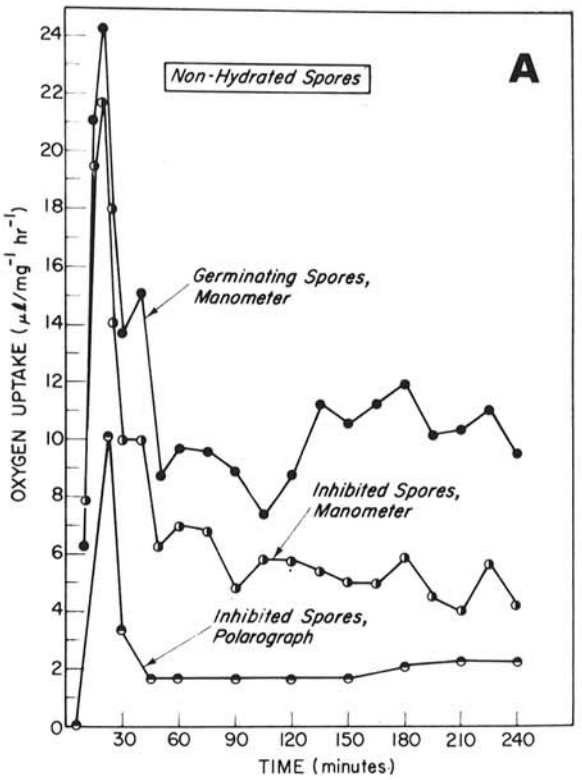
[d] Incubated in BNT for 15 min, then dried over $CaCl_2$ for 24 hr.

was enhanced subsequently but the rates remained irregular. After 240 min, approximately 70% of the urediospores had germinated and the germ tubes had adhered to the wall of the Warburg flasks and were exposed to partial drying.

Respiratory activity in hydrated urediospores, as measured with the Warburg apparatus, followed much the same pattern as that in nonhydrated spores (Fig. 3-B). The rates were not constant in individual experiments, even during short periods of time. In several experiments, following the initial burst in respiratory rate, another short-lived smaller increase was observed beginning 30 min after the start of incubation. The significance of this second burst, if any, is not understood. Comparisons show that hydration decreases the total oxygen uptake by urediospores (Table 2). The second and more prominent peak of respiratory activity observed in polarographic experiments did not occur in manometric experiments.

To determine if the rates observed in manometric experiments were affected by the restriction imposed on the metabolism of urediospores by the lack of carbon dioxide, we measured the rates in the presence of this gas. This was accomplished by use of 0.3 ml of Pardee's 0.4% carbon dioxide buffer (18), which was put in the center well of the Warburg flasks. In all experiments, using both hydrated and nonhydrated (Fig. 4) urediospores, the rates were more uniform. Furthermore, a small but steady increase in respiratory rate was found after the decline from the first peak.

Carbon dioxide production and respiratory quotient.—The rate of carbon dioxide production by hydrated, germinating urediospores is shown in Fig. 3-B. Immediately after coming in contact with liquid, urediospores show a rate of carbon dioxide production (Q_{CO_2}) approximately equal to the rate of oxygen uptake. A deficit in carbon dioxide production soon develops and



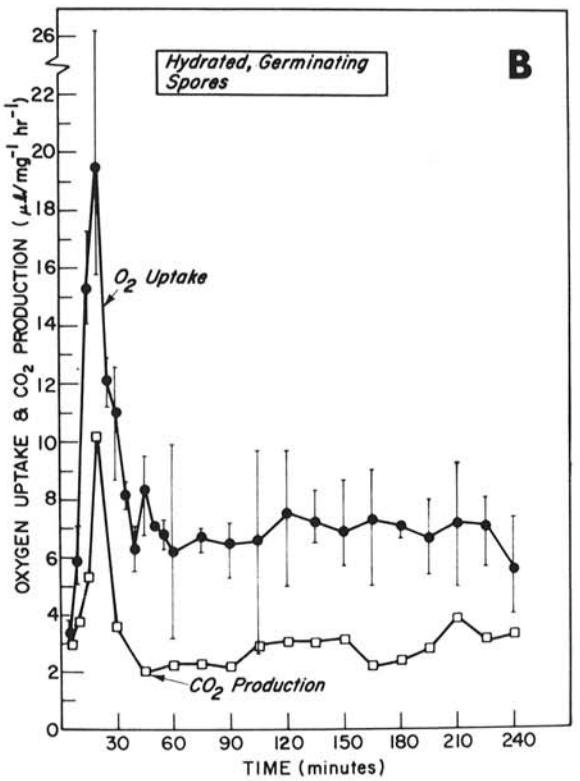


Fig. 3. A) Oxygen uptake of germinating and inhibited nonhydrated urediospores of *Puccinia graminis* f. sp. *tritici*. Manometric determinations are averages of 6 and 3 Warburg flasks, respectively. Germination was 70% in BNT and none in BNT + self-inhibitor. **B)** Manometric determination of Q_{O_2} and Q_{CO_2} of hydrated urediospores at intervals after contact with BNT. Values of O_2 uptake are average of 4 determinations involving 15 flasks; those for CO_2 production are of 2 determinations involving 6 flasks. Vertical lines indicate range of values. Urediospore germination at the end of experiments was 60-90%.

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is maintained during further incubation, when the Q_{CO_2} follows a course similar to the Q_{O_2}. The respiratory quotient (RQ) at intervals of 5, 10, 30, 60, 120, and 240 min is, respectively, 0.88, 0.64, 0.33, 0.37, 0.41, and 0.59. Because of the release of alkali-insoluble gas from nonhydrated urediospores, accurate measurements were hindered in the initial period, so these were not used in determining the Q_{CO_2}.

Effect of self-inhibitor of urediospore germination on respiration.—The addition of partially purified self-inhibitor to BNT at concn which totally inhibit urediospore germination did not affect the initial rise in respiratory activity (Fig. 3-A). The extent of the

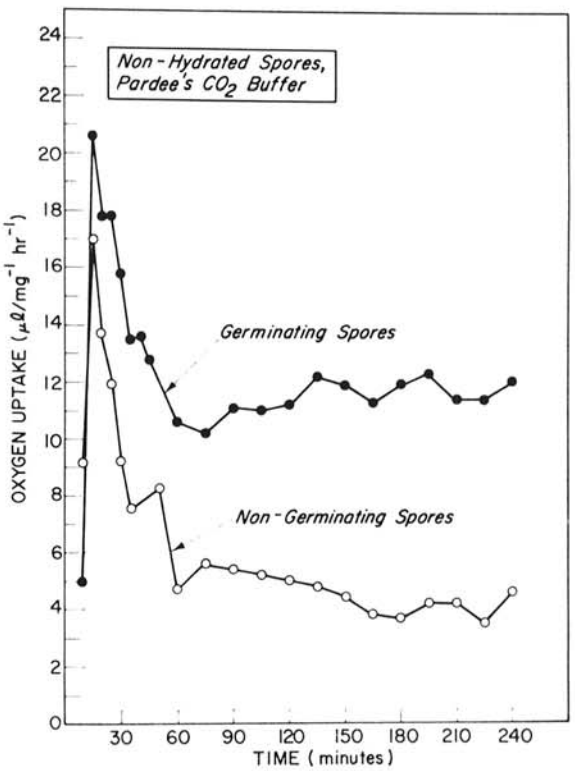

Fig. 4. Effect of Pardee's CO_2 buffer on oxygen uptake of nonhydrated urediospores of *Puccinia graminis* f. sp. *tritici* in the presence (●) and absence (○) of nonyl alcohol. Data are averages of 9 and 3 Warburg flasks, respectively. There was 70% germination when nonyl alcohol was present and none when it was lacking in the medium.

TABLE 2. Comparative rates of respiration of urediospores as measured by polarographic[a] and manometric[b] methods

Min after contact with liquid	Q_{O_2} (μliters oxygen/mg per hr)								
	Hydrated, germinating[c]			Nonhydrated, germinating			Nonhydrated, nongerminating[d]		
	Polarographic	Manometric		Polarographic	Manometric		Polarographic	Manometric	
		A[e]	B[f]		A[e]	B[f]		A[e]	B[f]
15	9.6	14.5	18.9	9.0	21.1	20.6	6.4	16.5	17.0
30	12.5	8.2	13.4	11.6	13.7	15.3	3.0	9.1	9.2
60	11.3	4.1	9.6	7.2	11.6	10.9	3.0	4.9	4.7
120	14.2	5.0	9.3	11.9	10.8	12.5	3.8	3.9	5.0
180	18.2	7.2	10.6	15.3	12.0	12.9	3.4	3.5	3.7
240	18.3	5.5	8.8	16.6	9.5	14.7	4.3	4.0	4.6

[a] Data from single experiments.
[b] Average of values for four flasks.
[c] Spores incubated in buffer + nonyl alcohol + Tween 20 (polyoxyethylene sorbitan monolaurate).
[d] Spores incubated in buffer + Tween 20.
[e] Flasks contained KOH.
[f] Flasks contained Pardee's CO_2 buffer.

initial rise in respiratory rate of treated urediospores, determined either polarographically or manometrically, was almost comparable to that of untreated spores. However, pronounced differences occurred after 20-30 min. Thus, when the rates of oxygen uptake of untreated spores measured polarographically (Fig. 2-A) are compared with those of treated spores (Fig. 3-A), it is noted that there is a sharper and greater decline in the rate of inhibitor-treated spores. Spores in the presence of self-inhibitor had a Q_{O_2} of 1.7 at 45 min which increased to only 2.3 during subsequent incubation. The Q_{O_2} of untreated spores declined initially to about 7 at 75 min, after which it increased maximally to about 15 (Fig. 2-A). Lower rates of oxygen uptake after 30 min in the presence of self-inhibitors were shown in manometric experiments as well. At the end of 240 min, the total oxygen uptake of these spores was 40% of control spores.

It should be pointed out that the polarographic and manometric rates of oxygen uptake of self-inhibited urediospores indicated in Fig. 3-A are not comparable because of 3 times more self-inhibitor added in the former case.

An experiment was performed to determine whether the effects of self-inhibitor on germination and respiration are proportional. The germination of these spores was inhibited by 70%, while their respiration, measured polarographically, was inhibited by 50%.

DISCUSSION.—The respiratory rates measured with the oxygen electrode and Warburg apparatus differ in the following respects: (i) Initially, as shown in Table 2, the manometric rates are up to 2 to 2½ times higher but, after 60 min, they become lower or equal to the polarographic rates. (ii) In experiments with the Warburg apparatus, the initial peak in oxygen uptake occurred at 15-20 min as compared to 30-45 min in polarographic measurements. These differences probably are due to limitations of the Warburg method and to the nature of the urediospores. Upon contact with the liquid in the Warburg flask, the hydrophobic urediospores float on the surface and are wetted only after some time. It is possible that the rate of oxygen consumption increases faster under these circumstances than when the spores are completely suspended. Also, the Warburg apparatus, in contrast to the oxygen electrode, measures net changes in air pressure rather than those of a specific gas.

The lower rates measured manometrically after 60 min may be due to suboptimal conditions of germination in Warburg flasks. Firstly, germinating urediospores adhered to the walls of the vessels, so exposure and drying of germinating spores occurred. This was probably due to the inadequate speed of shaking of respirometer flasks. Thus, as would be expected, respiratory rates of nongerminating spores, wherein the problem of exposure and drying of germ tubes does not exist, compare well in both methods. Secondly, Warburg's "direct method" of measurement requires that flasks be free of carbon dioxide. Accordingly, the absence of CO_2 may impose restrictions on the metabolism of urediospores, such as in CO_2-fixation reactions (13, 16). It has also been reported that higher CO_2 concn (up to 2.5%) improve the germination of urediospores under conditions of self-inhibition (1, 8). Indeed, we have noted a stimulatory effect of carbon dioxide (approximately 0.4%) on urediospore metabolism, as well as an improvement in the reproducibility of the results (Table 2). Thirdly, urediospores germinated more completely and synchronously in the method of incubation evolved for use with the oxygen electrode. Thus, the differences in the polarographic and manometric measurements of respiratory rates are due to different conditions of urediospore germination in the two methods. But for the reasons mentioned above, we believe that the polarographic measurements represent the respiratory characteristics of the urediospores more accurately.

In contrast to previous studies of the respiration of germinating urediospores (2, 8), our results show that respiration is characterized by the following four phases: Phase 1, the respiratory rate increases upon contact of urediospores with liquid before germination begins; phase 2, the respiratory rate declines and germ tube initiation occurs; phase 3, respiration in-

creases rapidly, germination is completed, and germ tube growth continues linearly; phase 4, a gradual decline in respiratory rate occurs after germination is complete and germ tubes have grown more than half of their maximal length.

Interesting parallels of such a respiratory pattern are found in some germinating seeds (20) and pollen (7, 12). On the other hand, these results do not corroborate those of Bush (3), who also used an oxygen electrode to measure respiration of wheat stem rust urediospores. He reported that the Q_{O_2} after 1 hr declined to 6, yet surprisingly, his other data show that during a 24-hr germination period, the Q_{O_2} actually declined only to 27.8, from 36.2 at the beginning of the experiment. It is not clear whether the measurements were of germinating spores, nor was it stated whether they were suspended in the medium or floating. Bush (3) also claimed that the polarographic measurements were 4 to 7 times greater than manometric ones, an observation different from ours.

The RQ of germinating (15) or nongerminating wheat stem rust urediospores incubated under conditions of self-inhibition (8, 19) has been reported to be 0.6 to 0.7. The time of measurement of the RQ in these studies has either not been mentioned or has ranged from 1 to 2 hr (19) to 3 days (15) after contact with liquid. These values have constituted evidence that the substrate for endogenous respiration in both germinating and nongerminating urediospores is lipid. In our study, the RQ was found to change from 0.88 after 5 min to values ranging from 0.33 to 0.59. These data are suggestive of a qualitative change in metabolism. In the light of Daly et al.'s observations (6) that, during the first hr, 60 to 80% of the ethanol-soluble materials disappear from urediospores whereas the ether-soluble ones do not change appreciably, it is likely that the respiratory phases observed are associated with a change from carbohydrate to lipid metabolism.

The lack of a precise knowledge of the pattern of respiratory development during urediospore germination has resulted in uncertainty regarding the effects of the self-inhibitor of urediospore germination on respiration. Farkas & Ledingham (8) found that respiration is inversely proportional to spore concn, and certain compounds which counteract self-inhibition prevent the early decline in respiration. Naito & Tani (11) obtained similar results with urediospores of *Puccinia coronata*. These authors, therefore, concluded that respiratory inhibition is the basis of action of the self-inhibitor. On the other hand, Williams & Allen (19) and Bush (3) found that oxygen uptake is independent of spore concn. We found that urediospores at lower concn respired at a faster rate, the difference being particularly marked in phase 3. In Williams & Allen's experiments (19), the oxygen uptake of spores induced to germinate by nonyl alcohol was not reduced by partially purified inhibitor unless the amounts were high enough to inhibit germination. We have found that although the self-inhibitor does not affect the initial rise in the respiration of germinating urediospores to any significant extent, it does inhibit the pronounced second increase (phase 3) which is characteristic of such spores. But the effects of the self-inhibitor on respiration and germination are not proportional, inasmuch as germination is more sensitive to inhibition than respiration.

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