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Stomatal Penetration of Wheat Seedlings by Stem and Leaf Rust: Effect of Light and Carbon Dioxide

Abstract. *Removal of atmospheric carbon dioxide enhanced penetration of seedling wheat by stem rust* Puccinia graminis *in light and dark but did not materially affect penetration by leaf rust* P. recondita. *A concentration of 5 percent CO_2 nearly suppressed penetration by* P. graminis *but not by* P. recondita. *Thus light may promote penetration by* P. graminis *through photosynthetic reduction of CO_2 within the leaf and* P. recondita *may penetrate independently of light because it is relatively insensitive to the effects of CO_2.*

Stem rust, *Puccinia graminis* Pers. f. sp. *tritici,* seldom penetrates stomata of seedling leaves of wheat (*Triticum* spp.) in the dark, while leaf rust, *P. recondita* Rob. ex Desm. f. sp. *tritici,* penetrates well in either light or darkness. Hart (*1*) concluded that *P. graminis* penetrates largely through open stomata, attributing the near failure of infection after inoculation in the dark to exclusion of the fungus by closed stomata. Germination of urediospores and appressorial formation by *P. graminis* occur in the dark, but light is required for appreciable development of substomatal vesicles (*2, 3*). Since there is a stimulatory effect of light on substomatal vesicle formation on artificial substrates (*4*), Sharp *et al.* (*2*) concluded that light-induced penetration by *P. graminis* resulted in part from direct stimulation of the fungus. The leaf-rust fungus penetrates wheat stomata equally well in light or dark and stomata, if originally open, close before penetration (*5*).

This study was made to elucidate further the mechanism of action of light on penetration by *P. graminis* and the very different responses to light by the two species of wheat-rust fungi. Seedlings of Axminster and Michigan Amber wheats were inoculated with urediospores of *P. graminis,* races 48A and 15B, and with *P. recondita,* races 2 and 104C. After an incubation period of 10 hours for stemrust and 4 hours for leaf rust in a dark moist-chamber, the seedlings were transferred to 2-quart Mason jars fitted with transparent plastic lids, and treated with combinations of light and dark, at different concentrations of CO_2. Aluminum foil wrapped around the jars provided the necessary darkness. Carbon dioxide was added by circulating a moistened mixture of 5 parts CO_2 and 95 parts air. It was removed for the "CO_2-free" experiments by absorption in a solution of KOH or $Ba(OH)_2$ or by circulating moistened air from which CO_2 had been absorbed. After exposure to experimental conditions for 9 to 10 hours, abaxial epidermal strips were taken from the first leaf of five plants in each group and the strips were microscopically examined for penetration. Each leaf was treated as one replication in the statistical analysis. Penetration is expressed as the percentage of appressoria from which substomatal vesicles had been produced. Stomatal opening was determined by observing living leaves and epidermal strips fixed in absolute alcohol. Germination and apressorial formation were normal and little penetration of either rust had occurred before the experiments were begun.

In the dark, little penetration by *P. graminis* occurred in normal air, while in CO_2-free air the average penetration increased to about ½ of that occurring in normal air in light (Table 1). In the light, penetration was somewhat greater in CO_2-free air than in normal air. Each of these differences between CO_2-free and normal air was significant ($p \leq .05$) for each trial and for the grand mean of trials with race 15B. The differences were also significant with race 48A in each of four trials in darkness, and in three of four trials in light. Similar and significant differences were obtained with the Michigan Amber wheat seedlings.

The capacity of stem rust to penetrate in light or in CO_2-free air in darkness might be interpreted as resulting from stomatal opening that occurs under these conditions. However, stomata, which were closed when occupied by appressoria of *P. graminis* in darkness, failed to open when exposed to light. Maximum opening was to narrow slits, and occurred in only 2 percent of the occupied stomata. Nearly all adjacent, unoccupied stomata opened widely. Thus the fungus can penetrate closed stomata.

Penetration of *P. graminis* in the light in 5 percent CO_2 was limited and comparable with that in darkness in normal air (Table 1). Results were similar with the Michigan Amber wheat.

The results with *P. recondita* (Table 1) contrast markedly with those for *P. graminis* and confirm the results obtained by Caldwell and Stone (*5*) in showing that abundant penetration occurs in darkness and through closed stomata. The addition of CO_2 did not significantly reduce penetration as it did with *P. graminis.* The removal of CO_2 had no significant effect on penetration of race 104C. A proportionately small, but statistically significant reduction resulted with race 2.

The results on the penetration of the Axminster wheat were corroborated for

Table 1. Percentage of penetration of *Puccinia graminis* f. sp. *tritici* and *Puccinia recondita* f. sp. *tritici* on Axminster wheat seedlings under different light and CO_2 treatments. The percentage penetrations are the grand means of four replicated trials with race 48A, and two trials with race 15B of *P. graminis* and three trials each with races 2 and 104C of *P. recondita.* The numbers in parentheses indicate the average number of pustules per predetermined 6-cm linear segments of the primary leaf in ten trials, replicated three times for *P. graminis;* and in one trial, replicated three times for *P. recondita.*

Race	Light			Dark		
	Normal air	CO_2-free	5 percent CO_2	Normal air	CO_2-free	5 percent CO_2
			P. graminis f. sp. *tritici*			
48A	59.8	70.4	2.4*	2.0	24.6*	
15B	49.8 (38.5)	73.8*	2.8* (4.4)	4.9 (3.1)	28.4* (3.2)	
			P. recondita f. sp. *tritici*			
2	(59.1)		(52.9)	64.3 (55.2)	42.3* (49.0)	50.8
104C				61.3	55.7	59.2

* A significant deviation ($p \leq .05$) of the grand mean from the grand mean in normal air, under the same light condition, as calculated by arc sine transformation.

both leaf and stem rust by the studies of pustule development (Table 1).

Thus CO_2 concentration of the atmosphere significantly affected stomatal penetration by *P. graminis* and neither stomatal opening nor direct stimulation of the fungus by light were major limiting factors. In contrast, neither removal of atmospheric CO_2 nor increase to a 5 percent concentration materially influenced penetration by *P. recondita*. Therefore it is possible that the major influence of light on penetration of *P. graminis* is exerted through its effect on the atmospheric CO_2 concentration within the leaf, which increases through respiration in darkness and is reduced by photosynthesis in the light, and that *P. recondita* penetrates independently of light, because of its insensitivity to the effects of CO_2 within the limits of concentration in the wheat leaf. Whether the effects of CO_2 on *P. graminis* are exerted directly on the rust fungus or indirectly through the host is not yet known (*6*).

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Suppression of the Development of Female Mating Behavior by Estrogen Administered in Infancy

Abstract. *The administration of estradiol benzoate subcutaneously to 4-day-old female rats resulted in reduced mating in response to estrogen and progesterone in adulthood.*

It is reasonably clear that sexual differentiation in mammals is influenced by fetal sex hormones. Male hormones, in particular, seem to be critical for the appropriate development of the Wolffian duct system, and, ultimately, of male external genitals. Only recently has the influence, on adult sexual behavior, of hormones secreted or administered during the embryonic, fetal, and neonatal periods begun to be studied.

Phoenix *et al.* (*1*) found that the administration of testosterone to pregnant guinea pigs resulted in female offspring which, in adulthood, exhibited reduced female sexual behavior and enhanced male sexual behavior. Using the rat, Barraclough and Gorski, and Harris and Levine (*2*) have shown that the application of testosterone to the 5-day-old female leads to the failure of spontaneous mating activity at maturity. Further, treated females fail to mate if castrated and treated with exogenous estrogen and progesterone. In addition, Harris and Levine have found that comparable effects prevail if the male rat is treated with estrogen during infancy. At maturity the male fails to copulate even if administered normally adequate doses of testosterone propionate.

These studies suggest that the neural structures which determine sexual behavior may be deleteriously affected by heterotypical hormones acting during a critical stage of their development. To date, the effects of homotypical hormones administered during infancy in a single injection have not been studied. The present investigation was designed to help fill this gap.

Nineteen female rats from a locally maintained, randomly bred, pigmented strain were treated on the fourth day after birth. Fourteen of the females were injected subcutaneously with 200 μg of estradiol benzoate in 0.2 ml of mineral oil. The five control females were given 0.2 ml of mineral oil (*3*). Subjects were reared in groups until 95 days of age, when they were ovariectomized and caged in pairs. Mating tests began at approximately 130 days of age.

Subjects were primed with estradiol benzoate, 50 μg at 72 hours and 25 μg at 48 hours prior to each set of mating tests. Five hours before testing they were given 1.0 mg of progesterone. This procedure has been found to reliably induce receptivity in spayed females. At the appropriate time after the final priming injection a female was placed with a male in a cylindrical glass observation cage for a 15-minute period. Each individual was tested twice, with an inter-test interval of 14 days. During each test the observer recorded, for the male, the frequency of mounts without intromissions, intromissions, and ejaculations, and for the female, the frequency of assuming the mating posture (lordosis) and the number of times the female kicked her hind legs at the male. The kicking response is an index of sexual refractoriness.

Table 1. Mating responses of female rats treated with estrogen in infancy. Abbreviations: χ^2, chi-square analysis of percentage data; F, analysis of variance of frequency data; *p*, probability; n.s., not significant.

Sexual responses	Estrogen treated ($N = 14$)		Oil treated ($N = 5$)		χ^2	F
	% of females	Mean frequency	% of females	Mean frequency		
By male:						
Mount	100	13.0	100	9.1	*	1.4 (n.s.)
Intromission	36	0.68	100	4.6	6.1 ($p < .025$)	35.6 ($p < .001$)
Ejaculation	21	0.11	80	0.80	5.4 ($p < .05$)	*
By female:						
Lordosis	29	0.46	100	12.8	7.5 ($p < .01$)	*
Kicking	100	5.2	40	1.0	10.0 ($p < .01$)	7.2 ($p < .025$)

* Not analyzed because of nonnormality of distributions.

Table 1 shows the percentage of each group which exhibited the various sexual responses *at least once* during the two tests, and the frequency of the responses averaged over both tests. Males mounted all females in both groups, and with similar frequencies, but were unsuccessful in achieving intromission with the females treated with estrogen in infancy. When mounted by males, the estrogen-treated females did not show lordosis; instead, on 40 percent of the mounts the female responded by kicking the male. The reduced lordosis frequency accounts for the low intromission and ejaculation frequencies among treated females. Control females, given only oil in infancy, assumed the lordosis posture when mounted by the male and permitted intromission and ejaculation. Except for the frequency with which the females were mounted by males, the two groups of females differed significantly in their sexual behavior according to analysis of variance and chi-square tests. The indiscriminate mounting by the males probably reflects only the extreme vigor of these animals. The absence of intromission does not reflect estrogen-induced changes in genital morphology as the vaginae of these animals appear normal.