Environmental and Host Effects on Colony Development of *Puccinia recondita* f. sp. *tritici*

J. R. Tomerlin, M. G. Eversmeyer, C. L. Kramer, and L. E. Browder

First author, formerly agricultural research technician, U.S. Department of Agriculture, Agricultural Research Service, Department of Plant Pathology, Kansas State University, Manhattan 66506 (present address: USDA-ARS, Field Crops Laboratory, Plant Genetics and Germplasm Institute, Beltsville Agricultural Research Center, Beltsville, MD 20705); second and fourth authors, USDA-ARS, Department of Plant Pathology, Kansas State University, Manhattan 66506; third author, Division of Biology, Kansas State University, Manhattan 66506.

Cooperative investigations of the USDA-ARS, and the Kansas Agricultural Experiment Station, Department of Plant Pathology, Contribution 82-267-J.

A portion of the Ph.D. dissertation of the first author.

Mention of a trademark or proprietary product does not constitute a guarantee or warranty by the USDA, and does not imply approval over other products that also may be suitable.

Accepted for publication 31 August 1983.

ABSTRACT

Tomerlin, J. R., Eversmeyer, M. G., Kramer, C. L., and Browder, L. E. 1984. Environmental and host effects on colony development of *Puccinia recondita* f. sp. *tritici*. Phytopathology 74: 225-229.

Plants of near-isogenic wheat lines Thatcher (TC), LR16(TC), and LR18(TC) were inoculated with urediniospores of culture UN02-64A of *Puccinia recondita* f. sp. *tritici* at seedling, heading, and anthesis stages. Following inoculation, seedlings were maintained at temperatures ranging from 15.6 to 29.4 C, and plants inoculated at heading or anthesis were maintained at temperatures ranging from 21.1 to 29.4 C. Rate of growth of colonies and uredinia was generally fastest at moderate temperatures on cultivar TC and on seedlings. Temperatures below 21.1 C greatly reduced fungal development on seedlings of LR18(TC). Colony growth was linear with time within each temperature at each growth stage. The number of urediniospores produced per unit area of uredinium was not a satisfactory way to evaluate environmental, host age, or host resistance effects. The number of urediniospores produced per unit area of colony was slightly more useful in evaluating those effects.

Additional key words: resistance, *Triticum aestivum*.

An important goal in wheat breeding is the development of disease-resistant cultivars. Historically, breeders have favored specific resistance, which is qualitative and can be readily described by various coding schemes (3,4). Such codes permit rapid evaluation of a great number of entries. Specific resistance generally results in death of host cells and pathogens (22), although some sporulation may occur before cessation of pathogen development.

Plant breeders have become interested in horizontal resistance, which is quantitative rather than qualitative. Horizontal resistance is characterized by one or more of the following: increased latent period, decreased spore production, decreased infectious period, decreased infection efficiency, and decreased lesion size (26). Slow-rusting cultivars allow the pathogen to develop, but at a much reduced rate. *Puccinia recondita* f. sp. *tritici* Rob. ex Desm. exhibits a longer latent period (10,15,20), smaller colonies, and smaller uredinia (8,9,13,16,24) on slow-rusting wheat cultivars than on fast-rusting (susceptible) cultivars. Fewer urediniospores are produced per uredinium on slow-rusting cultivars (8,20) and per unit uredinium (20). Recent studies indicate that some specific-resistance genes condition host responses that resemble slow rusting (14,25).

The purpose of the present study was to determine how colony size, uredinium size, and spore production per unit colony or per unit uredinium are associated with specific resistance to *P. recondita* in wheat (*Triticum aestivum* L. em Thell) and how these factors are influenced by temperature and host growth stage.

MATERIALS AND METHODS

Seeds of the wheat cultivar Thatcher (TC) (CI 10003) or the near-isogenic lines LR16(TC) (Thatcher 6*/Exchange, RL 6005) (1,5) or LR18(TC) (Africa 43/7* Thatcher, RL 6009) (2,5) were planted in plastic pots. Plants were grown in the greenhouse under a day/night temperature regime of 18–24/12–21 C until inoculation. Plants were subirrigated as necessary and fertilized at 3, 6, and 9 wk with ~5 g of 20-20-10 granular fertilizer. Plants with apparently equal vigor (determined by height and color) were selected for testing.

Test plants were inoculated with urediniospores of *Puccinia recondita* f. sp. *tritici* culture UN02-64A (ATCC PR3) as described previously (25). Culture UN02-64A is virulent on TC [infection type (IT) of 88] and avirulent on LR16(TC) and LR18(TC) (IT of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

23N and 56X, respectively) (3). Plants were inoculated at the seedling (Feekes scale 2) (11), heading (Feekes scale 10.0–10.1), or anthesis (Feekes scale 10.5.3–10.5.4) growth stage. After incubation in a moist chamber at 18 ± 1 C for 12–16 hr, inoculated plants were placed at random in environmental chambers.

In one series of experiments, seedling plants were maintained in environmental chambers at 15.6, 18.3, 21.1, 23.9, 26.7, or 29.4 C. In a second series of experiments, adult plants were inoculated at anthesis and maintained at 21.1, 23.9, 26.7, or 29.4 C. In a third series of experiments, plants were inoculated at seedling and heading growth stages and maintained at 21.1, 23.9, 26.7, or 29.4 C. Seedling plants were placed on stands to position infected leaves at the same level as infected flag leaves.

The 29.4 C temperature was actually a 29.4 light/21.1 C dark temperature regime. A lower night temperature was necessary to prevent premature senescence of infected leaves and to allow comparison of seedling and flag leaves. All other temperatures were held constant during day and night. The photoperiod was 12 hr at approximately 11,000 lux.

After incubation, the middle half of inoculated leaves was delineated with a nonphytotoxic nursery marker. At 84, 168, 252, and 336 hr postinoculation (PI), the third leaf of seedling plants or flag leaf of adult plants was collected, cleared, and stained as described by Rohringer et al (17), except that their procedure was modified by clearing leaf sections by autoclaving in a lactophenol-ethanol mixture for 3 min and by using Tinopal UNPS (Ciba-Geigy Corporation, Greensboro, NC 27409) as the fluorochrome.

After staining and mounting in 25% aqueous glycerol and ringing with Permount, whole-leaf sections were viewed with an Olympus microscope equipped with a UV lamp, two UG-1 exciter filters, a U (DM-400+1-410) dichromic mirror, and an L-420 barrier filter. The length and width of each noncoalescent colony and each erumpent uredinium within the designated area was measured with an ocular micrometer. Colony and uredinium areas were estimated by the formula for the area (A) of an ellipse:

$$A = 0.25 \times \Pi \times (\text{Length} \times \text{Width}).$$

Leaves collected at 336 hr PI also were monitored for urediniospore production. Urediniospores were collected from the adaxial surface of infected seedling and flag leaves from the beginning of sporulation until the leaves were harvested at 336 hr PI. Methods of collecting and counting urediniospores are described elsewhere (25). The number of urediniospores produced per square millimeter of colony or uredinium was also calculated.

The experiment with plants inoculated as seedlings or at anthesis had the following split-plot model:

$$Y_{ijkl} = \mu + R_i + C_j + \delta_{ij} + V_k + T_l + CV_{jk} + CT_{jl} + VT_{kl} + CVT_{jkl} + \epsilon_{ijkl}$$

in which i = one,···, four or six replications (R), j = one,···, four or six temperature treatments (C), k = one,···, three wheat cultivars (V), and l = one,···, four sampling times (T).

The experiment conducted with plants inoculated as seedlings and at heading had the following split-plot model:

$$Y_{ijklm} = \mu + R_i + C_j + \delta_{ij} + V_k + T_l + G_m + CV_{jk} + CT_{jl} + CG_{jm} + VT_{kl} + VG_{km} + TG_{lm} + CVT_{jkl} + CVG_{jkm} + CTG_{jlm} + VTG_{klm} + CVTG_{jklm} + \epsilon_{ijklm}$$

TABLE 1. Area of colonies and uredinia of *Puccinia recondita* on three wheat cultivars at three growth stages 336 hr after inoculation[w,x]

Cultivar	Colony area (10^{-3}·mm^2)[y]			Uredinium area (10^{-3}·mm^2)[y]		
	Seed	Head	Anth	Seed	Head	Anth
Thatcher	2,176 a[z]	1,417 e	1,457 f	329 a	101 d	106 f
LR18(TC)	1,600 b	1,063 d	889 g	225 b	151 e	69 fg
LR16(TC)	805 c	867 cd	741 g	64 c	49 c	38 g

[w] Seedling data are from an experiment in which plants at the seedling and heading growth stages were inoculated. Cultivar means are averaged over the temperatures 21.1, 23.9, 26.7, and 29.4 C.

[x] Colony and uredinium area estimated by the formula 0.25 × Π × (length × width).

[y] Plants inoculated at seedling (Seed), heading (Head), or anthesis (Anth) growth stage.

[z] Values in a column followed by a letter in common are not different according to Duncan's multiple range test, $P = 0.05$. Values for seedling and heading plants in a row followed by a letter in common are not different according to Student's t-test, $P = 0.01$.

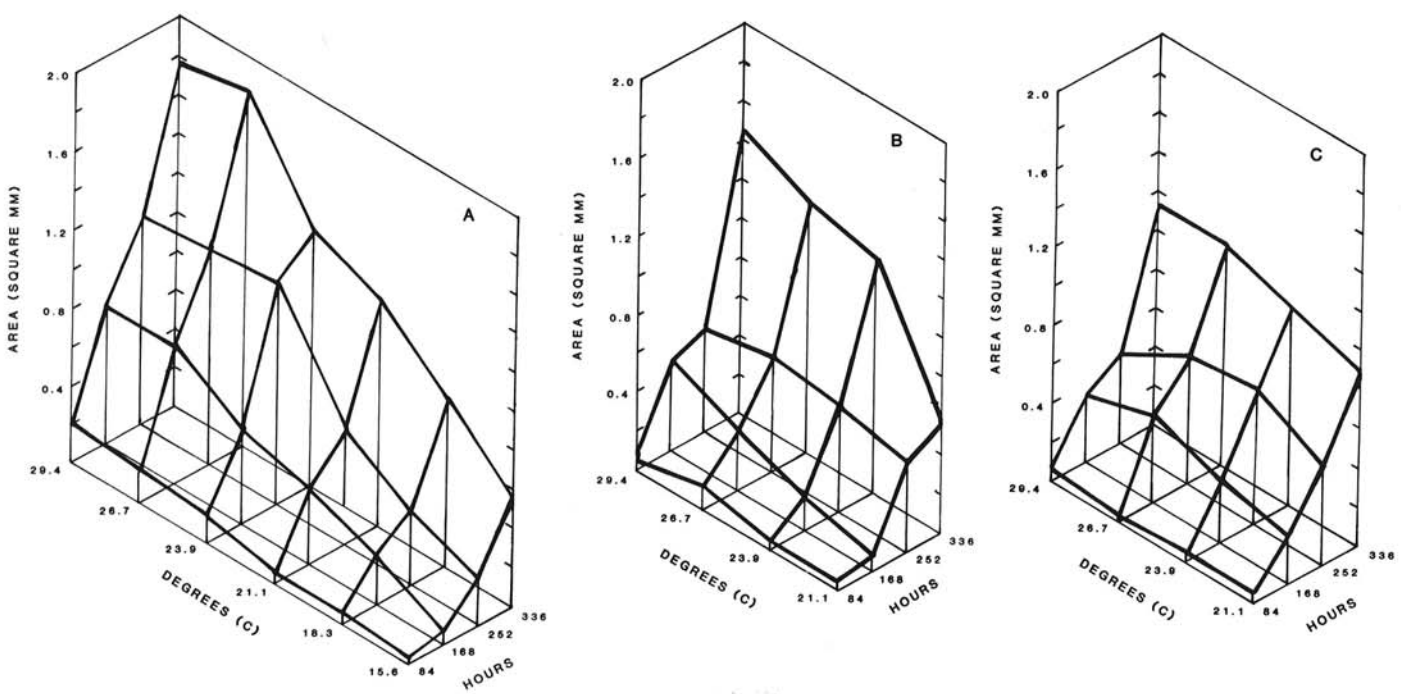

Fig. 1. Effect of temperature on colony growth of *Puccinia recondita* on wheat plants inoculated at: **A,** seedling; **B,** heading; and **C,** anthesis growth stages. Seedling data are from experiments conducted with seedlings only. Data are averaged over the cultivars TC, LR16(TC), and LR18(TC).

in which i = one,···, four replications (R), j = one,···, four temperature treatments (C), k = one,···, three wheat cultivars (V), l = one,···, four sampling times (T), and m = one or two growth stages (G). In each experiment, the number of replications equaled the number of temperature treatments, allowing each temperature to be tested in each of four or six different growth chambers. Preliminary analyses indicated that chamber effects were not significant. Sampling of leaf tissue was destructive, so sampling time was considered as a simple factor. Differences in rate of colony and uredinium growth were tested by comparing slopes using regression analysis (23).

RESULTS

Colony area. On seedlings, colony growth was faster at high than at low temperature (Fig. 1A). Linear trends of temperature were significant ($P \leq 0.01$) at all times, and quadratic trends of temperature were significant for 252 and 336 hr PI ($P \leq 0.10$ and 0.05, respectively). Colony growth on seedlings generally was fastest on TC and slowest on LR16(TC) (Fig. 2A). Colony growth was less rapid, however, on LR18(TC) at 15.6 and 18.3 C. The differences in rate of colony growth are reflected in final colony size. At 336 hr PI, colony sizes on seedlings were in the order TC > LR18(TC) > LR16(TC) (Table 1).

Regression analysis using cultivar as a qualitative variable and time as a quantitative variable showed that at temperatures ≥21.1 C, the rate of colony growth was more rapid on TC and less rapid on LR16(TC). The rate of colony growth on LR18(TC) was generally the same as on TC. At 15.6 and 18.3 C, however, the rate of colony growth was slowest on LR18(TC).

The rate of colony growth on plants inoculated at heading was linear within each temperature tested in the range 21.1–29.4 C. The rate of colony growth was more rapid at 29.4 C than at 21.1 C (Fig. 1B), which resulted in larger colonies 336 hr PI on plants maintained at 29.4 C. In addition, the regression of final colony size on temperature was significant.

Differences in rates of colony growth attributable to cultivars were only observed on plants maintained at 23.9 C. However, when averaged over all temperatures tested in the range 21.1–29.4 C, the rate of colony growth was faster ($P \leq 0.10$) for TC than for LR16(TC) and LR18(TC) (Fig. 2B). The faster rate of colony growth on TC resulted in larger colonies on TC 336 hr PI (Table 2).

Comparison of final colony size on seedlings and plants inoculated at heading shows that colonies were larger on TC and LR18(TC) seedlings than on plants inoculated at heading. Colony size on LR16(TC) was not affected by growth stage (Table 1).

On plants inoculated at anthesis, colony growth was linear within each temperature tested. However, the rate of colony growth was the same at all temperatures tested in the range 21.1–29.4 C (Fig. 1C). Cultivar differences, averaged over the range 21.1–29.4 C, were significant ($P \leq 0.10$), and the rate of colony growth on TC was faster than on LR16(TC) and LR18(TC) (Fig. 2C). When analyzed within temperature, cultivar differences in rate of colony growth were significant only at 23.9 C.

Uredinium area. Uredinia were not erumpent until at least 168 hr PI (Fig. 3). Once uredinia became erumpent, development was linear. Regression analysis showed that the rates of uredinium growth on seedlings were different among the different temperatures. The tendency was for development to be slower at

TABLE 2. Urediniospore production per unit area of colony and uredinium by *Puccinia recondita* on three wheat cultivars at three growth stages through 336 hr after inoculation[x]

Cultivar	Spores per mm² colony[y]			Spores per mm² uredinium[y]		
	Seed	Head	Anth	Seed	Head	Anth
Thatcher	1,176 a[z]	1,394 a	2,104 c	7,723 a	24,551 b	34,072 cd
LR18(TC)	1,728 a	952 a	4,196 c	15,062 ab	6,556 a	44,637 c
LR16(TC)	1,157 a	747 a	1,330 d	13,492 ab	14,856 ab	26,416 d

[x] Seedling data are from an experiment in which plants at the seedling and heading growth stages were inoculated. Cultivar means are averaged over the temperatures 21.1, 23.9, 26.7, and 29.4 C.

[y] Plants inoculated at seedling (Seed), heading (Head), or anthesis (Anth) growth stage.

[z] Values in a column followed by a letter in common are not different according to Duncan's multiple range test, $P = 0.05$. Values for seedling and heading plants in a row followed by a letter in common are not significantly different according to Student's t-test, $P = 0.01$.

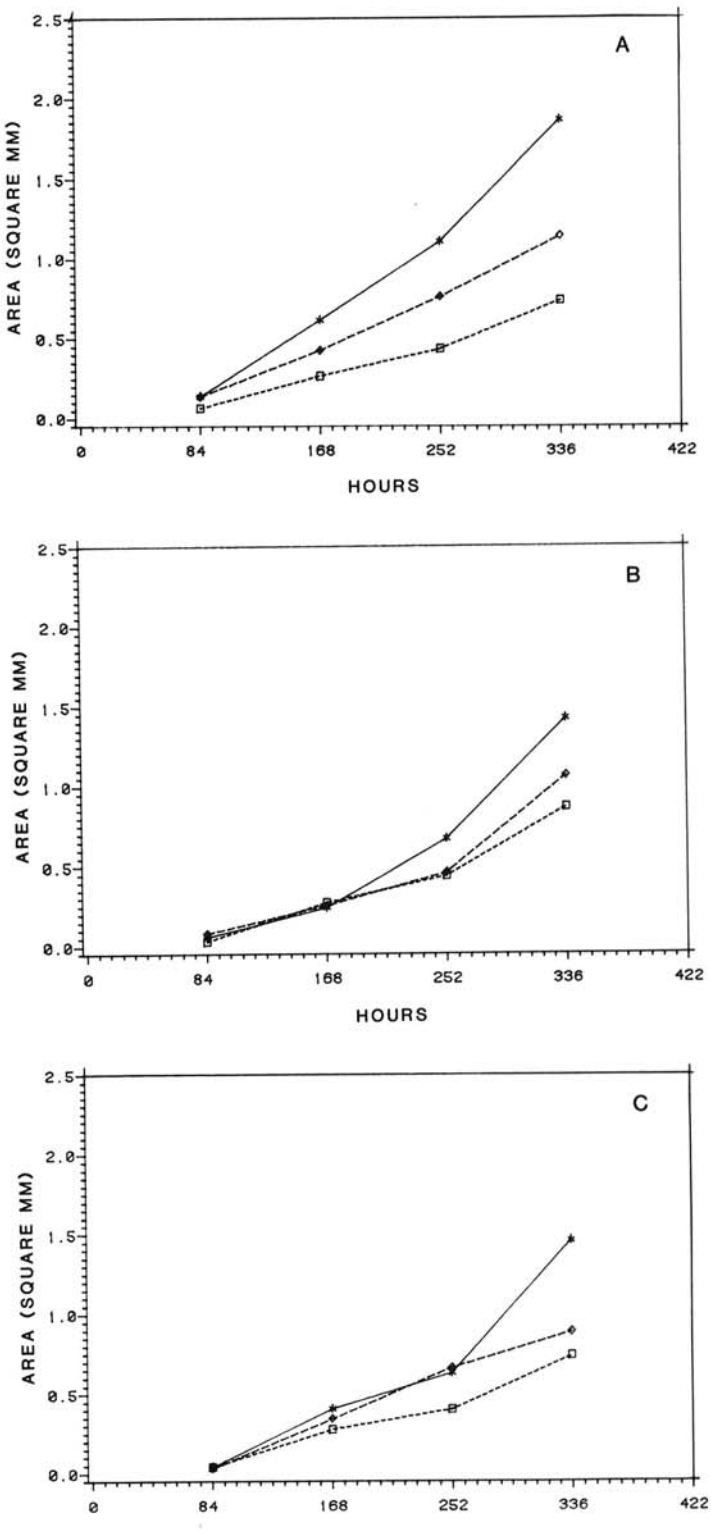

Fig. 2. Area of colonies of *Puccinia recondita* on three wheat cultivars inoculated at: **A,** seedling; **B,** heading; and **C,** anthesis growth stages. Seedling data are from experiments conducted with seedlings only. Data are averaged over temperature. * = TC, ◇ = LR18(TC), □ = LR16(TC).

low temperature than at high temperature.

The rate of uredinium growth was fastest on TC at all temperatures in the range 15.6–29.4 C and slowest on LR16(TC) at temperatures above 21.1 C. However, uredinium growth was slowest on LR18(TC) in the temperature range 15.6–21.1 C. When averaged over all temperatures, the rate of uredinium growth was fastest on TC and slowest on LR16(TC) (Fig. 4A).

On plants inoculated at heading or anthesis, uredinium growth was essentially linear with time for each temperature (Fig. 3B and C). The rate of uredinium growth was not significantly influenced by temperature. The rate of uredinium growth on plants inoculated at heading was fastest on LR18(TC) and slowest on LR16(TC) (Fig. 4B). However, on plants inoculated at anthesis, uredinia grew fastest on TC (Fig. 4C). The differences in growth rate are reflected in final uredinium size (Table 1). Uredinia on seedlings were larger than uredinia on plants inoculated at heading, except on LR16(TC) (Table 1).

Sporulation per unit area. In general, the erratic patterns observed with colony and uredinium area, particularly the occurrence of cubic and quadratic curvature, seemed to result from erratic progression of colony and uredinium width. This was magnified in the calculations for sporulation per square millimeter of colony and uredinium. The variability in these parameters generally precluded statistical separation of cultivar or growth stage response (Table 2). However, there was a tendency for more spores to be produced per unit area on seedlings than on plants inoculated at heading, particularly on LR18(TC). More spores per square millimeter of uredinium were produced on LR18(TC) than on either TC or LR16(TC) (Table 2).

DISCUSSION

Although it is difficult to compare our results with those of other researchers because of differences in host pathogen systems and experimental methods, our data pertaining to the effect of temperature on colony development are in general agreement with reports in the literature. Heagle and Moore (6) reported restricted pustule development of *P. coronata avenae* at low temperature. Samborski et al (19) reported smaller colonies of *Puccinia graminis tritici* on cultivars possessing the *Sr*6 gene at 20 C (*Sr*6 effective) than on the same lines maintained at 26 C (*Sr*6 ineffective). In our study, seedlings possessing the *Lr*18 gene had relatively small colonies and uredinia at temperatures below 21.1 C, whereas seedlings possessing *Lr*16 had relatively small colonies and uredinia over the temperature range 15.6–29.4 C. Thus, *Lr*18 is a resistance gene effective at specific temperatures similar to but having a different functional temperature than *Sr*6.

Temperature differences were reduced at heading and anthesis, as indicated by significance probabilities for temperature $\leqslant 0.01$ or 0.05 for seedlings and of $\leqslant 0.10$ for plants inoculated at heading or anthesis. Thus, the basic effect of temperature on colony growth was similar for all three growth stages, but more intense on seedlings.

Host growth stage often influences pathogen development. Formation of secondary elongating hyphae of *Erysiphe graminis tritici* was retarded on flag leaves (7), flag leaves supported smaller colonies of *E. graminis hordei* than seedling leaves (18), and rate of colony growth by *P. striiformis* was slower on flag leaves than on seedlings (12). However, *P. graminis avenae* colonies were larger on flag leaves than on seedlings (24). Ohm and Shaner (15) observed smaller pustules of *P. recondita tritici* on plants inoculated at heading than on the same cultivar inoculated at seedling or anthesis. In our study, colonies and uredinia were smaller on flag leaves than on seedlings, except for LR16(TC). It is not known whether this is due to the *Lr* genes or to other genes in the near isogenic lines.

Some of the variability in colony and uredinium area may have been the result of differential growth across leaf veins (24) or differences in inoculum density (21). The variation in spore production combined with variation in determining colony and uredinium area precludes the use of sporulation per unit area with present methods as an evaluation parameter in breeding programs.

The effect of cultivar on colony and uredium growth was temperature-dependent at the seedling growth stage. Temperature was less important at the two adult growth stages. In general, larger colonies and uredinia were observed on cultivars to which *P. recondita* UN02-64A is virulent. In greenhouse studies, Ohm and Shaner (15) and Shaner (21) observed smaller uredinia on slow-rusting cultivars than on fast-rusting cultivars. Shaner et al (20) also reported fewer spores per uredinium on one of two slow

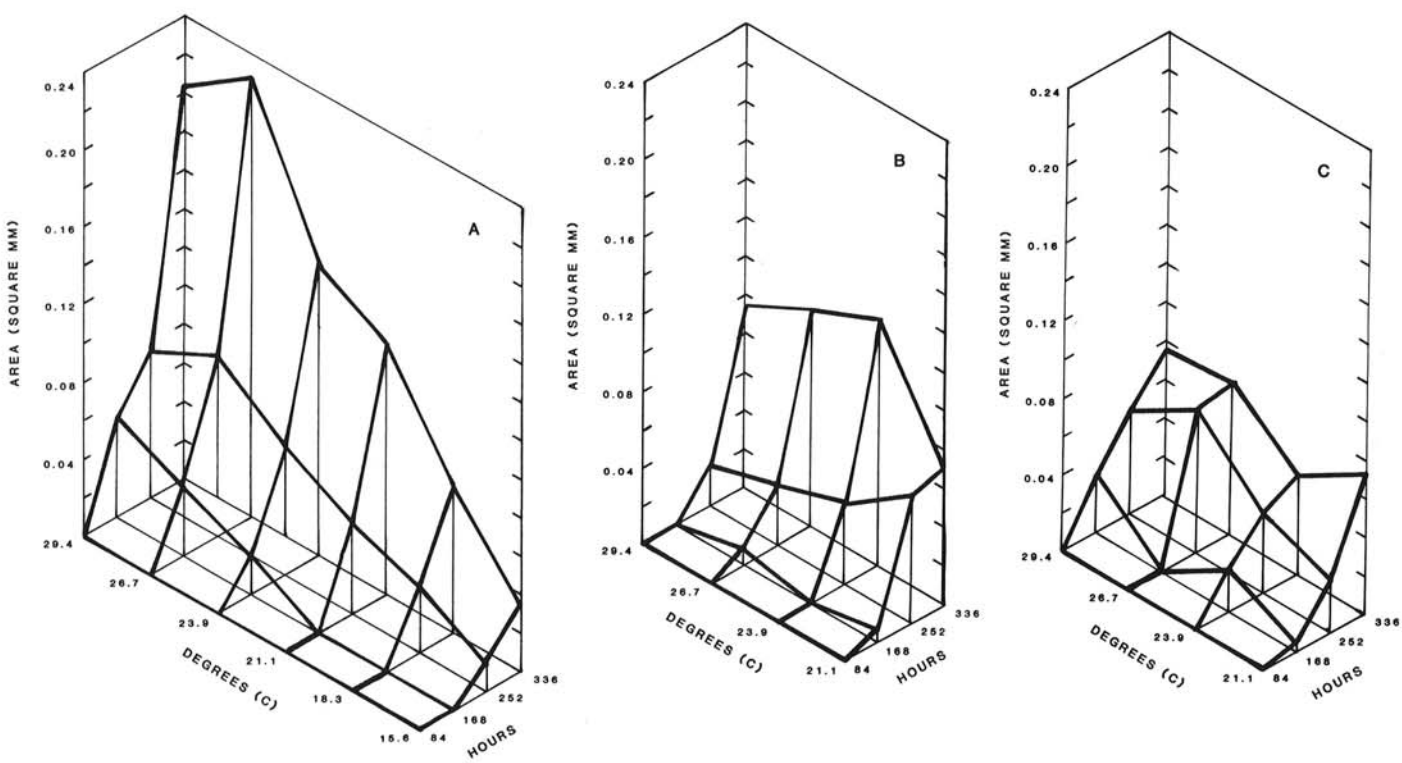

Fig. 3. Effect of temperature on uredinium growth of *Puccinia recondita* on wheat plants inoculated at: **A,** seedling; **B,** heading; and **C,** anthesis growth stages. Seedling data are from experiments conducted with seedlings only. Data are averaged over the cultivars TC, LR16(TC), and LR18(TC).

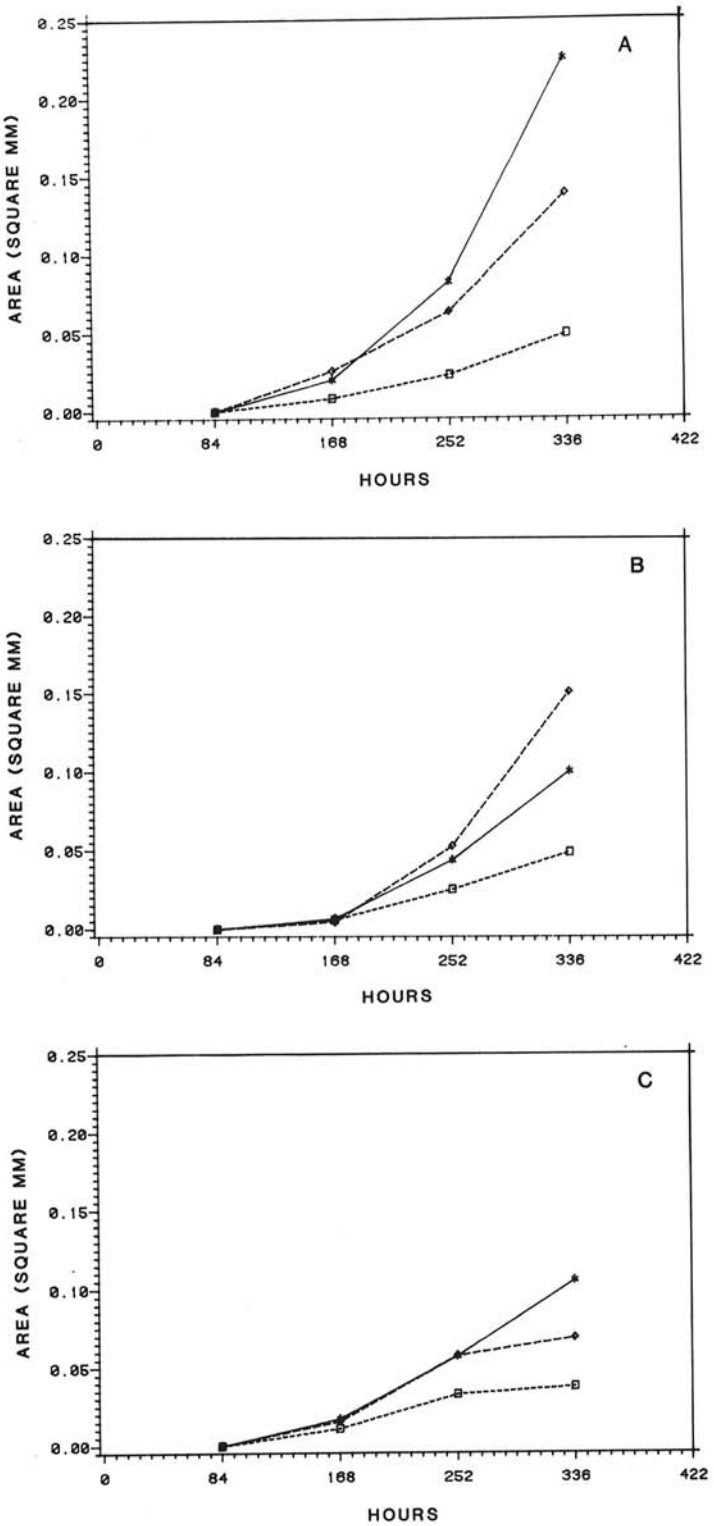

Fig. 4. Area of uredinia of *Puccinia recondita* on three wheat cultivars inoculated at: **A,** seedling; **B,** heading; and **C,** anthesis growth stages. Seedling data are from experiments conducted with seedlings only. Data are averaged over temperature. * = TC, ◊ = LR18(TC), □ = LR16(TC).

rusters.

Our study has some points in common with studies on slow rusting. However, slow rusting is often defined as reduced pathogen development with a susceptible infection type (10,16,21,24). Our study, however, was conducted with combinations of pathogen and host selected for high (TC) or low [LR16(TC) and LR18(TC)] infection types. In a previous study (25), we showed that other characters, such as latent period and spore production per uredinium, commonly associated with slow rusting, also were components of specific resistance. Reduced colony size, reduced uredinium size, and reduced spore production per unit area seem to be components of specific resistance, as well as of slow rusting.

LITERATURE CITED

1. Anderson, R. G. 1961. The inheritance of leaf rust resistance in seven varieties of common wheat. Can. J. Plant Sci. 41:342-359.
2. Anderson, R. G. 1966. Studies on the inheritance of resistance to leaf rust on wheat. Second Int. Wheat Gen. Symp. Lund, 1963, Hereditas Suppl. 2:144-155.
3. Browder, L. E. 1971. A proposed system for coding infection types of the cereal rusts. Plant Dis. Rep. 55:319-322.
4. Browder, L. E., and Young, H. C., Jr. 1975. Further development of an infection type coding system for the cereal rusts. Plant Dis. Rep. 59:964-965.
5. Dyck, P. L., and Samborski, D. J. 1968. Host parasite interaction involving two genes for leaf rust resistance in wheat. Proc. Third Int. Wheat Gen. Symp. Canberra, 1968:245-250.
6. Heagle, A. S., and Moore, M. B. 1970. Some effects of moderate adult resistance to crown rust of oats. Phytopathology 60:461-466.
7. Hyde, P. M. 1977. The effect of leaf age on infection of wheat seedlings by *Erysiphe graminis* and on subsequent colony development. Phytopathol. Z. 88:299-305.
8. Johnson, D. A., and Wilcoxson, R. D. 1978. Components of slow-rusting in barley infected with *Puccinia hordei*. Phytopathology 68:1470-1474.
9. Kochman, J. K., and Brown, J. F. 1975. Host and environmental effects on post-penetration development of *Puccinia graminis avenae* and *P. coronata avenae*. Ann. Appl. Biol. 81:33-41.
10. Kuhn, R. C., Ohm, H. W., and Shaner, G. E. 1978. Slow leaf-rusting resistance in wheat against twenty-two isolates of *Puccinia recondita*. Phytopathology 68:651-656.
11. Large, E. C. 1954. Growth stages in cereals: Illustration of the Feekes scale. Plant Pathol. 3:128-129.
12. Mares, D. J., and Cousen, S. 1977. The interaction of yellow rust (*Puccinia striiformis*) with winter wheat cultivars showing adult plant resistance: Macroscopic and microscopic events associated with the resistant reaction. Phys. Plant Pathol. 10:257-274.
13. Martin, C. D., Littlefield, L. J., and Miller, J. D. 1977. Development of *Puccinia graminis* f. sp. *tritici* in seedling plants of slow-rusting wheats. Trans. Br. Mycol. Soc. 68:161-166.
14. Milus, E. A., and Line, R. F. 1980. Characterization of resistance to leaf rust in Pacific Northwest wheats. Phytopathology 70:167-172.
15. Ohm, H. W., and Shaner, G. E. 1976. Three components of slow-rusting at different growth stages in wheat. Phytopathology 66:1356-1360.
16. Parlevliet, J. E. 1976. Evaluation of the concept of horizontal resistance in the barley/*Puccinia hordei* host-pathogen relationship. Phytopathology 66:494-497.
17. Rohringer, R., Kim, W. K., Samborski, D. J., and Howes, N. K. 1977. Calcofluor: An optical brightener for fluorescence microscopy of fungal parasites in leaves. Phytopathology 67:808-810.
18. Russell, G. E., Andrews, C. R., and Bishop, C. D. 1976. Development of powdery mildew on leaves of several barley varieties at different growth stages. Ann. Appl. Biol. 82:467-476.
19. Samborski, D. J., Kim, W. K., Rohringer, R., Howes, N. K., and Baker, R. J. 1977. Histological studies on host-cell necrosis conditioned by the *Sr6* gene for resistance in wheat to stem rust. Can. J. Bot. 55:1445-1452.
20. Shaner, G. 1983. Growth of uredinia of *Puccinia recondita* in leaves of slow and fast-rusting wheat cultivars. Phytopathology 73:931-935.
21. Shaner, G., Ohm, H. W., and Finney, R. E. 1978. Response of susceptible and slow leaf-rusting wheats to infection by *Puccinia recondita*. Phytopathology 68:471-475.
22. Skipp, R. A., Harder, D. E., and Samborski, D. J. 1974. Electron microscopy studies on infection of resistant (*Sr6* gene) and susceptible near-isogenic wheat lines by *Puccinia recondita*. Can. J. Bot. 52:2615-2620.
23. Snedecor, G. W., and Cochran, W. G. 1967. Statistical Methods, 6th ed. Iowa State Univ. Press, Ames. 593 pp.
24. Sztejnberg, A., and Wahl, I. 1976. Mechanisms and stability of slow stem rusting in *Avena sterilis*. Phytopathology 66:74-80.
25. Tomerlin, J. R., Eversmeyer, M. G., Kramer, C. L., and Browder, L. E. 1983. Temperature and host effects on latent and infectious periods and on urediniospore production of *Puccinia recondita* f. sp. *tritici*. Phytopathology 73:414-419.
26. Vanderplank, J. E. 1963. Plant Disease: Epidemics and Control. Academic Press, New York and London. 349 pp.