

Greenhouse Evaluation of the Adult Plant Resistance of *Sr2* to Wheat Stem Rust

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ABSTRACT

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Plants of the wheat cultivars Hope, Hopps, and H-44 (possessing resistance gene *Sr2*) and Line E, and McNair 701 (susceptible) were inoculated in a settling tower at four growth stages; ie, first-node, second-node, boot, and anthesis. The resistance to *Puccinia graminis* f. sp. *tritici* conditioned by *Sr2* was non-race specific to the races 15-TLM, 15-TNM, and 151-QSH, and was characterized by reductions in number, size, and location of uredia. The resistance was best expressed after anthesis. A reduction in number of uredia (compared to those formed on a susceptible host) occasionally occurred as early as the second-node stage. Uredial numbers on plant lines with *Sr2* were significantly lower than those of

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susceptible hosts at the boot stage. At anthesis, the greatest reduction in uredial numbers on lines with *Sr2* occurred on the peduncle, but not on the spike. A reduction in uredial size usually was apparent on plants inoculated after the boot stage. The greatest reduction in size of uredia occurred on the peduncle and on the internode directly beneath it, but not on the spike or the lower internodes. The infection types on the peduncle and internode below the peduncle generally decrease in size, with the type 4 infections occurring nearest to the nodes and the type 0 two-thirds of the way up or one-third of the way down the internode.

There is ample evidence to support the fact that Hope, H-44, and several other cultivars of wheat (*Triticum aestivum* L.) have adult plant resistance to stem rust incited by *Puccinia graminis* Pers. f. sp. *tritici*. The genetic nature of this adult plant resistance is not fully known, but the *Sr2* gene is recognized as a major component. At present, the adult plant resistance to stem rust in Hope and H-44 is considered to be monogenically inherited (7,8), but universally effective. The effects of environmental conditions on the expression of this resistance are largely undetermined, which may partly explain the conflicting reports concerning the resistance of Hope and H-44 (17).

Little effort has been expended to study *Sr2* in wheat cultivars. Since *Sr2* did not condition a specific infection type and was expressed only in adult plants, there was a lack of techniques to screen for it in the greenhouse. In recent controlled experiments it has been shown that adult plants of Hopps have low receptivity to wheat stem rust (13). The objectives of this study were to extend our knowledge of the manner in which *Sr2*: conditioned resistance to stem rust, was affected by host growth stage, and was affected by differing host genetic background. A better understanding of the response conditioned by *Sr2* would permit screening for this resistance in breeding programs. Furthermore, the pathogen population could be screened for virulence which is currently unknown for *Sr2*.

MATERIALS AND METHODS

Twenty-six cultivars of wheat were used to study adult plant resistance to stem rust, conferred by *Sr2*. Hope (CI 8178), H-44 (CI 8177), H-44-24 (PI 351220), and Hopps (PI 268325), which are known to possess *Sr2* (7,8,10,12), were compared with the susceptible hosts, Line E and McNair 701 (CI 15288). The remaining cultivars were tested for *Sr2*-type adult plant resistance by comparing them with a line known to have *Sr2* and with a susceptible host. Tests were done with a race virulent on seedlings of the cultivars and inoculations were done after heading.

Three wheat stem rust races: 15-TLM, 15-TNM, and 151-QSH, commonly found in the field (14), were used. They were selected because at least one of them was virulent on seedlings of the cultivars studied.

The wheat stem rust races were initially identified on the Cereal Rust Laboratory single genes wheat differentials (15). Inoculum was increased weekly on McNair 701 seedlings kept in isolation cubicles in the greenhouse. Inoculum was collected in gelatin capsules via cyclone spore collector (1). Cultures periodically were checked for contaminants during the course of the experiments.

Plants were grown in 10-cm diameter pots in soil treated with methyl bromide. Fluorescent lighting, 15 cm above the plants, supplemented daylight. A water-soluble fertilizer (23-19-17, N-P-K) was applied at a rate of 0.4 gm/pot 17 days after planting. Plants were thinned to three tillers 1 day before inoculation and were fertilized a second time with 0.4 gm/pot the day following inoculation.

Plants were inoculated in a settling tower in which eight plants were placed on individually rotating supports that were attached to a revolving table at the base (11). The inoculum load consisted of 28-32 mg of uredospores mixed with 120 mg of talc. The inoculum was dispersed into the top of the settling tower with a blast of CO₂ from a modified pellet gun. Five minutes were allowed for the spores to either impact on the plants or settle to the chamber floor (11). Uredospore density at the base of the tower was estimated by counting spores deposited on Vaseline-covered glass slides. Deposition of seven-to-ten spores per square millimeter produced large numbers of uredia on susceptible checks (11).

Uredospore germination and fungal penetration occurred in an adult plant incubation chamber. Moisture was maintained on the plants during 12-14 hr of darkness followed by slow drying during 3 hr of light. Glass-distilled water was constantly atomized on the plants throughout the dark period. Metalrac lamps were the light source; the intensity varied from 3,850 lux at the base to 6,050 lux at the top of the plants. The maximum temperature was kept below 27 and 32 C for the dark and light periods, respectively. Plants were slowly dried in the chamber before being moved to the greenhouse.

Stem rust severity was evaluated in the greenhouse 14 days after inoculation. Individual pustules were counted and infection type was recorded as a measure of infected tissue. Only four infection

types were used due to the variability of maturity of plant tissue. These corresponded to Stakman's 0, 1, 2, and 4 infection types (16). Location of uredia was determined for each internode, peduncle, and spike. These were designated: head = HD, peduncle = P, first internode below peduncle = P-1, second internode below peduncle = P-2, third and fourth internodes below peduncle = P-3+4. The P-3+4 internodes were treated as a single internode since the P-4 internode often was beneath the soil. The extent of reduction in numbers of uredia and their restriction to certain locations on the wheat tiller were evaluated in two replications when plants were inoculated at the boot and anthesis growth stages. Each replication consisted of six test plants with *Sr2* and two Line E plants inoculated with race 15-TNM.

The stage of maturity at which adult plant resistance, conferred by *Sr2*, was expressed was determined by inoculation of plants at four growth stages (first-node, second-node, boot, and anthesis) with race 15-TNM. Six Hopps and two Line E plants per growth stage were inoculated simultaneously in two replications.

The significance of differences in uredial numbers between cultivars was tested by using an analysis of variance in which $P = 0.05$. Student's *t*-test was used for comparing two cultivars and the

F-test for three or more cultivars. When replications were performed on different days, uredial numbers were converted to percentages of uredia present on the common susceptible host.

RESULTS

Stem rust races 15-TLM, 15-TNM, and 151-QSH, were virulent on 7-day-old seedlings of Hopps, while only 15-TNM was virulent on Hope. In trials with progressively older plants, the uredial numbers per plant were not different ($P > 0.05$) among cultivars inoculated at the first-node stage. In plants inoculated at the second-node growth stage, there was a difference ($P < 0.05$) among cultivars in one replication, but not the other. However, the mean number of uredia on Hopps was less than on Line E at both growth stages. The greatest difference in number of uredia per plant between Hopps and Line E was observed in plants inoculated at anthesis (Fig. 1). A similar pattern existed for other cultivars having *Sr2*. Stem rust races 15-TLM, 15-TNM, and 151-QSH produced fewer uredia ($P < 0.05$) on Hopps than on Line E when inoculated at the boot stage (Fig. 2). The resistance response conditioned by *Sr2* to these three races was similar in both numbers of uredia (Fig. 1) and infection type.

Hopps and Line E were inoculated with 15-TNM at anthesis in five experiments. Uredial number varied between plants, cultivars, and replicates with means of 288 and 81 uredia for 17 plants of Line E and 24 plants of Hopps, respectively. The standard deviation for uredia per plant for Line E was 118 (range 160 to 383) and for Hopps, 54 (range 24 to 159) (Fig. 3).

Another expression of *Sr2* resistance was a reduction in uredial size that was not apparent until approximately the boot stage of host development, but was best expressed at anthesis. The infection type (in part based on uredial size) conditioned by *Sr2* did not fit into a category like those conditioned by most of the other single genes for resistance. On most Hopps plants that were inoculated at anthesis, type 4 uredia developed on the glumes, awns, top 0.5-0.8 cm of the peduncle, 0.5 to 2.0 cm above and below the top two

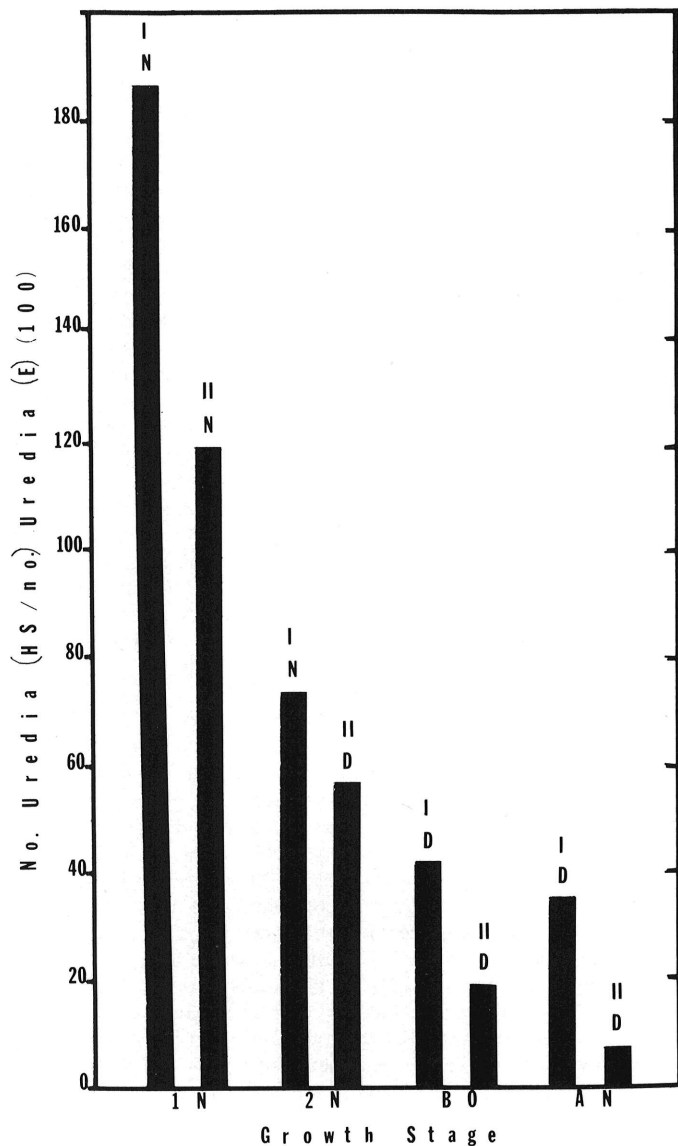


Fig. 1. Relative numbers of *Puccinia graminis* f. sp. *tritici* uredia on Hopps (HS) wheat expressed as percentage of uredia on susceptible Line E (E) when inoculated at the first-node stage (1N), second-node stage (2N), boot stage (BO), and anthesis (AN) for replications I and II. Uredial numbers between cultivars at each growth stage were N = not different ($P > 0.05$), or D = different ($P < 0.05$).

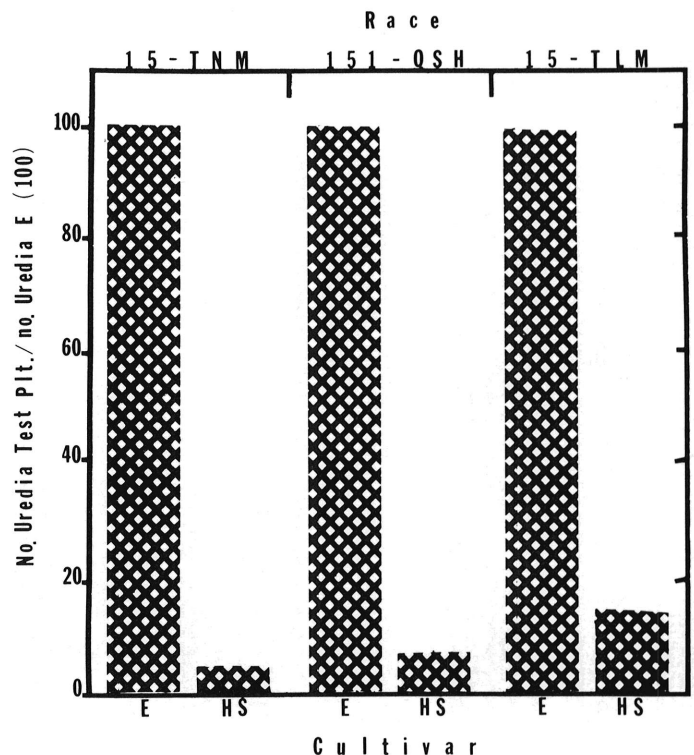


Fig. 2. Number of uredia on Line E (E) and Hopps (HS) wheats expressed as percentages of uredial number on Line E (susceptible) when inoculated at the boot stage with three races of stem rust. Percentages for Hopps were not significantly different ($P > 0.05$) from each other, but were different ($P < 0.05$) from Line E.

nodes, and at random on the three lower internodes. Infection types 0, 0₁, 1, and 2 developed throughout the top two internodes. The variation in infection type on individual plants was great. In each experiment a few Hopps plants exhibited a 0 fleck infection type on the upper three internodes. Occasionally, there were no uredia on the entire stem of all Hopps plants in a replication, although type 4 uredia occurred in the spike. Line E, inoculated in the same manner, had type 4 uredia on the glumes, on the peduncle, and on all internodes.

A third expression of *Sr2* resistance was a change in the distribution of uredia on the host plant. Varying results were obtained when Hopps and Line E were inoculated at boot stage. In the first replication, Hopps had fewer uredia on the peduncle than any other plant part. Line E had nearly equal numbers of uredia on both the peduncle and P-1 internode (Fig. 4A). In the second replication of this test, both Hopps and Line E had more uredia on the peduncle than on any other plant part (Fig. 4B). Hopps had fewer ($P < 0.05$) uredia than Line E in both replications.

When inoculated at anthesis, Hopps had more uredia on the head than on the peduncle or any internode. Although differences were statistically nonsignificant ($P > 0.05$), there were more uredia on the P-2 and P-3+4 internodes than on the peduncle or P-1

internode (Fig. 4C). Line E had more ($P < 0.05$) uredia on the peduncle than on any internode.

The effect of *Sr2* resistance in H-44-24 on location of uredia was somewhat different. The heads of H-44-24 had fewer uredia than on the peduncle or the P-1 and P-2 internodes. No difference ($P > 0.05$) existed for uredial numbers on the peduncle, or the P-1 and P-2 internodes. Line E, however, had more ($P < 0.05$) uredia on the peduncle than on any other portion of the plant (Fig. 4D).

Some Hopps plants, when inoculated at anthesis and left in the greenhouse 28-35 days, developed type 4 uredia at the nodes, which resulted in a banded appearance. In contrast, Line E, similarly inoculated, had a uniform distribution of uredia over the stems. Hopps did not exhibit this response at 14 days after inoculation in the greenhouse and only 10-15% of the plants exhibited this banded appearance in the field.

When other cultivars with *Sr2* were susceptible to a race of *P. graminis* f. sp. *tritici* as seedlings, the adult plants responded like Hopps and those without *Sr2* responded like Line E.

DISCUSSION

The only gene reported to confer resistance to stem rust in wheat cultivar Hopps was *Sr2* (10). Recent evidence indicates, however, that *Sr9d* also is present but provides no resistance to races 15-TLM, 15-TNM, or 151-QSH (A. P. Roelfs and D. V. McVey, unpublished). Numerous stem rust races have been reported avirulent at anthesis, but were virulent on seedlings of Hopps, Hope, and H-44 (3,4,7,8). At present, *Sr2* can be considered an effective adult plant gene for wheat stem rust resistance which reduced ($P < 0.05$) the number of uredia on adult plants of Hopps, H-44. A similar response on Hopps also was found by Mortensen and Green (12). The problem of distinguishing disease escapes and resistant plants in cultivars with *Sr2* was eliminated by using an inoculum treatment of 30 mg of uredospores (65-90% germination) mixed with talc. However, significant ($P < 0.05$) variation in number of uredia occurred among plants of the same cultivar in different replications. Thus, direct comparison of absolute uredial numbers between cultivars in different replications was impossible. However, the analysis was completed by converting the data to percentage of uredia present on the susceptible host.

The distribution of uredia on Hopps and H-44 differed from that on Line E when the cultivars were inoculated at anthesis (Fig. 4). These data confirm previous reports (4,5,8). For Line E, uredial number on the peduncle was greater ($P < 0.05$) than for any other part of the plant (Fig. 4). This pattern of uredial distribution on Line E was expected, since uredospores that impacted on the upper portion of the plants had no chance to impact the lower parts. The presence of fewer uredia on the spike than the peduncle of Line E was due to the smaller size of the spike. The less succulent tissue toward the base of the plant, as well as decreased light intensity on the lower internodes in the dew chamber, also could result in reduced uredial numbers on successively lower internodes.

Awned spikes of Hopps may have created a more favorable microclimate for uredospore germination and penetration in the dew chamber. This could partially explain the large uredial numbers in the spikes. The internodes of greenhouse-grown Hopps were larger both in length and diameter compared to those of Line E. If data had been adjusted for these factors, the contrast in response to stem rust between the cultivars would have increased.

The previously reported (3,4,7,8,12) adult plant resistance of Hope, H-44, and Hopps to race 15-TNM was confirmed in the greenhouse. DePauw and Buchanan (2) found this response expressed as susceptible to mesothetic and then to moderately susceptible when inoculated at the fourth-leaf stage with races EA4(295) and EA8(40). The fourth-leaf stage occurs 14-21 days before the second-node development on the modified Feekes' Scale. It was only possible to distinguish a reduction of numbers of uredia at that stage in this experiment.

Our results agree with those of Johnson and Newton (6) that infection types become progressively lower as plant maturity of Hope at inoculation time increased from the early jointing to the heading stage. Hopps and Hope inoculated at anthesis had

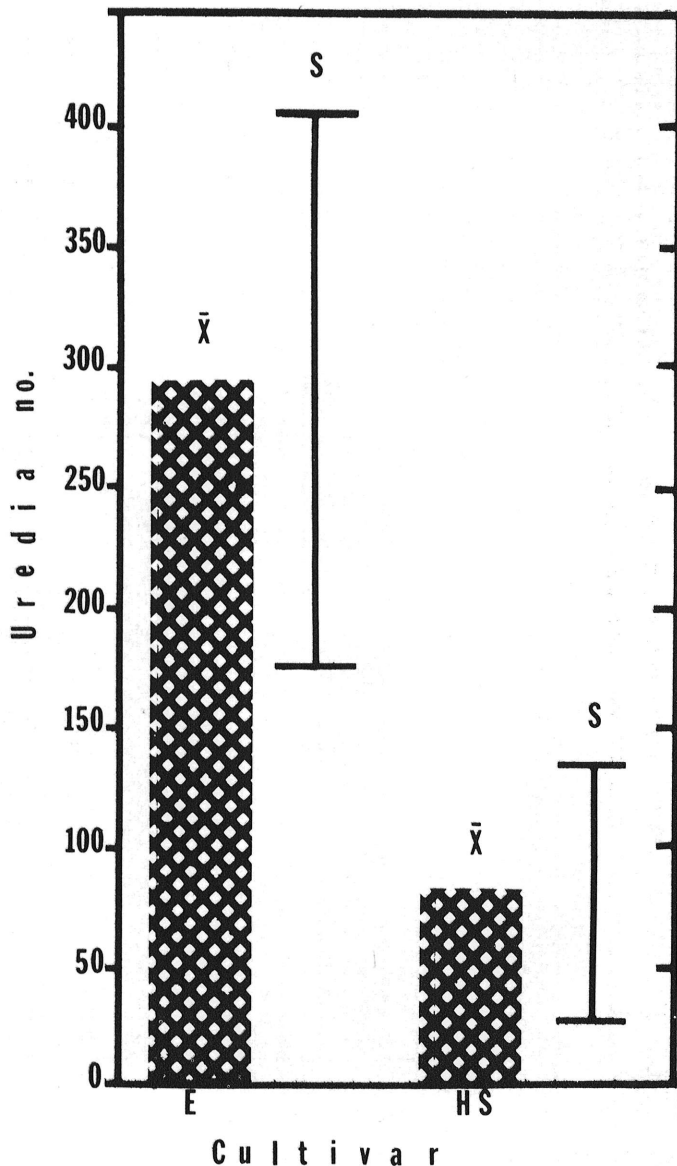


Fig. 3. Mean (\bar{X}) and standard deviation (S) for numbers of uredia on 17 Line E (E) and 24 Hopps (HS) wheat plants inoculated with stem rust race 15-TNM at anthesis.

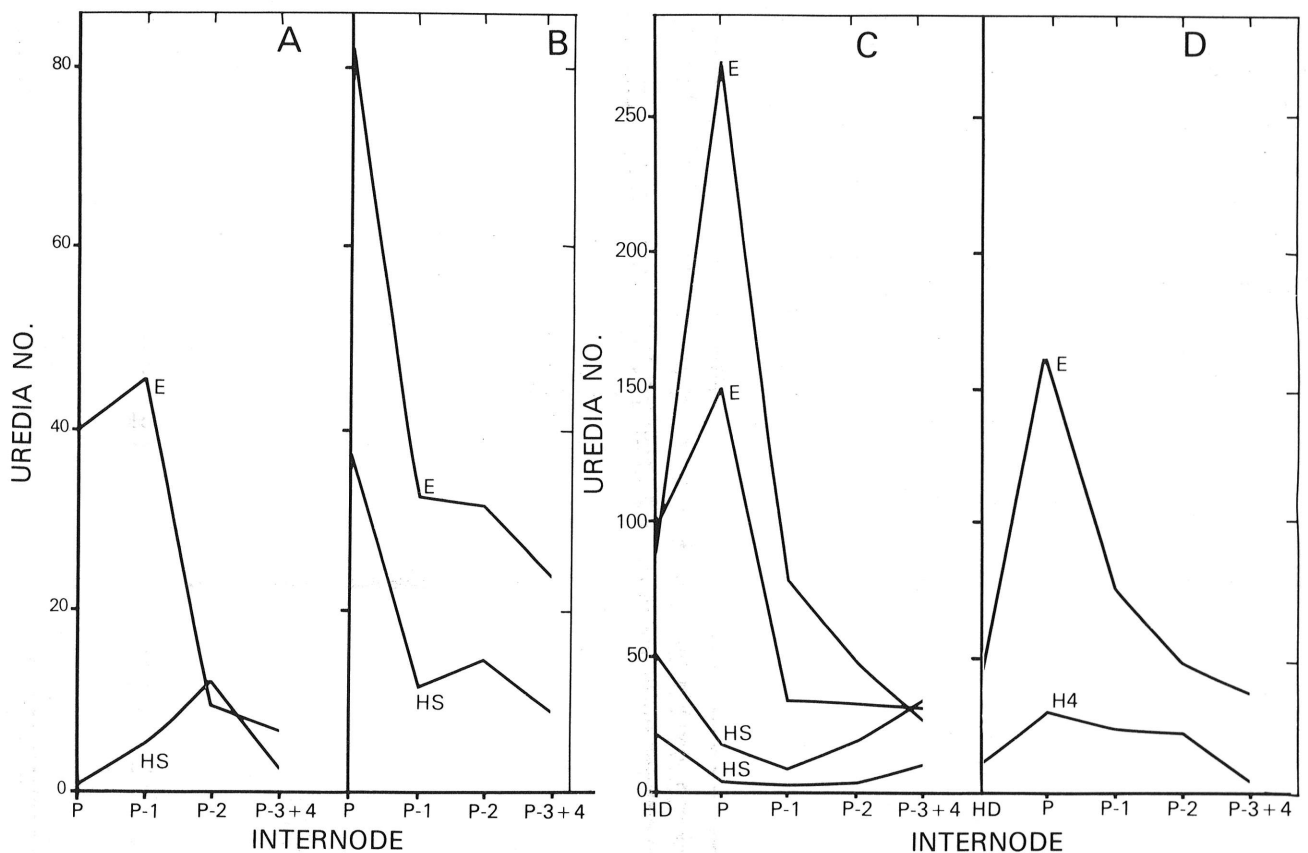


Fig. 4. Stem rust of wheat. Mean uredial number on peduncle (P), first internode below peduncle (P-1), etc.; **A**, and **B**, for six Hopps (HS) and two Line E (E) plants inoculated at boot stage, **C**, for six Hopps (HS) and two Line E (E) plants inoculated at anthesis, replicated twice, **D**, for six H-44-24 (H-4) and two Line E (E) plants inoculated 2 wk after anthesis. The third and fourth internodes below the peduncle (P-3+4) were treated as a single internode.

infection types 0, 1, 2, and 4. The infection type 4 occurred primarily on the three lower internodes, near the top two nodes and on the spike, while infection types 0, 1, and 2 occurred between the top two nodes. In our experiments, Hopps produced large uredia (infection type 4) just above the nodes, similar to earlier reports with Hope (4,5,8,9).

A reduction in infection type was consistently observed in Hope inoculated at the early boot stage, but Hopps inoculated at this stage was more variable. This suggests that the expression of *Sr2* may be modified by other host genes at slightly different growth stages. Anthesis was the earliest growth stage for reliable observation of *Sr2*-conferred adult plant resistance in the cultivars studied. Therefore, we recommend the evaluation of *Sr2* at anthesis. *Sr2* is a useful gene for stem rust control in areas where the logarithmic increase of the rust epidemic begins after wheat has reached the boot growth stage. It should also be useful in combinations with other genes as a back-up resistance in high-hazard areas for stem rust.

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