Histological Aspects of Dwarf Bunt Resistance in Wheat

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Scientific Paper 5008, Washington State University, Pullman; this research was supported in part by the Old West Regional Commission and the Montana Wheat Research and Marketing Committee through a grant to the second author and the College of Agriculture Research Center Project 0283.

We wish to thank Agricultural Research, Science and Education Administration, (SEA), U.S. Department of Agriculture (USDA), Regional Cereal Laboratory, Pullman, Washington, for supplying the wheat cultivars and J. A. Hoffmann, Agricultural Research, SEA-USDA Logan, UT 84332, for the smut collection used in this study.

Accepted for publication 20 April 1978.

ABSTRACT

FERNÁNDEZ, J. A., R. DURÁN, and J. F. SCHAFER. 1978. Histological aspects of dwarf bunt resistance in wheat. Phytopathology 68:1417-1421.

Four wheat cultivars differing in resistance to race D-2 of *Tilletia controversa* were all initially infected following artificial inoculation. Following penetration of the coleoptile in plants of the highly susceptible cultivar Red Bobs, intercellular hyphae ramified throughout primordial leaf and nodal tissue, and reached cells of the growing point. The fungus was assumed to sporulate in plants so infected, provided hyphae reached the growing point before internodal elongation. This assumption was based on results of sectioning 40 Red Bobs seedlings at various intervals, 37 of which were shown to be infected, and on demonstrating bunt in 57 of 63 plants grown to maturity. In the resistant cultivars Requa and Nugaines, hyphae of the fungus followed a similar route, but almost always failed to invade cells of the growing point and thus failed to sporulate. In the highly resistant cultivar P.I. 178383, the few hyphae detected usually were confined to the first and second leaves well away from the growing point.

Dwarf bunt of wheat, which is caused by *Tilletia controversa* Kühn, is endemic in areas where the fungus and disease development are favored by significant snow cover. Because standard seed treatments which control the common bunts do not control dwarf bunt, resistant wheat cultivars thus far have been the only means of control. Although considerable progress has been made in studies of the genetics of resistance to bunt (5, 6), the mechanisms by which genes confer resistance in certain wheat cultivars have yet to be explained. To date, 10 bunt resistance (*Bt*) genes have been identified in wheat (7, 11, 16).

To determine if dwarf bunt resistance could be explained on a morphological basis, Hansen (4) studied the histopathology of artificially inoculated wheat plants, using both resistant and susceptible wheat cultivars. She concluded that hyphae of the fungus in resistant cultivars generally were retarded, compared to development and spread of the fungus in tissue of susceptible cultivars. Her findings and conclusions generally were comparable to those of Woolman (17) in his studies of common bunt resistance. In neither study, were races of the fungus nor resistance genes of the cultivars indicated.

We describe here the histopathology of host-parasite interactions in four artificially-inoculated wheat cultivars known to differ in resistance to race D-2 of *T. controversa*.

00032-949X/78/000257$03.00/0

MATERIALS AND METHODS

Inoculum of *T. controversa* was prepared by germinating teliospores of race D-2 on 2% soil extract agar as described by Meiners (8). Seeds of the wheat cultivars were germinated on moist filter paper at 20-23 C; developing seedlings were subsequently inoculated by placing masses of basidiospores on the coleoptiles when they were 2-5 mm long.

After inoculation, seedlings were transplanted into sterile vermiculite at a depth of 2.5 cm and incubated in darkness for 3-4 wk at 3 C. Thereafter, the seedlings were incubated in continuous light for 1-2 wk at 5 C. At the end of this time, developing plants were transplanted to pots 15 cm in diameter containing a sand and soil mixture (1:1, v/v) and allowed to mature in a greenhouse at 20-25 C.

To determine the time and nature of infection in coleoptile tissue and spread of the fungus in developing plants and heads, infected plant tissue was sampled at various intervals from two groups of plants, one grown during April-July 1976 and another during August 1976-February 1977. In the first group of infected plants sampled for histological study, tissue was taken 1 wk after inoculation and peridodically thereafter up to 90 days; in the second group, tissue samples were collected for study beginning 50 days after inoculation and continued until heads formed and matured in the greenhouse (approximately 140 days). In the second group, which was grown during winter, plants required supplemental light to induce head formation. Although not critically studied, there was no indication that the host-parasite

interaction was influenced by seasonal variations in light intensity; thus, our interpretations are based on a histological study of both groups of infected plants.

For histological study, infected tissue of the four cultivars was fixed in FAA (1), dehydrated in an ethanol-toluene series, and embedded in paraffin. Longitudinal sections (8-12 μm thick) were treated as follows: (i) deparaffinated in a graded xylene-alcohol-water series, (ii) treated in a 1% buffered solution (pH 7.0) of diastase for 15 min to prevent host starch granules from reacting with iodine, (iii) treated for 3 min in Lugol's iodine (1), (iv) washed in running water, (v) stained 3-5 min in 0.5-1.0% aqueous crystal violet, (vi) washed in running water, (vii) returned to Lugol's iodine for 1-2 min, (viii) washed in running water, (ix) rinsed in 95% ethanol, (x) destained in 1:1 ethanol (95%) and acetone, (xi) rinsed in 95% ethanol, (xii) counterstained in 5% picric acid in 95% ethanol, (xiii) rinsed in absolute ethanol, (xiv) rinsed in three changes of xylene, and (xv) mounted in synthetic mounting medium.

Race D-2 of *T. controversa* was used throughout the study. Invasion of host tissue by the fungus was studied in cultivar Red Bobs (C.I. 6255), a highly susceptible spring wheat with no known *Bt* genes for resistance; Requa (C.I. 11554), a winter wheat with variable resistance that originated from a farmer's field selection; Nugaines (C.I. 13968), a winter wheat resistant to race D-2 with *Bt* genes 1, 3, and 4; and P.I. 178383, a winter wheat resistant to all known races of common and dwarf bunt with *Bt* genes 8, 9, and 10 (9, 10).

RESULTS

Red Bobs.—Ten days after inoculation, penetration had occurred (Fig. 1-A), and fungal hyphae were observed in both the coleoptile and in the space between it and the first true leaf (Fig. 1-B). Ingress of the fungus appeared to be intercellular; however, tissue immediately around the point of entry stained poorly, making a precise interpretation difficult. All development and spread of the fungus after infection was intercellular. Within 10-45 days after inoculation, mycelia reached the first and second leaf primordia (Fig. 1-C). At this stage, hyphae were seen in the leaf bases and entered the nodal regions below the main growing point (Fig. 1-D). It frequently appeared that the fungus had grown downward between the coleoptile and the first leaf primordium and penetrated tissues at the leaf bases. After 50 days, the fungus was seen in nearly all juvenile leaf tissue, had ramified throughout the nodal regions, and had reached the main growing point (Fig. 1-E) and tiller initials in most plants. Plants in this stage showed pronounced flecking of newly emerged leaves in which hyphae were subsequently seen.

Well before elongation of internodes was complete, hyphae proliferated in all parts of developing heads (Fig. 1-F). Plants in this stage of development were dwarfed, leaf flecking was severe, and excessive tillering was evident. Immediately before emergence of heads from the boot, mycelia began to invade ovarian tissue intracellularly. Initial stages of sporulation (Fig. 1-G) began before heads emerged from the boot as previously described by Hansen (4).

Requa.—Early stages of infection in this cultivar followed a sequence similar to that described for Red Bobs. For example, 10 days after inoculation, hyphae were seen between the coleoptile and leaf primordia. After 30 days, hyphae had entered first and second leaf primordia (Fig. 2-A) and were in the immediate proximity of the main growing point. At the end of 50 days, hyphae were present in nearly all leaf tissue; however, in only one sample had hyphae grown into nodal tissue. Subsequent examinations of numerous sections failed to reveal hyphae in tissues near the growing point, presumably, because the fungus was unable to invade this tissue. Unlike Red Bobs, at no time did Requa exhibit disease symptoms, nor did mature plants show any traces of sporulation.

Nugaines.—Development of the fungus in Nugaines generally proved to be similar to that described for Requa. As in Red Bobs and Requa, penetration of the coleoptile occurred readily, usually within 10 days. Thirty days after inoculation, hyphae were seen in the second leaf primordium which appeared to be growing toward the main growing point (Fig. 2-B). After 50 days, hyphae in a few sections were seen in nodal tissue below the growing point (Fig. 2-C). However, numerous examinations failed to reveal hyphae in host cells proximal to the growing point, presumably because this tissue is resistant to the fungus. Only one inoculated plant among hundreds of Nugaines developed bunt, and none exhibited symptoms between the time of inoculation and maturity.

Wheat line P.I. 178383.—Development of the fungus in this cultivar differed markedly from the other three cultivars. Examination of sectioned material 10 days after inoculation showed darkly stained material in coleoptile and first leaf tissues which could not be positively identified. However, 50 days after inoculation, parasitic hyphae were seen in first and second leaf tissues (Fig. 2-D). But even among the few plants in which infections were detected, hyphae were sparse, usually confined to first and second leaf tissue, and were well away from the growing point. Of several hundred plants that were inoculated, none showed disease symptoms during vegetative growth and none was bunted at maturity.

DISCUSSION

Our interpretation of the course of development of *T. controversa*, race D-2, in the highly susceptible cultivar (Red Bobs) is similar to that described by Hansen (4) in her studies of other susceptible cultivars. In Red Bobs, hyphae reached the growing point most readily by following the spaces between the coleoptile and the first true leaf and between leaf primordia. Hyphae following this route reached the leaf bases and ramified in the nodal tissue below the growing point (usually within 50 days or before). From these infected sites, mycelia became permanently established in the growing point and tiller initials, always before internodal elongation, and thus assured sporulation of the fungus. It appears likely that in all highly susceptible cultivars hyphae follow the route described for Red Bobs. Although there is no proof that this is invariably the case, the similarity in anatomy and development of wheat plants would seem to dictate a similar path.

Not all hyphae in the infected leaf primordia reached

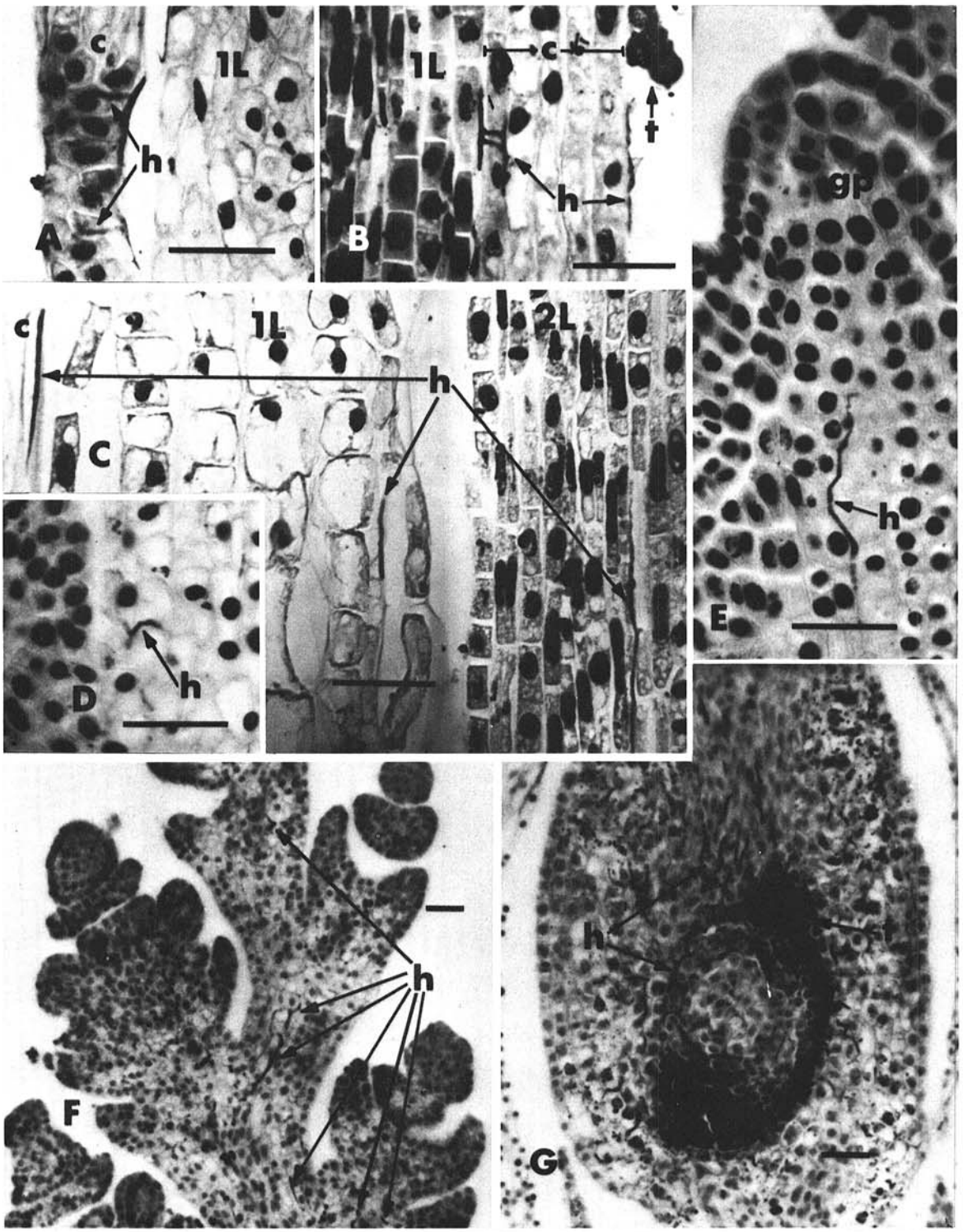

Fig. 1-(A to G). Development of *Tilletia controversa* in the susceptible wheat cultivar Red Bobs. **A)** Intercellular hyphae in the coleoptile 10 days after inoculation. **B)** Hyphae on the surface of the coleoptile and entering the space between the coleoptile and the first leaf 10 days after inoculation. **C)** Hyphae in the coleoptile, first leaf, and second leaf primordia 45 days after inoculation. **D)** Hypha entering tissue just below the growing point via the base of the first leaf 30 days after inoculation. **E)** Hypha approaching the growing point 45 days after inoculation. **F)** Pervasive intracellular hyphae in developing inflorescence 90 days after inoculation. **G)** Onset of sporulation in developing ovarian tissue 115 days after inoculation. Bars represent ~50 μm. Legend: h = hyphae; c = coleoptile; 1L = first leaf; 2L = second leaf; gp = growing point; and t = teliospores.

the apical meristem. Instead, some were diverted from the growing point during growth and moved out with the emerging leaves. These mycelia did not sporulate but apparently caused the leaf-flecking associated with highly susceptible cultivars, especially under greenhouse conditions.

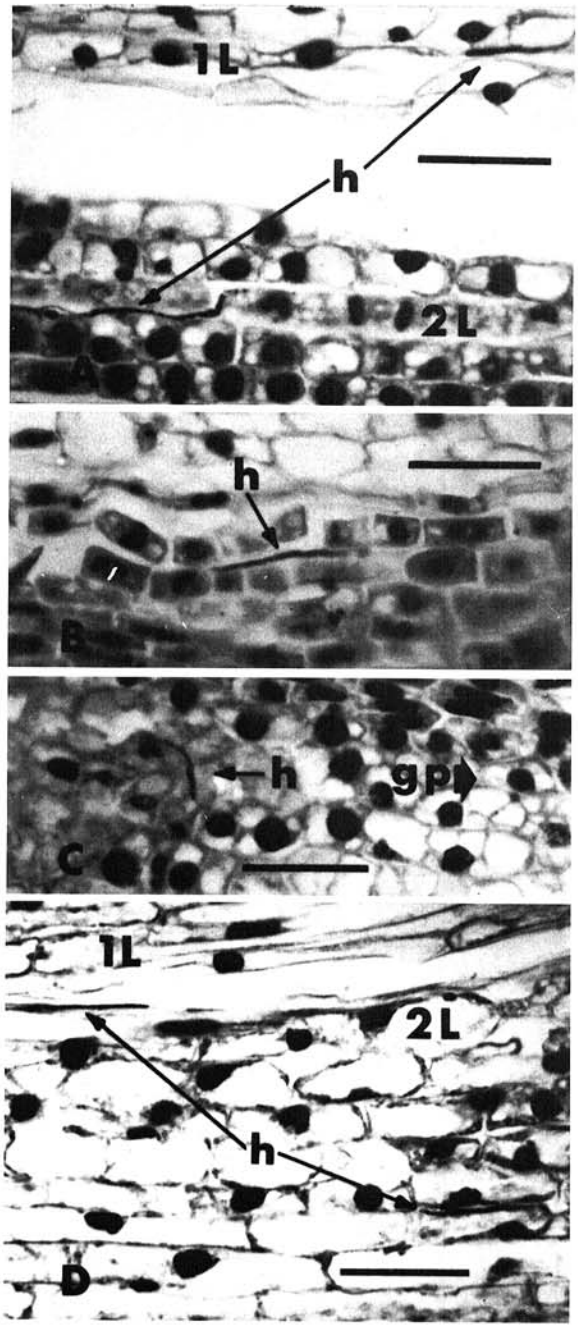

Fig. 2-(A to D). Hyphae of *Tilletia controversa* in the resistant wheat cultivars Requa, Nugaines, and P.I. 178383. **A)** Hyphae in the first and second leaf of Requa 30 days after inoculation. **B)** Hypha in the second leaf primordium near the growing point of Nugaines 30 days after inoculation. **C)** Hypha below the growing point of Nugaines 50 days after inoculation. Large arrow indicates direction of growing point. **D)** Hyphae in first and second leaf tissue of P.I. 178383, well away from the growing point. Bars represent ~50 μm. Legend: h = hyphae; 1L = first leaf; 2L = second leaf; and gp = growing point.

Based on our findings, we concur with Hansen (4) and Swinburne (14) that in susceptible cultivars hyphae of the cereal bunt fungi must reach the growing point and/or tiller initials before internodal elongation in order to sporulate. Why the fungus is unable to establish itself in the growing point of resistant cultivars is still unknown. In Requa and Nugaines, for example, mycelia became established in the coleoptile tissue soon after inoculation and subsequently (30 days) in the first and second leaf primordia. But further development of the fungus in these cultivars was somehow arrested at this stage. Few plants showed mycelia in nodal or growing point tissues. Thus, while considerable ramification of mycelia occurs in juvenile tissue following inoculation, the fungus cannot continue its development. In these cultivars, the host resistance response thus seems not to be initiated until well beyond the coleoptile stage in and/or around tissue of the growing point. Fungal development simply appeared to wane as hyphae approached these tissues. Presumably some hyphae are carried out with the emerging leaves. We noted that cessation of fungal development in Requa and Nugaines coincided with the time when these cultivars were moved from cold to warmer temperatures of the greenhouse. Perhaps resistance in Requa and Nugaines is influenced by temperature changes and is expressed when infected plants overwintering in the field resume growth during rising temperatures in spirng. Prolonged incubation of infected plants at cold temperatures, conceivably, might negate such resistance by allowing additional time for the fungus to reach the growing point and ultimately infect ovarian tissue where sporulation occurs. The influence of temperature on expression of host resistance and sporulation of *T. caries* (DC.) Tul. in Baart 38 has been reported (2, 3).

Unlike in the other cultivars, development of race D-2 in P.I. 178383 was arrested from the outset. Mycelia were seldom seen despite examination of hundreds of sections and, when they were, they were sparse and found only in leaf tissues away from the growing point. At no time were mycelia seen in other host parts. Resistance appeared to be intrinsic and not influenced by temperature. Trione (15) suggested that perhaps in tissues of resistant cultivars, the parasitic dikaryophase dissociates to non-pathogenic monokaryotic elements, although we saw no histological evidence in our study to support this hypothesis.

Resistance in different cultivars to the cereal bunts has been discussed by Pope and Dewey (12) who stated, "...expressing dwarf smut infection as a head percentage..ignores...phenotypic differences that express increasing degrees of successful opposition by wheat...to development of the smut pathogen." Admittedly, other factors in addition to percentage of infected heads should be used as criteria to assess resistance, although it seems clear that histological findings should finally correlate in all cases with results of field resistance studies, if environmental factors are not mitigating.

We conclude that two types of resistance can be described in the cultivars studied: one in Requa and Nugaines that is possibly influenced by temperature

changes, in which mycelia are established in host tissue soon after inoculation but which are arrested before reaching the growing point, and the other in cultivar P.I. 178383 that is not influenced by temperature changes, in which development of the fungus is essentially minimal in all tissues after infection.

In view of the different *Bt* gene combinations in the large number of commercial cultivars, the number of dwarf bunt races, and environmental influences, it is likely that resistance in other cultivars may be manifested in ways other than those described here. Nevertheless, we believe development of the fungus in other susceptible cultivars is apt to be similar to that described for Red Bobs. Although conjectural, this seems likely, since there is little variation in the anatomy of wheat seedlings and the time and place of infection is generally similar, regardless of cultivar.

With some effort the information obtained here could be utilized to identify or evaluate the incorporation of additional resistance genes to presently resistant cultivars. For example, Nugaines is an established commercial cultivar in the Pacific Northwest. Through a backcrossing program resistance of P.I. 178383 could be transferred to a Nugaines-type cultivar. However, the transfer of this resistance could not be verified by a final test of smutted vs. nonsmutted plants at the sporulation stage, as neither cultivar would show smut with the presently used race D-2. In contrast, histological examination should show the presence of the P.I. 178383 level of resistance, as identified by the more limited ramification of mycelia, in a Nugaines genotype background. Nugaines possesses genes *Bt* 1, 3, and 4. Plant line P.I. 178383 possesses genes *Bt* 8, 9, and 10 plus additional unassigned resistance. This technique would thus provide a mechanism to combine one or more of these latter genes with genes *Bt* 1, 3, and 4.

Further, it is conceivable that recombinations of resistance genes, such as those of Requa and Nugaines operating at a similar level, could be identified if there is an interaction in their effects. Such interactions have been previously demonstrated in *Puccinia recondita* Rob. ex Desm., the causal organism of wheat leaf rust (13).

LITERATURE CITED

1. AINSWORTH, G. C. (ed.). 1968. Plant pathologist's pocketbook. Commonw. Mycol. Inst., Kew, Surrey, England. 267 p.
2. GRIFFITH, R. B., F. P. ZSCHEILE, and J. W. OSWALD. 1953. Resistance of wheat to bunt associated with inability to produce chlamydospores. Phytopathology 43:473 (Abstr.).
3. GRIFFITH, R. B., F. P. ZSCHEILE, and J. W. OSWALD. 1955. The influence of certain environmental factors on expression of resistance to bunt in wheat. Phytopathology 45:428-434.
4. HANSEN, F. 1958. Anatomische Untersuchungen über Eindringen und Ausbreitung von Tilletia-Arten in Getreidepflanzen in Abhangigkeit vom Entwichlungszustand der Wirtspflanze. Phytopathol. Z. 34:169-208.
5. HOFFMANN, J. A., E. L. KENDRICK, and J. P. MEINERS. 1962. Pathogenic races of Tilletia controversa in the Pacific Northwest. Phytopathology 52:1153-1157.
6. HOFFMANN, J. A., E. L. KENDRICK, and R. J. METZGER. 1967. A revised classification of pathogenic races of Tilletia controversa. Phytopathology 57:279-281.
7. HOFFMANN, J. A., and R. J. METZGER. 1976. Current status of virulence genes and pathogenic races of the wheat bunt fungi in the northwestern USA. Phytopathology 66:657-660.
8. MEINERS, J. P. 1959. Methods of infecting wheat with the dwarf bunt fungus. Phytopathology 49:4-8.
9. METZGER, R. J., C. R. ROHDE, and J. A. HOFFMANN. 1977. Inheritance of resistance to common bunt in Triticum aestivum L., 'P.I. 178383'. Am. Soc. Agron. (69th Ann. Meeting). Agron. Abstr. p. 64.
10. METZGER, R. J., C. R. ROHDE, and E. J. TRIONE. 1962. Inheritance of resistance to common bunt, Tilletia caries, in a cross of P.I. 178383 × Elgin wheat. Phytopathology 52:743 (Abstr.).
11. METZGER, R. J., and B. A. SILBAUGH. 1971. A new factor for resistance to common bunt in hexaploid wheats. Crop Sci. 11:66-69.
12. POPE, W. K., and W. G. DEWEY. 1975. Quantitative resistance to dwarf smut (Tilletia controversa Kühn) of wheat as influenced by gene interactions and non-specific genes. A paper presented at the Second International Winter Wheat Conference, June 9-11, Zagreb, Yugoslavia.
13. SCHAFER, J. F., R. M. CALDWELL, F. L. PATTERSON, and L. E. COMPTON. 1963. Wheat leaf rust resistance combinations. Phytopathology 53:569-573.
14. SWINBURNE, T. R. 1963. Infection of wheat by Tilletia caries (DC.) Tul., the causal organism of bunt. Trans. Br. Mycol. Soc. 46:145-156.
15. TRIONE, E. J. 1974. Morphology and cytology of Tilletia caries and T. controversa in axenic culture. Am. J. Bot. 61:914-919.
16. WAUD, J. L., and R. J. METZGER. 1970. Inheritance of a new factor (Bt 8) for resistance to common bunt in wheat. Crop Sci. 10:703-704.
17. WOOLMAN, H. M. 1930. Infection phenomena and host reactions caused by Tilletia tritici in susceptible and non-susceptible varieties of wheat. Phytopathology 20:637-652.