THE EFFECT OF STEM RUST INFECTION ON THE SOLUBLE PROTEINS OF WHEAT LEAVES

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Summary

The soluble proteins of wheat leaf and of stem rust uredospores were resolved into about 28 and 34 components, respectively, by disk electrophoresis. The fungus-containing and the fungus-free areas of infected wheat leaves were examined. The electrophoretic pattern of an extract of the lesions was markedly different from the pattern of an extract of uninfected leaves. Comparison with the pattern of an extract of uredospores suggested that some of these differences were due to contamination with fungal proteins. For susceptible varieties, extracts of the extra-lesion areas of infected leaves showed different electrophoretic patterns from extracts of uninfected leaves. This was not so for resistant wheat varieties.

I. Introduction

Early suggestions that specific plant proteins are responsible for resistance and susceptibility of cereal leaf to fungal infection followed from observations on the effect of nitrogenous fertilizers on rust susceptibility (Gäumann 1950). More recently, electrophoretic and chromatographic studies have demonstrated changes in the protein composition of wheat and bean leaves after fungal and bacterial infections (Barrett and McLaughlin 1954; Staples and Stahmann 1964; Rudolph and Stahmann 1964). As these studies were performed on the whole diseased leaf, it is difficult to distinguish between changes induced in the leaf proteins and apparent changes due to contamination with proteins of the parasite. In the present study, samples of non-infected leaves served as controls. For infected leaves, two samples were examined: the lesion itself (where the leaf had been penetrated by the fungal hyphae), and the extra-lesion area (where the fungus was absent). The protein composition of extracts of these samples has been compared by disk electrophoresis. This technique resolves wheat leaf proteins into a large number of clearly demarked components (Wrigley, Webster, and Turner 1966).

II. Methods

(*a*) *Leaves*

In the studies on rust infection, three isogenic lines of the cultivar Marquis of wheat (*Triticum aestivum*) were used in addition to cv. Marquis itself: Kenya 58–Marquis^6, carrying the gene for stem rust resistance *Sr* 6; Kenya 117A–Marquis^6, carrying *Sr* 9*b*, and Egypt Na95–Marquis^4 carrying *Sr* 10 (Green *et al.* 1960). Plants

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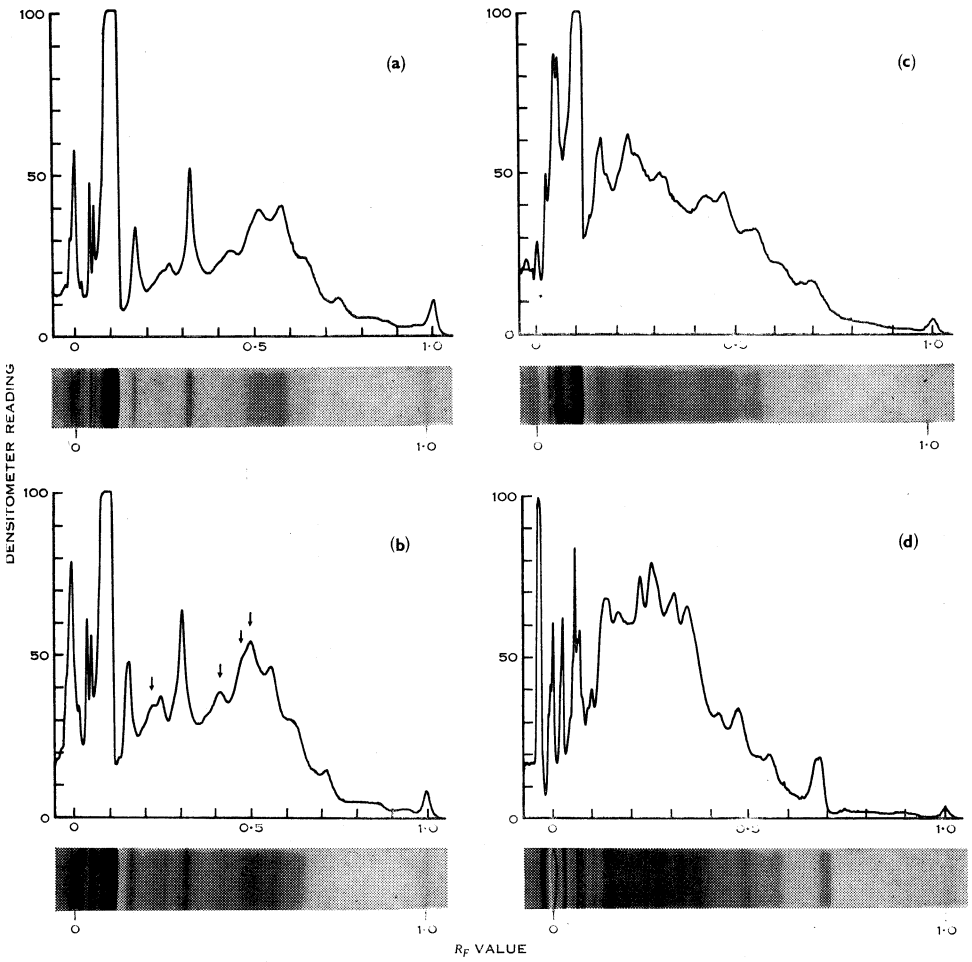
were grown in pots of sterilized soil to the two to three leaf stage at a day temperature of 15–22°C. Primary leaves (1 g) were picked and immediately frozen. After 30 min, the leaves were ground at 4°C with an equal weight of sand (acid-washed) and an equal volume of buffer [Tris 0·041M, glycine 0·32M, glucose 12·5% (w/v), pH 8·5].



Figs. 1(*a*)–1(*d*).—Photographs and densitometer scans of electrophoretic separations of extracts of: (*a*) healthy wheat leaf, cv. Marquis; (*b*) extra-lesion area of leaf infected with race 126-Anz-6; (*c*) lesion of infected leaf; and (*d*) uredospores, race 126-Anz-6. The beginning of the separation gel and the fastest band are designated 0 and 1·0, respectively. The Photovolt densitometer (model 530) was set on response 5. Densitometer readings 0 to 85 correspond linearly to absorbances of 0 and 0·80, thence non-linearly to reading 100 (infinite absorbance).

The extract, freed of sand and fibre by centrifugal filtration, was centrifuged at 37,000 *g* for 30 min. The clear amber supernatant was used for electrophoresis within 3 hr of preparation. Protein concentrations were estimated by the method of Lowry *et al.* (1951) using bovine serum albumin (Commonwealth Serum Laboratories, Melbourne) as standard.

(*b*) *Uredospores*

Electrophoretic analyses were also performed on extracts of germinated and ungerminated stem rust uredospores (*Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn., race 126-Anz-6). Germination was carried out on the surface of 0·005% sodium lauryl sulphate at 22°C for 10 hr in the light, followed by 14 hr of darkness. About 60% germination was obtained. Uredospores (germinated or ungerminated) were ground with four parts of sand and four parts of extraction buffer and rinsed from the mortar with a further four parts of buffer. The preparation was centrifuged at 105,000 *g* for 30 min and this produced a clear, almost colourless supernatant.

(*c*) *Electrophoresis*

The disk-electrophoresis procedure of Davis (1964) was used with only minor modifications. The extract was introduced into the gel column by layering it under the electrode buffer (180 μg protein for leaf extracts and 130 μg for uredospore extracts). The conditions of electrophoresis and staining with amido black 10B have previously been described (Wrigley, Webster, and Turner 1966).

III. Results

(*a*) *Uninfected Leaf*

The soluble leaf proteins of the wheat cultivar Marquis were resolved into about 28 visually identifiable bands [Fig. 1(*a*)]. Most of these bands can be distinguished in the densitometer tracing. A number are not reproduced in the photographic process, a discrepancy which has been reported by other workers (e.g. Steward and Barber 1964). An increase in the acrylamide concentration of the separation gel from 7·5 to 10% reduced the mobility of all components and provided better resolution in the region of higher mobility. No cationic components were observed in 7·5% acrylamide when the polarities of the electrodes were reversed using the "reversed unidirectional" procedure of Clarke (1964).

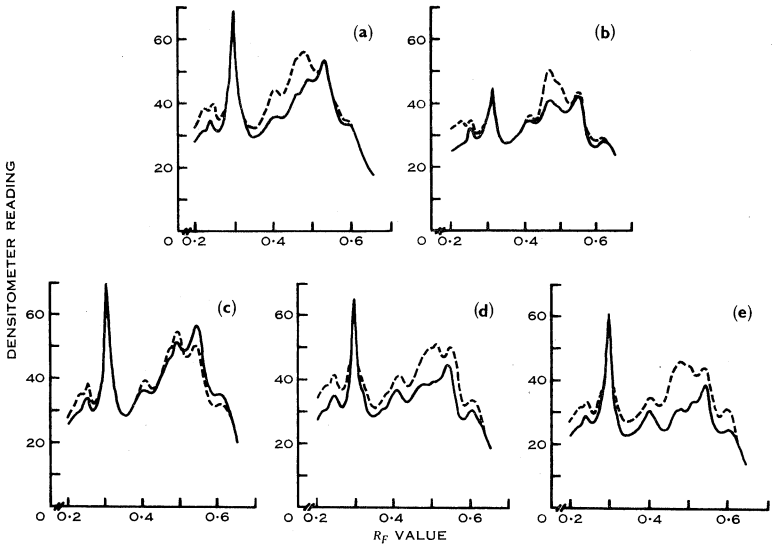
The reproducibility of the whole procedure was satisfactory. Duplicate separations were routinely performed for each extract and these agreed well. Extracts prepared on the same day from the same leaf sample produced similar patterns. Variations were sometimes evident in the R_F region between 0·4 and 0·5 when comparing leaf extracts of the same cultivar prepared on different occasions [compare Fig. 1(*a*) with Figs. 2(*a*) and 2(*b*)]. In order to remove any possible difficulty in reproducibility, a rusted leaf extract was always examined together with an identically treated extract of uninfected leaf grown under similar conditions. When the material remaining after the routine single extraction was further extracted for a second and third time, similar electrophoretic patterns were observed in all three supernatants. These three extracts contained about 60% of the total nitrogen of the leaf sample.

No consistent differences were observed between the electrophoretic patterns of leaf extracts of different commercial cultivars of *T. aestivum*, such as Marquis, Chinese Spring, Gabo, Koda, Federation, and three lines of Marquis carrying the respective genes *Sr* 6, *Sr* 9*b*, and *Sr* 10. On the other hand, minor differences were noted between the patterns of cv. Einkorn, W10 (*T. monococcum*), cv. Vernal Emmer, W11 (*T.*

dicoccum), and *T. aestivum*. Considerable pattern differences were apparent between extracts of wheat, Goldfoil barley, B167 (*Hordeum vulgare*), Ruakura oats, O526 (*Avena sativa*), and black winter rye (*Secale cereale*).

(*b*) *Infected Susceptible Leaf*

About 12 days after inoculation with stem rust (race 126-Anz-6), heavily infected first leaves of the susceptible cultivar Marquis were dissected to separate the lesion, including the chlorotic area, from the extra-lesion area. Pattern variations were visible between extracts of uninfected leaf and of the extra-lesion area of infected leaf. The main areas of the differences [arrowed in Fig. 1(*b*)] occur at R_F values of 0·22, 0·42, 0·46, and 0·49. Although these changes were relatively small,



Figs. 2(*a*)–2(*e*).—Relevant portions of densitometer scans of electropherograms obtained from extracts of uninfected leaves (solid lines) and extra-lesion area of infected leaves (broken lines) for cv. Marquis [(*a*) and (*b*)], the *Sr* 10 line of Marquis [(*c*) and (*d*)], and the *Sr* 9*b* line of Marquis [(*e*)].

they were consistently reproducible. Figures 2(*a*) and 2(*b*) show that despite variations, mentioned above, in the patterns of extracts of healthy leaves, pattern differences between extracts of healthy leaves and of the extra-lesion area of rusted leaves were still consistent with the differences arrowed in Figure 1(*b*). These differences were similar for a susceptible line of Marquis carrying gene *Sr* 10 [Figs. 2(*c*) and 2(*d*)]. Samples of the extra-lesion area, taken close to (within 1 cm) or at some distance from (4–5 cm) the infection court, gave similar patterns. More pronounced differences were observed between extracts of uninfected leaf and extracts of the actual lesion area [Fig. 1(*c*)] of infected leaf. No differences were demonstrated between extracts of infected and uninfected whole leaf at any stage of infection or colonization prior to the onset of sporulation. Patterns similar to those shown in Figures 1(*b*) and 1(*c*) were observed for extracts of the extra-lesion and lesion areas of the susceptible line of Marquis carrying gene *Sr* 10.

(c) *Inoculated Resistant Leaf*

The possession of gene *Sr* 6 by another line of Marquis confers on it resistance to race 126-Anz-6 below about 22°C. No differences in electrophoretic patterns were observed between extracts of inoculated and uninoculated leaves of this line at any stage up to 14 days after inoculation. A slight deficiency in the major band with R_F of 0·11 was observed in extracts of the hypersensitive fleck area, dissected from leaves about 12 days after inoculation. However, extracts of the extra-lesion area of these leaves were electrophoretically identical with extracts of uninoculated leaves. The *Sr* 9*b* line of Marquis is moderately resistant to race 126-Anz-6 and shows type 2 infection. Extracts of the extra-lesion area of leaves of this line usually showed a pattern [Fig. 2(*e*)] similar to that of extracts of the extra-lesion area of fully susceptible leaves.

(d) *Uredospores*

As it seemed likely that some of the pattern changes at the lesion arose from contamination with fungal proteins, an extract of uredospores was submitted to electrophoresis. The pattern [Fig. 1(*d*)] showed about 34 bands and was completely different from the pattern for wheat leaf extract. The pattern for germinated spores differed only slightly from that of ungerminated spores.

IV. Discussion

Resolution of the leaf proteins was slightly better than that already published (Wrigley, Webster, and Turner 1966), largely due to technical improvements suggested by Davis (1964). The patterns differ in some respects from those of Strobel and Sharp (1965) for the proteins of wheat leaf and show considerably more bands than those for extracts of bean leaf (Staples and Stahmann 1964). Resolution of the proteins of the wheat rust uredospores was much better than with proteins of germinated bean rust uredospores (Staples and Stahmann 1964). Though considerable changes in enzyme activity following germination of fungal spores have been observed by Gottlieb (1964) and by Staples and Stahmann (1964), the electrophoretic patterns showed only minor differences.

The procedure failed to show any differences in the protein composition of uninfected leaves of a number of wheat varieties, irrespective of rust susceptibility. This finding contrasts with the marked varietal differences in the composition of the endosperm proteins of wheat grain shown by polyacrylamide and starch gel electrophoresis (Graham 1963; Lee and Wrigley 1963). However, varietal differences in the endosperm proteins were more marked in the water-insoluble gluten proteins than in the albumins and globulins. The genetic difference between the triploid endosperm and the diploid leaf tissue may also be significant in this respect. In contrast to the present results, Strobel and Sharp (1964), using varieties not examined in the present study, briefly reported different disk-electrophoretic patterns for the leaf proteins of several wheat varieties.

Certain bands of the spore extract pattern appear to coincide with bands in the pattern of the pustular extract (e.g. bands with R_F values of 0·02, 0·05, 0·06, 0·23, 0·48, and a double band at 0·68). Although the situation is confused by the large number of bands, this observation suggests that the fungal proteins may be responsible

for some of the bands appearing in the pattern of the pustular extract. It is realized that the pattern shown for the spore extract is only a rough approximation to the pattern of the total fungal proteins of infected leaf, since a great part of the fungus is in mycelial form. The absolute dependence of mycelial growth on the presence of leaf cells precludes isolated study of the mycelial proteins. It can only be stated that the transition during germination is associated with only small changes in pattern.

Some of the pattern changes in the pustular extract represent a decrease in peak height compared with the pattern of a mixture of spore and leaf extracts, e.g. bands with R_F values of 0·11 and 0·31. This suggests that there may be changes in the leaf protein composition at the pustule as distinct from the additive effects of fungal proteins. It is possible that other changes in the leaf proteins are obscured by the proteins of the parasite. A band (R_F of 0·11) which stained strongly for an extract of healthy leaves showed a marked decrease in an extract of the pustule. A smaller decrease in the amount of this band was noted in an extract of the hypersensitive fleck of a resistant variety. It has been suggested (Wrigley, Webster, and Turner 1966) that this band is fraction I (Wildman and Bonner 1947). In view of its probable role in photosynthesis and pigmentation (Trown 1965), a fall in the concentration of fraction I agrees well with the observations of chlorosis at the infection centre and reduced photosynthetic activity of the infected leaf (Livne 1964).

More pronounced changes in leaf protein composition after rust infection were observed in this study than in previous work, probably because of the superior resolution of the fractionation procedure. Barrett and McLaughlin (1954) reported mobility changes for several electrophoretic components in extracts of whole wheat leaves infected with rust compared with uninfected leaves. More specifically, changes in the mobility of fraction I have been reported in extracts of whole rice leaves as a result of blast infection (Anon. 1964). In the present work, no mobility changes associated with rust infection could be observed. It is possible, however, that the large number of bands may obscure mobility changes in minor bands. Furthermore, the use of acrylamide at concentrations lower than 7·5% may provide a better medium for observing mobility changes in fraction I in view of its large molecular weight (Trown 1965).

As the fungal hyphae do not spread beyond the chlorotic area surrounding the pustule, variations (although minor) in the pattern of the extra-lesion area compared with the pattern of uninfected leaf can only be interpreted as changes in the leaf proteins themselves. As these changes are not observed in an inoculated leaf of a resistant variety, the change appears to be dependent on the presence of the actively colonizing fungus. In view of the large number of bands, it is difficult to determine whether these differences are due to changes in the normal leaf proteins or to newly synthesized proteins.

The production of this systemic effect remote to the pustule could be due to the response of the whole leaf to the chemical changes occurring at the site of infection. For example, Shaw and Samborski (1956) using radioactive techniques have demonstrated the accumulation of a range of metabolites at the rust lesions of infected wheat leaves. The differences in protein composition may reflect a change in the metabolism of the leaf cells directed towards restoring the metabolic balance

disturbed by the drain of metabolites to the pustule. On the other hand, the observed effect may be interpreted in the light of reports of toxin production from uredospores (Bushnell and Allen 1962) and from rust colonies on infected leaves (Silverman 1960). In the present case, metabolically active substances, originating from either the fungal hyphae or the leaf cells in the infection court, may produce the observed effect by direct contamination or by stimulating a response in the uninfected cells.

V. Acknowledgments

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