# Studies on Wheat Stem Rust Resistance Controlled at the Sr6 Locus.
## I. The Role of Phenolic Compounds

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### ABSTRACT

Leaves of healthy or inoculated paired near-isogenic lines of wheat carrying either the *Sr6* allele for resistance or the *sr6* allele imparting susceptibility to race 56 of *Puccinia graminis tritici* were analyzed for total, combined, and bound phenols. A number of extraction procedures and several chemical methods were employed. Contrary to previous reports, no significant differences in total phenolic compounds were found among healthy or inoculated, resistant and susceptible, plants at any stage of disease development. Analysis of the concn of individual aromatic compounds by paper, thin-layer, or gas chromatography also failed to reveal any correlations with resistance or susceptibility. It is concluded that, if aromatic compounds are involved in the expression of resistance controlled at this gene locus, such involvement cannot be demonstrated by techniques employed previously in studies of other host-parasite combinations. Phytopathology 60: 1322-1328.

*Additional key words: Triticum,* obligate parasitism.

Antonelli & Daly (1), amplifying earlier work on indoleacetic acid (IAA) metabolism in rust diseases (5, 8, 26), provided evidence for a metabolic lesion which appeared to be correlated with development of resistance in near-isogenic lines of wheat (21) carrying the *Sr6* allele for resistance to race 56 of *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn. Lines possessing this allele show a resistant infection type (28) of 0; when grown at 19-21 C but produce a susceptible reaction (infection type 4) when grown at 25-26 C. Paired near-isogenic lines carrying the recessive allele show susceptibility at either temp. On the basis of studies in which reciprocal transfers between these temp were made at various times after inoculation, it was concluded that resistance becomes operative approximately 70 hr after infection occurs (1).

At approximately this time, tissue sections from resistant lines grown at 20 C showed a marked increase (4- to 8-fold) in ability to decarboxylate exogenous IAA, presumably through the action of IAA "oxidase" (13, 14, 32). The effect persisted for at least 13 days after inoculation. The same line grown at 26 C, or susceptible lines grown at either 20 or 26 C, showed a moderate increase in IAA degradation during the first 3-4 days, but by the time sporulation had occurred (6-7 days), rates of IAA degradation were lower than for noninoculated plants. Thus, there was not only a correlation with the final infection type in all cases, but the effect was observed at the time when resistance mechanisms apparently are being developed in resistant lines prior to visible expression of disease reaction.

Although hormones have been suggested to play a role in disease development (6) and also in resistance (26), it was pointed out that the correlation of resistance with IAA degradation might only be fortuitous (1), reflecting other fundamental changes in metabolism associated more directly with resistance. One

suggested possibility was a change in phenolic compounds, some of which are known to affect the activity of IAA oxidase in vitro (13). Hare (14) compiled many instances in which levels of aromatic compounds in vivo have been suggested to alter IAA, IAA oxidase, and growth.

The possible involvement of aromatic compounds in disease resistance is covered in a number of reviews (4, 19, 20, 24). In the case of stem rust of wheat disease, the earlier work of Newton & Anderson (22) was re-examined by Király & Farkas (18), who reported that infected resistant varieties showed increased tissue concn of phenols at an earlier stage of disease development than did varieties classed as susceptible. Rohringer et al. (23) and Fuchs et al. (12) reported more active synthesis of certain aromatic compounds in infected lines possessing the *Sr6* allele when compared to the paired susceptible near-isogenic line. Elnaghy & Linko (9) have claimed that resistant wheats possess higher concentrations of 4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one which is hydrolyzed to toxic products during infection. Elnaghy & Shaw (10) found some correlation in concn of this compound and varieties showing resistance to a number of rust races.

MATERIALS AND METHODS.—*Plant materials.*—The near-isogenic lines were furnished by W. Q. Loegering. The historical data on the lines is given (21, Table 1) for the wheat lines listed as I $Sr_6$-Ra and I $Sr_6$-Sa, except that the lines we used had been selfed only for six generations. In previous studies the lines used had been selfed for only three generations.

The plants were grown under essentially the same conditions as in earlier studies (1). The temp was 20 C with a photoperiod of 14 hr/day. Inoculation with race 56 of *Puccinia graminis tritici* was accomplished by dusting a mixture of talc and uredospores at a time after planting when the second leaf was just visible.

Near the end of a photoperiod, the plants were dusted and placed in a humidity chamber for approximately 12 hr. In the text, "day 1" refers to the day when the inoculated plants were removed from the chamber.

In contrast to the isogenic lines previously used, there was some additional growth of the primary leaf after inoculation. Consequently, the infection was localized in the top 4 to 5 inches. In sampling tissue for analysis, the top 5 inches were used routinely and the basal 0.5 to 1 inch of tissue was discarded.

*Estimation of disease reaction.*—Disease reaction was based both on the infection types of Stakman et al. (28) and on pustule numbers. With the normally heavy infections we obtained, susceptible reactions were more closely akin to an infection type 3 rather than the infection type 4 observed with isolated pustules on susceptible leaves. At 19-20 C, resistant leaves developed typical 0; reactions, but occasionally a few pustules in the $1^-$ to 1 range of infection were observed.

An accurate determination of infection intensity in terms of pustules per leaf was necessary, and estimation of the numbers of infection sites per leaf presented several problems. Flecks and resistant pustules were most distinctive when observed from the underside of the wheat leaf, but sporulation was most readily detected on the upper surface. Since both the total number of sites and an estimation of sporulation were necessary for interpreting data on the reversion of resistant reactions to susceptible reactions, the upper surface was used for measuring both parameters. It should be noted that such a procedure probably underestimated slightly the total number of infected sites, especially in resistant reactions. Usually two or three observers counted pustules on a minimum of 10 leaves each for a total of 20 or 30 leaves.

*Extraction of tissues.*—In preliminary studies, tissue was blended in methanol with a Waring Blendor and the methanol extract reduced to dryness under vacuum at 35 C. The dry residues were then re-extracted with smaller volumes of methanol for subsequent analysis. Although similar concentration procedures are used routinely, the data we obtained were frequently erratic. Several modifications of the extraction and reduction procedure were examined in an attempt to decrease data variability. Various tissue to methanol ratios, partial reduction, and no reduction of extracts were tested. Optimum extraction procedures for detecting phenols consisted of blending for 2 min the top 5 inches of primary leaves in sufficient methanol so as to give a ratio of approximately 100 mg fresh wt of tissue/ml of methanol. After centrifugation or filtration, the extracts were assayed without reduction in volume. Variations from this procedure are given in the text. All samples in a given experiment were harvested at the same time into the photoperiod, usually 2-3 hr after the start of the photoperiod.

*Assay for total phenols.*—Estimation of phenols was accomplished with three widely used methods. A modification (27) of the Folin-Ciocalteu reaction was used with caffeic acid as a standard. The procedure used by Johnson & Schaal (16) based on the Folin-Denis

TABLE 1. Absorbance values for 100-μg (except as indicated) samples of representative phenolics in Arnow's reaction

| Compound | A at 520 nanometers |
| --- | --- |
| Caffeic acid | 0.350 |
| Catechol (40 μg) | 0.710 |
| Chlorogenic acid | 0.340 |
| Quercitin | 0.250 |
| Sinapinic acid | 0.050 |
| p-Coumaric acid | |
| Ferulic acid | |
| Scopoletin (40 μg) | |
| Pyrogallol | |
| Shikimic acid | |
| Tyrosine | |

reagent (11) was scaled down so as to give a final volume of 10 ml. Chlorogenic acid was used as the reference phenolic compound. Arnow's procedure for the estimation of dihydric aromatic compounds was carried out as described (2), except that 2 ml of NaOH and 2 ml of $H_2O$ were used. Even with optimum extraction procedures, the Arnow method gave variable data. In an attempt to determine the cause of the variability, spectra were taken of a number of common plant phenolics reacted with the Arnow reagent. Although the method has been used in the determination of plant phenolics, its specificity for dihydroxy phenolics had not been tested to any extent. Table 1 shows that absorption maxima of dihydroxy phenolics appear at approximately 520 nanometers, and is relatively specific for dihydroxy phenolics. This suggested that the variability in data was possibly due to interfering compounds.

*Gas chromatography.*—Gas chromatographic examination of extracts and hydrolysates was carried out as follows. Extract volumes equivalent to approximately 0.2 g fresh wt were placed in vials and reduced to dryness under a stream of dry N. The vials were then capped with serum stoppers and 0.1 ml of silylating reagent injected. Two different reagents were used in separate determinations, N,O-bis(tri-methylsilyl)acetamide and N-trimethylsilyl imidazole. Residues were solubilized within 5 min by each reagent. Five-μliter samples of the reacted material were then injected into the gas chromatograph.

The instrument was a Hewlett-Packard F and M 5750 Research Gas Chromatograph utilizing dual columns and hydrogen flame ionization detectors. Columns were 2 ft × ⅛ inch outside diam stainless steel, containing Anakrom ABS, 90-100 mesh, coated with 3% SE 52. Packard columns were cured a min of 18 hr at 225 C with continuous N flow through the columns.

The column temp was programmed to provide a 6-min postinjection interval at 100 C, then a 4 C/min rise to 250 C followed by 21 min at the upper limit. Injection port temp was 250 C, detector temp 300 C. Nitrogen was used as the carrier gas, and gas flow was adjusted for optimum dual column operation. This was usually around 20 ml/min for the analytical column. The auxiliary gas was air, with a flow rate of 300 ml/min.

RESULTS.—*Free phenolic compounds.*—In four different inoculation series of plant tissue, we were not

TABLE 2. Caffeic acid equivalents (μmoles/g fresh wt) of healthy or rust-infected wheat leaves determined by the Lowry modification of the Folin-Ciocalteu Reaction

| Tissue[a] | Days after inoculation | | | | | | |
|---|---|---|---|---|---|---|---|
| | 3 | 4 | 5 | 6 | 7 | 10 | 12 |
| RC | 24.5 | 23.0 | 19.0 | 17.8 | 18.5 | 23.4 | 20.0 |
| RI[c] | 26.0 | 20.3 | 19.6(F)[b] | 17.5 | 19.6 | 19.7 | 21.0 |
| SC | 19.4 | 20.6 | 21.6 | 19.5 | 18.7 | 17.7 | 22.6 |
| SI[c] | 22.6 | 20.1 | 21.1 F | 21.0(S)[b] | 23.0 | 24.3(80S) | 24.5 |

[a] RC = Resistant leaves, noninoculated. RI = Resistant leaves, inoculated. SC = Susceptible leaves, noninoculated. SI = Susceptible leaves, inoculated.
[b] (F) = Faint flecks; F = strong flecks; (S) = initial sporulation; 80S = 80% sporulation.
[c] Final infection; 104 type 3+ pustules/leaf on SI, 26 type 0; pustules on RI.

able to show clearly any significant differences in phenolic compounds in methanol extracts of infected and healthy leaves using either the Lowry modification of the Folin-Ciocalteu procedure or Arnow's procedure. Table 2 presents data chosen because they show the maximum differences observed. On days 7 and 10, the susceptible reaction types showed sporulation of the fungus and there was an increase of 5 to 7 μmoles of caffeic acid equivalents/g of fresh wt, corresponding to an increase of approximately 30% over the healthy control. This is much less than either the absolute or percentage increases reported by Király & Farkas (18) to occur in both susceptible and resistant reactions by the 6th day after inoculation. Although they did not report data at earlier periods of the infection process, in our experiments no differences from controls were observed during the crucial early stages of infection (days 2–5) when resistant or susceptible characteristics are being developed.

The intensity of infection, which in our experiments averaged over 125 pustules/leaf for the susceptible reaction, is one biological variable that could have accounted for the differences with previous reports. It is possible that the level of infection influences phenolic accumulation. Low levels of infection might allow the synthesis and accumulation of phenolics in noninvaded tissues adjacent to infected sites, while at high intensities of infection, accumulation is prevented because of utilization or turnover in invaded areas.

It should be noted that in all our experiments the number of observable infection sites which developed with susceptible reactions was always greater than the number of sites observed with resistant reactions (Table 2). Antonelli & Daly (1) observed the same phenomena, and speculated that it resulted from a statistical distribution in the development of fungus

colonies as a consequence of resistance mechanisms. It seems probable that in both resistant and susceptible types of infections equivalent inoculum results in the same number of initial infection sites, but that development of individual sites is variable in time as evidenced by the fact that the percentage of sporulating pustules even in susceptible reactions increases over several days. In the case of resistant reactions, the same distribution in development results in only a small percentage of the infection sites proceeding to a point where they become visible.

Table 3 shows that failure to detect an increase in phenolics cannot be attributed to any influence of infection intensity for either resistant or susceptible reactions. In some instances, susceptible lines of wheat supporting over 200 pustules/leaf likewise show no significant changes in phenolics at any stage of the infection process.

All of the data given above were obtained with the Lowry modification of the Folin-Ciocalteu reagent for phenolic compounds. Since individual phenolic compounds show considerable variation in reactivity and in extinction coefficients with this reagent, it was possible that changes in certain phenolics important to resistance were not being detected by the procedure. Accordingly, the Folin-Denis reaction was used with chlorogenic acid as the reference compound. Table 4 presents a comparison of the methods on the same tissue extracts. As with previous tests, no significant differences were apparent in free phenolic compounds.

Since previous workers have used solvents other than absolute methanol for extracting phenolic compounds, and since phenolic compounds peculiar to disease reaction may have unusual solubility properties, a number of extraction media were employed; 50 and 80% methanol or ethanol, acetone, ether and ethyl

TABLE 3. The influence of infection intensity on concn of phenolic compounds in rust-infected wheat leaves

| Tissue[a] | No. visible sites | μmoles caffeic acid equivalents/g fresh wt on day | | | | | |
|---|---|---|---|---|---|---|---|
| | | 3 | 4 | 5 | 6 | 8 | 10 |
| RC | | 10.1 | 10.9 | 11.0 | 10.9 | 11.7 | 12.6 |
| RIL[b] | 13 | 11.0 | 9.6 | 10.4 | 10.8 | 11.2 | 11.0 |
| RIH[b] | 108 | 10.4 | 10.0 | 10.3 | 11.2 | 11.4 | 11.7 |
| SC | | 10.3 | 11.1 | 10.6 | 11.9 | 10.7 | 10.3 |
| SIL | 67 | 11.3 | 10.4 | 10.3 | 10.4 | 11.1 | 11.7 |
| SIH | 183 | 10.6 | 9.8 | 10.1 | 10.9 | 11.3 | 11.6 |

[a] RC = Resistant leaves, noninoculated. RI = Resistant leaves inoculated. SC = Susceptible leaves, noninoculated. SI = Susceptible leaves, inoculated.
[b] L = light infection; H = heavy infection.

TABLE 4. Comparison of methods in determination of free and combined phenolics in healthy and rust-infected wheat leaves

| Tissue | μmoles chlorogenic acid equivalents[a] /g fresh wt on day | | | | | μmoles caffeic acid equivalents[b] /g fresh wt on day | | |
|---|---|---|---|---|---|---|---|---|
|  | 3 | 4 | 5 | 6 | 8 | 4 | 6 | 8 |
|  | *Free phenols* | | | | | *Free phenols* | | |
| RC[c] | 3.7 | 3.6 | 3.8 | 3.7 |  | 15.0 | 13.5 | 13.6 |
| RI | 3.8 | 3.4 | 3.6 | 3.7 |  | 15.2 | 14.2 | 15.1 |
| SC | 3.9 | 3.6 | 3.5 | 3.9 |  | 13.9 | 13.1 | 15.6 |
| SI | 4.1 | 3.4 | 3.5 | 3.8 |  | 14.9 | 15.7 | 16.1 |
|  | *After acid hydrolysis*[a] | | | | | *After alkaline hydrolysis*[b] | | |
| RC |  | 7.4 |  | 6.5 | 6.8 | 15.7 | 13.6 | 13.8 |
| RI |  | 7.8 |  | 6.3 | 6.4 | 15.4 | 14.7 | 14.8 |
| SC |  | 8.1 |  | 7.2 | 5.7 | 13.5 | 14.7 | 14.6 |
| SI |  | 7.5 |  | 6.8 | 6.3 | 14.7 | 14.4 | 15.4 |

[a] Determined by the Folin-Denis reaction.
[b] Determined by the Lowry modification of Folin-Ciocalteu reaction.
[c] RC = Resistant leaves, noninoculated. RI = Resistant leaves, inoculated. SC = Susceptible leaves, noninoculated. SI = Susceptible leaves, inoculated.

acetate extractions provided no evidence for an increase in free phenolic compounds.

*Combined and bound phenolic compounds.*—To check on the possibility that phenols of importance in disease might exist in a combined form as glycosides in the methanol extracts, they were subjected to acid and alkaline hydrolysis. An aliquot of the methanol extract was reduced to dryness. Acid hydrolysis was carried out in sealed evacuated tubes in 5 ml of 2 N HCl for one hr at 100 C. After cooling, the solutions were neutralized and the concn of phenolics was determined by the Folin-Denis reaction (Table 4). Acid hydrolysis resulted in some release of combined phenols, but the increase was equivalent, within error, in all tissues. Alkaline hydrolysis for the same samples was performed in 5 N NaOH at room temp for 2 hr. Longer time periods apparently cause degradation. The results also are given in Table 4 as caffeic acid equivalents from the Lowry procedure. In one experiment, bound phenolics were determined on the cellular residue from the methanol extract using alkaline hydrolysis as above. The data for both the methanol extract and residue from tissues through the 4th day are given in Table 5.

Examination of the tables shows that, although considerable quantities of phenolics exist either as combined forms especially sensitive to acid hydrolysis (Table 4) or as bound phenolics (Table 5), there are no significant changes in pool size that can be related to the infection process.

*Gas chromatography.*—It was possible that there were significant changes in the amounts of individual aromatic compounds which might not be reflected in the concn changes of total phenols, but comparisons of gas chromatographs of extracts prepared in several ways did not reveal any differences. Figure 1 presents some typical chromatographs. Ethyl acetate extracts of acid hydrolysates of equivalent (10 mg) amounts of tissue from the same experiment shown in Table 4 were used. Although analysis was performed on extracts from each day indicated in Table 4, only 2 days are represented in Fig. 1. These days were chosen since they represented stages of infection when re-

sistance mechanisms become operative (day 4), and when the fungus has reached maximum development in a susceptible reaction (day 8). The peak positions of some reference aromatic compounds are also shown in Fig. 1. Full scale deflection would result from 3 to 10 μg quantities for any of the reference compounds given.

Although there are changes in the levels of some components as the tissue matures, the chromatographs from the four types of tissue are almost superimposable. Paper and thin-layer chromatography also did not reveal any major differences in types or concn of phenolic compounds.

A final attempt was made to fractionate components which might be involved in disease reaction by extracting 2 g of R, S, RI, and SI tissue with absolute methanol at several stages during infection. Each methanol extract was divided in half and reduced to dryness. Half of the dried residues of each sample was treated with 2% sodium bicarbonate to extract acid phenolics, the pH was lowered to 2.0, and this solution extracted successively with ethyl ether and butanol. The other half of the residue was extracted

TABLE 5. Free and residue bound phenolics in healthy and rust-infected wheat leaves during the early stages of infection

| Tissue | μmoles of caffeic acid/g fresh wt on day | | | |
|---|---|---|---|---|
|  | 1 | 2 | 3 | 4 |
|  | *Methanol extract* | | | |
| RC[a] | 12.3 | 11.0 | 10.8 | 10.8 |
| RI[b] | 10.3 | 13.8 | 11.3 | 11.4 |
| SC | 11.6 | 11.3 | 10.9 | 11.1 |
| SI[c] | 10.9 | 10.8 | 11.2 | 11.8 |
|  | *Alkaline hydrolysis of residue* | | | |
| RC | 7.0 | 7.5 | 8.0 | 8.4 |
| RI | 9.3 | 6.6 | 8.3 | 7.9 |
| SC | 8.1 | 7.6 | 8.7 | 8.9 |
| SI | 7.5 | 7.4 | 8.4 | 8.0 |

[a] RC = Resistant leaves, noninoculated. RI = Resistant leaves, inoculated. SC = Susceptible leaves, noninoculated. SI = Susceptible leaves, inoculated.
[b] 80 type 0; infection per leaf.
[c] 146 type 3+ infections per leaf.
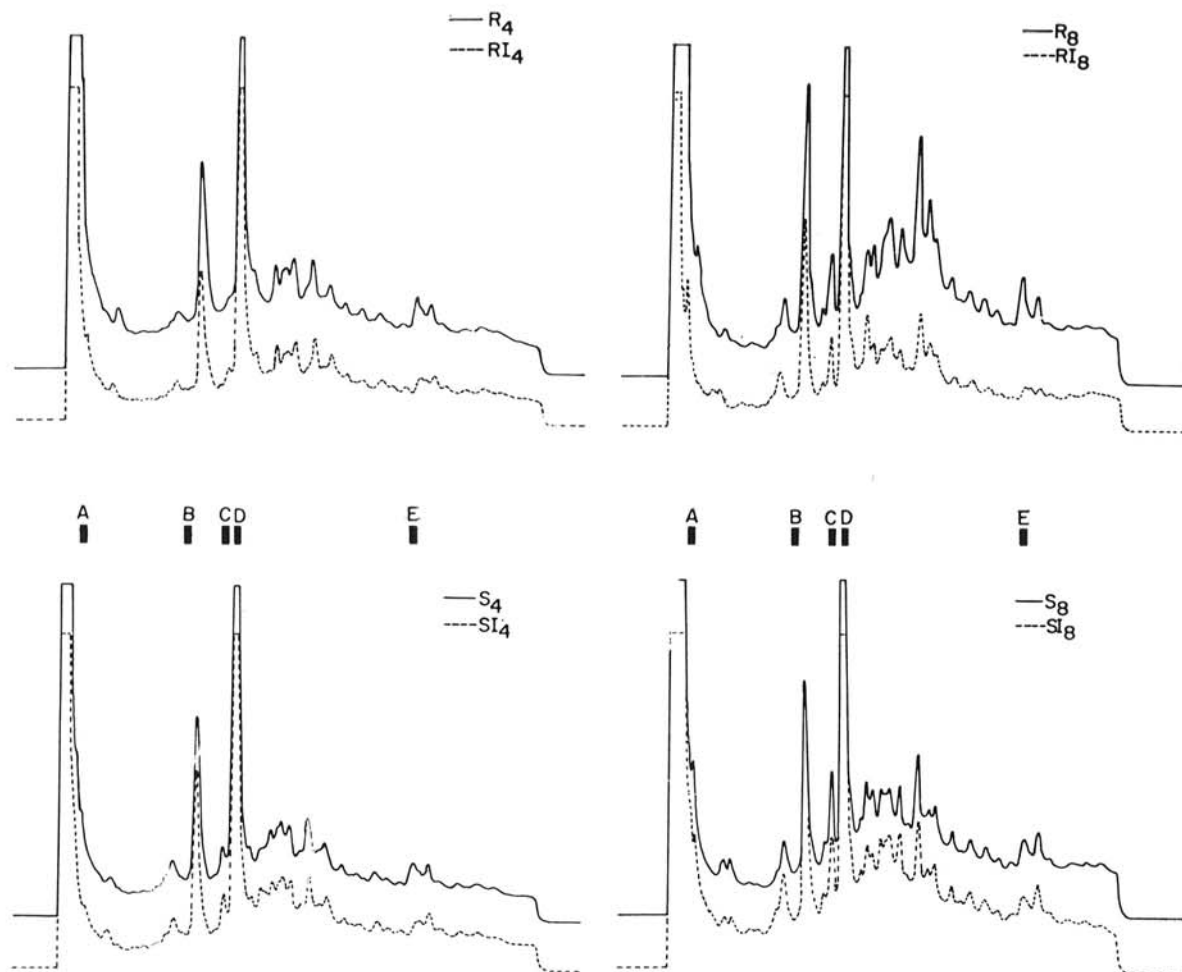
**Fig. 1.** Gas chromatographs of healthy resistant (R) or susceptible (S) leaves and the corresponding inoculated (RI and SI) leaves of wheat. The subscripts refer to the days after inoculation when extracts were made. The peak positions and relative widths of some reference compounds are indicated by the black bands: (A) *trans*-cinnamic acid; (B) *p*-coumaric acid; (C) ferulic acid; (D) caffeic acid; (E) chlorogenic acid.

with 0.1 M $KH_2PO_4$ to remove basic compounds, the pH raised to 8.0, and the solution extracted with ethyl ether and *n*-butyl alcohol.

All of the ether and butyl alcohol fractions were subjected to gas and thin-layer chromatography. The thin-layer solvents were chloroform-ethyl acetate-formic acid (50:40:10, v/v) in one dimension and isopropyl alcohol-ammonium hydroxide-water (10:1:1, v/v) in the other. Phenols were detected by fluorescence or ferric chloride-ferricyanide and diazotized sulfanilic acid reagents.

There was no evidence for a qualitative or quantitative change in the components detected by either gas or thin-layer chromatography.

DISCUSSION.—The results obtained in the present study are anomalous in several respects and are in contrast to most of the available data on host-parasite interactions. Despite the fact that higher rates of metabolism develop as a consequence of either resistant or susceptible reactions in rust diseases (1,

25), the greater metabolic activity was not reflected in any net synthesis of aromatic compounds. The pathways by which aromatic compounds are formed occur universally in higher plants and fungi, and are apparently activated readily in many disease situations, but their role in resistance is still controversial. Considering the metabolic rates, failure to detect net synthesis in the case of infections of the susceptible type especially is unusual, since rates of metabolism measured as respiration rates increase to values 200 to 300% greater than values for healthy controls. The higher metabolic rates are associated with synthesis and accumulation (17) of other metabolic intermediates. Failure to detect increases in phenols in resistant infections is not surprising, since the metabolic rates for this particular host-parasite combination do not reach as high a level as do susceptible reactions (1). In light of some current concepts of disease resistance (4, 19, 20) and the available data on other combinations of wheat varieties and races of stem rust

fungi (18), however, the data obtained on phenolic compounds during disease development in resistant tissue in the present study was unexpected.

It is possible that a fundamental difference in analytical techniques from the techniques of previous workers may explain our failure to detect any increases in phenolic compounds. The procedures followed are basically the same as those used for wheat stem rust disease (18) or cases of facultative parasitism (3) where large increases were observed. In the latter instance, the original extraction of tissue was more exhaustive than ours, but the solutions upon which analysis finally was performed were equivalent, in terms of the solubility of phenolics, to the procedures used for extraction in this study. Possible interference by ascorbic acid (16) in the assay does not seem likely, since Király & Farkas (18) reported large differences between susceptible and resistant infected wheat leaves, which therefore should have been reflected in some way in our data. In addition, the phenolic assay employed by them was not corrected for contributions of ascorbic acid.

Canadian workers (12, 23) have presented evidence for greater incorporation of intermediates of the shikimic acid pathway into certain phenolic fractions and individual compounds using the same host-parasite combinations as those we employed. It is possible to reconcile our results with their data by the assumption that turnover of phenolic compounds is greater during infection but results in the formation of insoluble end-products such as cell-wall lignin rather than low molecular wt, free aromatic compounds, or glycosides.

The Canadian group (23) has shown that $^{14}C$ from quinic or shikimic acid is incorporated into insoluble residue or insoluble esters to a greater extent in resistant inoculated plants than in susceptible inoculated leaves. The differences are not so apparent when more immediate precursors, such as phenylalanine, are used (12) and, when replicated, there is some variability in the magnitude of the incorporation of $^{14}C$ from experiment to experiment. The only compounds whose actual concn were measured in their studies were shikimic, quinic, phenylalanine, and tyrosine, but these probably represent only a small fraction of the possible aromatic components of wheat leaves. Furthermore, the increased activity of the shikimic acid pathway may have little influence on the total amounts of aromatic compounds detected, since other major pathways of biosynthesis are known. The proportion of the compounds detected by the methods we used for analysis and which are synthesized by the shikimic acid pathway may be minor.

A second alternative to reconcile our results with those of other workers is that increased concn of phenolic compounds formed are sequestered in uredospores. With our procedures, it is doubtful that phenolic components in uredospores would be extracted. Spore breakage does not occur with 1 or 2 min of blending uredospores with alcohol in a Waring Blendor, and a brief exposure of spores to ethanol does not solubilize appreciable quantities of material from intact spores (7). Visual examination of the residue from

extractions of susceptible sporulating tissue showed large numbers of unbroken spores. Van Sumere et al. (31) reported a number of aromatic compounds in uredospores of the wheat stem rust organism. It is perhaps of interest that significant increases (18) in phenolics were not observed until the time during infection when spores would be formed. It should be noted also that in this report the resistant reaction was a type 1 infection where sporulation does occur. Studies of $^{14}C$ incorporation (12, 23) were conducted at 5 to 6 days after inoculation when sporulation would have been initiated on the susceptible leaves.

Environmental variables might have caused some of the differences from previous results. An examination of Tables 2, 3, 4, and 5 shows that, although the phenolic concn were consistent in successive daily samples in any one experiment, they varied from 10 to 24 µmoles caffeic acid equivalents among experiments. The effect of photoperiod was checked by determining phenols at the start and end of the light periods, but no differences were noted either in healthy or infected plants. It is probable that soil N levels can regulate the levels of phenolics in leaf tissue, since the amino acids, tyrosine, and phenylalanine are intermediates in one of the biosynthetic pathways necessary for formation of some aromatic compounds.

In any event, our data does not indicate that resistance, in this instance, can be ascribed to any phenolic compound or group of phenols of the general type previously reported to be involved in resistance. It might be argued that the concn necessary to impart resistance by inhibition of fungal growth are beyond the limits of detection of the analytical methods employed.

The $ED_{50}$ values for individual compounds believed to be associated with resistance [e.g., Rishitin (30), Pisatin (4), and 3-methyl-6-methoxy-8-hydroxy-3,4-dihydroxy isocoumarin (15)] are greater than $10^{-4}$ M. Molal tissue concn of these materials are in the same order of magnitude, but cannot be achieved in aqueous media.

In our studies, the highest concn of total phenols observed was $2.6 \times 10^{-2}$ molal (expressed as caffeic acid equivalents) in uninfected tissue when determined by the Lowry modification of the Folin-Ciocalteu reaction. The highest concn of any individual phenol detected was caffeic acid at a maximum concentration of $3 \times 10^{-3}$ molal. Amounts of other known or unidentified aromatic compounds detected by gas chromatography apparently were somewhat less than this, assuming equal analytical sensitivity. Estimations of concn of individual phenolic compounds through color development of thin-layer or paper chromatography gave values of approximately 2-10 µg/g of tissue for the three or four components which could be detected. It appears that any phenolic compound responsible for resistance in the present instance would possess a higher order of toxicity than any known naturally occurring inhibitor associated with disease.

It should be remembered that the resistance mechanism in the host-parasite combination we have em-

ployed is somewhat unusual in its temp sensitivity. Some of the apparent conflicts in data on such phenomena as phenol accumulation or differences (1) in the timing and absolute rates of respiration between resistant and susceptible reactions (25) may mean only that the factors controlled by the *Sr6* allele are unique and therefore may not be operative in other instances of resistance of wheat to stem rust fungi. It is becoming increasingly clear, however, that there is no single case where resistance and biosynthesis of aromatic compounds can be unambiguously correlated, let alone proven. As has been pointed out elsewhere (29), resistance may be expressed through more subtle metabolic processes than by growth inhibition due to synthesized toxicants.

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