Sterol Changes in Maize Leaves Infected with Helminthosporium carbonum

P. H. Jennings, F. P. Zscheile, Jr., and B. L. Brannaman

Department of Agronomy and Range Science, University of California, Davis, California 95616

Abstract

Results of studies with polyene antibiotics have pointed to an interaction with membranes (1). These interactions were reported to occur specifically with sterols (2), and a sparing effect has been noticed upon addition of exogenous sterols (4). It has also been noted that certain phytotoxins produced by plant pathogens interact with plant cell membranes, affording their permeability and occasionally causing them to rupture (7, 12, 15). Still other work has implicated sterols in membrane structure (14), and the addition of exogenous sterols was found to exhibit a sparing effect against the action of certain chemicals which induce a loss of electrolytes from plant cells (5).

Since permeability may be involved in some mechanisms of disease resistance, it appeared logical to study a particular host-pathogen combination which involved a toxin (13) and to determine what differences, if any, existed in sterol content between healthy and inoculated resistant and susceptible host plants. For this purpose maize and Helminthosporium carbonum (Ullstrup) were chosen as the host-pathogen combination. Susceptible hybrids (Pr x K61) and resistant hybrids (Pr1 x K61) of maize were referred to hereafter as Pr and Pr1, respectively. The latter is resistant to both races 1 and 2 of this pathogen but Pr is susceptible to race 1 and resistant to race 2.

Culture and inoculation conditions are reported fully elsewhere (6). Forty-eight hours after incubation the plants were harvested. Only infected areas of leaves or comparable areas of control leaves were used. Twenty-five grams of fresh tissue were homogenized in 100 ml of acetone in a Virtilis blender, the extract was filtered, and the residue was extracted twice more with 100-ml volumes of acetone. Additional acetone extracts contained no sterols. The acetone extracts were combined and the acetone was evaporated in vacuo, as was done in all other evaporation operations. The remaining aqueous extract was then made 33% with respect to acetone and extracted four times with 100-ml portions of diethyl ether.

The ether phase was washed twice with 50-ml volumes of water, dried over Na2SO4 for 30 min, and evaporated to near dryness. Saponification was accomplished by addition of 50 ml of 95% ethanol and 5 ml of 60% KOH, followed by refluxing for 1 hr. After cooling, 200 ml of water were added and the mixture was then extracted thrice with 100-ml portions of CHCl3. The combined CHCl3 extract was washed twice with 50-ml volumes of water, dried over Na2SO4 for 30 min, evaporated to dryness, and dissolved in 2 ml of CHCl3. After adsorption on 0.5 g of alumina (activity III) the CHCl3 was evaporated. The alumina and adsorbed sample were applied to the top of a column (22 mm in diameter) of 12.5 g of alumina adjusted to Brockman activity III by addition of 6% (w/w) water. The column was eluted successively with 200 ml of hexane, 200 ml of 4% ether in hexane, 150 ml of 40% ether in hexane, and 150 ml of ether. The ether fractions showed that the sterols were contained in the 4 and 40% ether fractions. These were combined, evaporated to dryness, and dissolved in 2 ml of CHCl3 (fraction I). The aqueous phase from the ether extract was concentrated to 75 ml, and 25 ml of concentrated HCl were added. After refluxing for 3 hr, it was cooled and extracted thrice with 100-ml volumes of benzene. The benzene was evaporated to near dryness, and the residue was saponified as for fraction I. After saponification the solution was extracted thrice with 100-ml portions of benzene; the combined benzene extract was washed twice with 50-ml volumes of water, dried over Na2SO4, evaporated to dryness and the residue was taken up in 2 ml of CHCl3 (fraction II).

Gas-liquid chromatography of fractions I and II was accomplished on columns of 1% QF-1 and 3% SE-30. However, the former failed to separate campisterol and stigmasterol, and only the results from the 3% SE-30 column will be reported. The extracts from 0.5 ml of CHCl3 solution were allowed to react at room temperature with 0.1 ml of Na2SO4. The reaction was complete in 15 min under these conditions. One microliter was injected into a stainless steel column (153 x 0.32 cm) under the following conditions: injector 260 C, column 245 C, detector 255 C, Ne flow rate of 30 ml/min. The chromatograph was equipped with a flame ionization detector and the sterols were quantitated by computing peak height times width at half height. Cholesterol, stigmasterol, and β-sitosterol were run as standards for comparison of the relative retention times of the unknowns. Campesterol was identified from the position of its peak in relation to those of known sterols.

Two phenomena were noted in relation to sterol content when comparisons were made between healthy and inoculated leaves. First, there was a reduction in total sterol content in inoculated leaves which was more pronounced with race 1 than with race 2 (Table 1); indeed, race 2 produced equal percentage reductions in both hosts, and fraction I appears to have gained at the expense of fraction II in the case of Pr1. The susceptible reaction between Pr and race 1 also resulted in a lower total sterol content and a greater percentage reduction than any of the other host-pathogen interactions.

The second observation concerned percentages of the individual sterols (Table II). In composition, the two fractions are similar in all cases. The percentage of campesterol remained constant among all the samples. In the resistant reactions the

\[ 
\text{Fraction I:} \quad \text{Pr1} > \text{Pr1} > \text{Pr} \quad \text{Fraction II:} \quad \text{Pr} > \text{Pr1} > \text{Pr1} 
\]

\[ 
\text{Fraction I:} \quad \text{Pr1} > \text{Pr1} > \text{Pr} \quad \text{Fraction II:} \quad \text{Pr} > \text{Pr1} > \text{Pr1} 
\]

\[ 
\text{Fraction I:} \quad \text{Pr1} > \text{Pr1} > \text{Pr} \quad \text{Fraction II:} \quad \text{Pr} > \text{Pr1} > \text{Pr1} 
\]
Table I. Sterol Contents of Healthy and Inoculated Maize Susceptible and Resistant to Helminthosporium carbonum

<table>
<thead>
<tr>
<th></th>
<th>Fraction I</th>
<th></th>
<th>Fraction II</th>
<th></th>
<th>Total</th>
<th>mg/25 g fresh wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pr1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>1.343</td>
<td>0.662</td>
<td></td>
<td></td>
<td>2.005</td>
<td></td>
</tr>
<tr>
<td>Race 1 (susceptible)</td>
<td>0.635 (47)</td>
<td>0.132 (20)</td>
<td>0.767 (38)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Race 2 (resistant)</td>
<td>0.822 (61)</td>
<td>0.752 (114)</td>
<td>1.574 (78)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pr2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>0.818</td>
<td>0.820</td>
<td></td>
<td></td>
<td>1.638</td>
<td></td>
</tr>
<tr>
<td>Race 1 (resistant)</td>
<td>0.514 (63)</td>
<td>0.408 (50)</td>
<td>0.922 (56)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Race 2 (resistant)</td>
<td>1.154 (141)</td>
<td>0.108* (13)</td>
<td>1.262 (77)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Percentage of healthy in parentheses.
2 Average of three experiments.
3 Average of two experiments.
4 Results of a single experiment.

Table II. Sterol Composition of Healthy and Inoculated Maize Susceptible and Resistant to Helminthosporium carbonum

<table>
<thead>
<tr>
<th></th>
<th>Fraction I</th>
<th></th>
<th>Fraction II</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Campesterol</td>
<td>Sigma-sterol</td>
<td>beta-Sitosterol</td>
<td>Campesterol</td>
<td>Sigma-sterol</td>
</tr>
<tr>
<td>Pr1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>14 19a</td>
<td>67a</td>
<td></td>
<td>13 19a</td>
<td>68a</td>
</tr>
<tr>
<td>Race 1 (susceptible)</td>
<td>14 38 bc</td>
<td>48 bc</td>
<td>17 38 bc</td>
<td>45 bc</td>
<td></td>
</tr>
<tr>
<td>Race 2 (resistant)</td>
<td>16 24 ac</td>
<td>60 ac</td>
<td>15 22 ac</td>
<td>63 ac</td>
<td></td>
</tr>
<tr>
<td>Pr2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>16 20</td>
<td>64</td>
<td></td>
<td>14 15</td>
<td>71</td>
</tr>
<tr>
<td>Race 1 (resistant)</td>
<td>17 25</td>
<td>58</td>
<td>16 24</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Race 2 (resistant)</td>
<td>15 26</td>
<td>59</td>
<td>17* 22a</td>
<td>61a</td>
<td></td>
</tr>
</tbody>
</table>

1 Means for each sterol followed by different letters differ significantly at the 5% level.
2 Mean of three experiments.
3 Mean of two experiments.
4 Results of single experiment.

percentage of stigmasterol increased and that of beta-sitosterol decreased slightly when compared with the healthy controls. The susceptible reaction showed a large increase in percentage of stigmasterol and a large decrease of beta-sitosterol; in absolute amounts, beta-sitosterol decreased most. Although percentages are similar for all resistant reactions, absolute amounts and their changes with infection are widely variable.

In a study of the histology of infection by race 1 of H. carbonum it was reported that no difference existed in time of host reactions in resistant and susceptible leaves (7). It was observed that the pathogen was confined to epidermal cells in resistant leaves but ramified through parenchyma cells in susceptible leaves. Unfortunately, the length of time necessary from inoculation to the development of these differences in degree of penetration between susceptible and resistant leaves was not mentioned. In the present study visible symptomatic differences between the two host reactions were just developing at the time of sampling (48 hours after inoculation).

In consequence, the differences in total sterols between healthy, resistant, and susceptible reactions might be the result of degree of penetration, even at the early stage of disease development sampled. If the loss of sterols were a nonspecific response, then the percentage sterol composition should remain the same. However, this was not the case in the susceptible reaction where significant changes in sterol composition were detected.

Malca and Zscheile (10) reported no consistent differences in content of several organic acids between susceptible and resistant maize infected with H. carbonum. However, the level of malic acid was approximately 50% lower in seedlings exhibiting the susceptible reactions as compared with the noninoculated controls. Thus, it would appear that changes in organic acids and sterols, may, in some instances, be specific, at least during the early stages of infection.

Although Richardson et al. (11) reported no differences in sterol content or composition between normal and tobacco mosaic virus-infected tobacco plants, altogether different mechanisms may exist for expression of resistance to viral and fungal infections in plants. It has also been shown that certain sterol glucosides isolated from plants exhibit biological activity against some microorganisms (3, 9).

Now that changes in sterol content and composition have been observed in diseased tissues, it would be desirable to determine when these changes were first initiated and what role the fungal toxin may play in eliciting these changes. More information is also needed on the sterol composition of the pathogen itself.

An interaction, such as binding, between the toxin of the pathogen and sterol component of the host cell membranes is not sufficient to explain the changes in sterol content and composition observed in inoculated plants. A possible explanation might be that the toxin produced by H. carbonum interacts with sterols of the host, changing the host cell permeability and ultimately leading to loss of sterols during the catabolic action of disease development.

Acknowledgment—The cultures of Helminthosporium carbonum and the genetic lines of maize used in this study were kindly supplied by A. J. Ultsch, Department of Botany and Plant Pathology, Purdue University.

LITERATURE CITED