

## Some Speculations on the Role of Dopamine in the Resistance of Sugarbeets to *Cercospora* Leaf Spot<sup>1</sup>

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In recent years there has been increased interest in the role of phenolic compounds in the physiology of disease resistance. We believe that in the resistance of sugarbeets to *Cercospora* leaf spot a specific phenolic compound, dopamine (3-hydroxy-tyramine), has a prominent function. In this paper we present a few ideas as to the complex response that enables some varieties of sugarbeets to manifest resistance to attacks of *Cercospora beticola* (Sacc.).

A correlation has been found between the amount of dopamine in the leaves of sugarbeets and their resistance to attacks of *Cercospora beticola* (9,12). Although there is a significant relationship between dopamine content and resistance, a few susceptible varieties are relatively high in dopamine. Hence the mechanism of resistance is not a simple phenomenon as in the case of resistance to onion smudge. If the role of dopamine in disease resistance were elucidated it might lead to understanding and control of leaf spot disease and might have implications in other fungal diseases.

In previous papers we have discussed some of the aspects on infection of sugarbeets with *Cercospora beticola* (6,8,9,11,12) and recent studies indicate that the resistance may be due to specific biochemical properties of the host. One of the best documented characteristics of resistance to fungal diseases in general is a sharp, post-infectious raise in phenol concentrations (5,17,20). Dopamine and other phenolic compounds have been detected and identified in the leaves of sugarbeets (6,9). There are considerable quantities of dopamine in the leaves of healthy, resistant sugarbeets and the amount of dopamine is further increased upon mechanical injury to the leaves (8). When dopamine is oxidized it inhibits the growth of the fungus (9). The correlation between the dopamine content of sugarbeet leaves and their disease resistance has been reported (12).

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Several seemingly unrelated investigations in different plants may have implications in the resistance mechanism of sugarbeets. Auxin may be formed from tryptophan through the action of polyphenolic compounds (7) and this pathway might be considered as a latent mechanism for auxin production. Mace and Solit (13) while investigating *Fusarium* wilt of bananas, reported interesting interactions between indolacetic acid (IAA) and dopamine which could apply to other diseases (7). Their findings pertinent to our investigations may be summarized as:

1. IAA is responsible for increased meristematic activity.
2. Neither dopamine nor its oxidation products inactivate IAA, even though oxidized catechol does inactivate IAA.
3. Dopamine can protect IAA from oxidation by inhibiting IAA oxidase.
4. There is some evidence that oxidized dopamine may function in the conversion of tryptophan to an auxin which appears to be IAA.

Sugarbeet leaves resistant to *Cercospora beticola* have higher polyphenol content and higher activity of polyphenol oxidase than susceptible leaves, according to Trzebinski (21,22,23). Work in our laboratory, however has shown high dopamine levels correlated with low polyphenol oxidase activity (11). This discrepancy in results might be due to the fact that Trzebinski extracted all of the water-soluble phenols, whereas we extracted only dopamine by using selective solvents. Furthermore the polyphenolase enzyme may have been activated by Trzebinski's technique.

Kovács (10) found the low incidence of local lesions on the leaves of a sugarbeet variety resistant to *Cercospora* leaf spot correlated with the presence of diffusible substances on healthy leaves which inhibited spore germination. We have also observed that fewer spores of the fungus germinated on the resistant leaves or in washings from resistant leaves (9). Dopamine has been detected in the washings from resistant leaves in greater concentrations than in washings from susceptible leaves, but this does not prove that the substances described by Kovács was dopamine.

Superimposed upon the natural mechanism of the host's resistance is the existence of races or biotypes of *Cercospora beticola* (14,15,16), which differ in their virulence. A possible chemical basis for this difference in virulence was reported by Schlösser (18) where he isolated a chloroform-soluble, yellow substance from cultures of *Cercospora beticola*. This substance was reported to contain phenolic hydroxy groups associated with a

possible tropolone nucleus, and when applied to the leaf surfaces produced symptoms similar to those caused by the pathogen. This unidentified substance appeared to be associated with the virulence of the fungus (19).

Cunningham (3) completed a histological study of the leaves infected by *Cercospora beticola*. He observed that some distance from the lesion there was renewed meristematic activity in the cells of the mesophyll which resulted in complete occlusion of the intercellular spaces and the formation of a definite cicatrice. Canova (2) has also reported the development of a secondary periderm around the infected area.

### Experiments and Results

In relation to the observations of Cunningham (3), we stained leaf sections containing lesions of *Cercospora* leaf spot and observed much the same results. Sections of infected leaves were stained by the method of Dinkel (4) which utilizes a  $\text{HNO}_2$  solution followed by immersion in KOH. A bright red color is indicative of *ortho*-dihydroxyphenolic compounds. This color was produced wherever the vascular system of the leaves was visible. The small veins appeared to stain more intensely than the larger veins, possibly because of a higher concentration of phenolic compounds. A few cells immediately surrounding the *Cercospora* lesions were also colored, but to a lesser degree. The color observed could be due to dopamine or other *ortho*-dihydroxyphenolic compounds in these structures, but dopamine would seem likely since it has been found in relatively high concentrations in the leaves and gives the observed reactions with the reagents. The same colors and structures were observed around mechanical injuries and may represent a general wound response.

The success of the disease depends ultimately on the production of spores. Abundant spores are formed when the fungus is grown on beet leaf agar, but on other agars it is sometimes difficult to obtain spores. The simplest agar of definite composition is Czapek's medium and this was chosen to test the effect of several phenolic compounds on spore formation. The number of spores produced in agar containing the phenolic compounds was compared with a control of the same media not containing the phenolic compounds. The number of spores per square centimeter of culture was determined according to a method described by Calpouzos (1). The results are averages of six different areas. The same isolate of the fungus was used for comparison in all cases. The phenolic compounds were incorporated

into the agar before autoclaving, and thus some degree of oxidation occurred due to the heating. These data are presented in Figure 1.

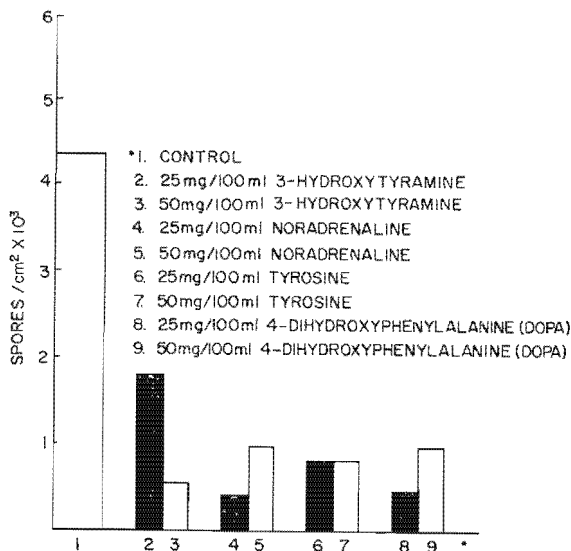


Figure 1.—The effect of various phenolic compounds upon sporulation of *Cercospora beticola* grown in Czapek solution agar for 4 days at room temperature.

In another experiment the spore-producing ability of single-spore isolates from resistant varieties was compared with single-spore isolates from susceptible varieties. Single spores were selected at random from dried, infected leaves of a susceptible variety of sugarbeet (R. and G. Pioneer) and from a resistant variety of sugarbeet (SP 5481-0). The isolated spores were grown on various agar media, and the number of spores per square centimeter of culture determined. The leaves from which the isolates were made were grown in the same field under the same conditions. The results are presented in Table 1.

Table 1.—Sporulation of *Cercospora beticola* isolates from susceptible and resistant sugarbeet leaves, grown at room temperature and counted four days after inoculation.

Spore source	Media		
	Beet leaf agar	Corn meal agar	Czapek's agar
Susceptible plants	18.9*	8.6	4.8
Resistant plants	7.8	4.2	2.3

\* Each value represents the average of fifteen single spore isolates. Grown in culture as spores/cm<sup>2</sup> × 10<sup>3</sup>.

The single spore isolates from the susceptible plants produced nearly twice as many spores as those from resistant plants. Each value represents the average of colonies from fifteen single spores taken from susceptible or resistant leaves. New single spores were isolated from the infected leaves and used each time. Three different areas were sampled from each culture of a single spore and averaged. In every instance the spores from susceptible leaves produced more spores than those from resistant leaves. There did not appear to be any correlation between the number of spores and the cultural characteristics (color, shape, growth rings, etc.). A few isolates, mostly from resistant leaves, produced no spores. These non-sporulating isolates were usually white, but other white isolates produced spores. The spore counts were made after allowing the fungus to grow for 4 days at room temperature.

It would appear that the resistant varieties either select out a strain of fungus that produces fewer spores, or which produce either a temporary or permanent genetic change in the fungus leading to less spores. In either case the effect is to lower the number of spores available for reinfection and hence to reduce the intensity of the disease. This may be as a result of higher dopamine levels in the resistant leaves as shown in Figure 1.

### Summary

On the basis of these data and the observations of other workers, some factors contributing to the resistance of sugarbeets to *Cercospora beticola* are summarized below:

1. Dopamine, or its oxidation products, are exuded onto the leaf surface and may inhibit the germination of the spores of the pathogen.
2. Single-spore isolates from susceptible plants produced nearly twice as many spores as those from resistant plants. This would lower the amount of inoculum.
3. There is an increase in the dopamine content of the leaves as a result of fungus infection or mechanical injury.
4. Most resistant sugarbeet varieties have higher dopamine contents in their leaves than do susceptible varieties. Further studies are needed to elucidate the reason why the high dopamine content in a few susceptible varieties is not effective in resisting *Cercospora beticola*.
5. A large number of phenolic compounds closely related to dopamine mediate the conversion of tryptophan into indolacetic acid (7).

6. Dopamine inhibits IAA oxidase, and this causes a further increase in the IAA concentration (13). If similar mechanisms are operating in the sugarbeet, which seems likely since the reactions have been shown to operate *in vitro*, the IAA would be present in sufficient quantity to initiate new meristematic activity. This would form a protective barrier around the infected area, and the accumulation of phenolic compounds in this protective barrier would contribute to arresting the advancement of the pathogen.

Thus the mechanism of resistance to *Cercospora beticola* in sugarbeets seems to operate at two levels. In resistant varieties entry and infection are reduced and subsequent events limit the activity of the pathogen after entry has been achieved. It is unlikely that any single mechanism exists which can account for all the disease resistance of sugarbeets to leaf spot. This could account for the few susceptible varieties that contain high levels of dopamine. A better understanding of the role of dopamine in the resistance mechanism may lead to a more efficient selection of resistant varieties, as well as to a more fundamental approach to the chemical nature of disease resistance in plants.

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