CERTAIN ASPECTS OF TOXICOLOGICAL STUDIES WITH SPECIAL REFERENCE TO *FUSARIUM VASINFECTUM* ATK.*

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Most of the workers on toxicological studies in the genus *Fusarium* have based their conclusions primarily upon the production of toxins by these pathogens in synthetic media and *in vitro* studies of these toxins. Recent evidence of *in vivo* experiments done in this laboratory (Kalyanasundaram, 1953 and 1954; Subba Rao, 1954) largely seems to support the conclusions of *in vitro* studies done here (Kalyanasundaram, 1953) and elsewhere (Gäumann, Naef-Roth and Kobel, 1952). A detailed study of the symptomatology of cotton plants infected by *Fusarium vasinfectum* (Kalyanasundaram, 1954; Subba Rao, 1954), as well as biochemical studies on these infected host plants (Kalyanasundaram, 1953) has led us to the logical conclusion that wilting is more due to chemical agents than mere physical causes. This will be considered briefly here before entering into other aspects of this toxicological problem.

The indispensability of ascorbic acid in plant metabolism and its role as a phytohormone is well-known (Virtanen, 1936). In *Fusarium vasinfectum* infected cotton plants, between the period of infection in the root region and symptom appearance in the aerial parts—this period was found to vary from 5 to 8 days—the ascorbic acid content was very much above the normal. This period, when there was no visual toxic symptom (Satyanarayana and Kalyanasundaram, 1952), could be called the period of incipient toxæmia as Hageman, Hodge and McHargue (1942) have indicated the possibility of using ascorbic acid measurements in plants as an index of the health of the plants. Both in this stage and in the subsequent stage of the disease, when the first visual symptom had made its appearance on the host, there was inhibition of starch synthesis along the regions of vein clearing, coupled with lowered ascorbic acid metabolism. There was, however, considerable transport of synthesised food materials from the leaves to the root region (Kalyanasundaram, 1954) until the necrosis of the stem set in and the plant collapsed. In fact, Waggoner and Dimond (1952) have shown in tomato

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plants infected by *Fusarium lycopersici* that in the earlier stages of the disease, when browning of the vascular strands was seen, there was upward transport of phosphate to the leaves from the roots. Hence this apparently normal activity of an infected plant in the earlier stages of toxæmia—normal in those regions of the leaves which are not yet affected by the lethal concentration of the toxins—could possibly be visualised in toxic wilting as the pure toxins are known to act specifically on certain host tissues (Gäumann, 1951), leaving other tissues unaffected. Moreover, the period of plant life following symptom appearance before complete death of these plants, which was seen to vary from 12 to 260 hours, depending on the age of the host at the time of infection, could be interpreted only on the basis of toxic wilting, as it is well known that any interruption in the supply of toxin could bring the plant to normal life and in fact, Varadarajan (1953) has demonstrated that Fe/Mn amendments to soils infected with *F. vasinfectum* could bring the plants to normal conditions even though the vein clearing symptom had made its appearance on the infected plants. The exact mechanism of resistance offered by Fe/Mn treated plants is, however, not clear.

The resistant strains of cotton plants (*Gossypium hirsutum*) were resistant to the wilt disease caused by *F. vasinfectum* in spite of their being infected by the pathogen under field conditions of pathogenicity. A study of the ascorbic acid and carbohydrate metabolisms of these infected plants indicated that it was a case of metabolic resistance to the disease (Kalyanasundaram, 1953). Such cases of metabolic resistance have been reported by Davis and Dimond (1952) in the case of tomato plants infected by *F. lycopersici*, brought about by treatments with chemotherapeutants. These instances of plant infection not culminating in the disease itself could not but happen in toxic wilting. Moreover, Gäumann and Naef-Roth (1953), working with the wilt toxin lycomarasmin on cut shoots of tomato, have shown that maximum toxic damage was seen in winter months and not in summer months. This they ascribed to the greater ascorbic acid synthesis of these shoots during summer months with greater light period.

Most of the work done in this laboratory has been on the wilt of cotton caused by *F. vasinfectum*, although wilt of tomato produced by *F. lycopersici* has been investigated widely in Europe (Plattner and Clauson-Kass, 1945; Gäumann *et al.*, 1952) and America (Gottlieb, 1944; Scheffer and Walker, 1953; Dimond, Waggoner and Dimond, 1954). All the same, both these pathogens are known to produce fusaric acid as one of their toxins and evidence of *in vitro* and *in vivo* studies presented here based on this toxin could be taken to explain the mechanism of wilting caused by these vascular Fusaria in general, unlike the studies on the wilt toxin lycomarasmin alone,

since it is highly specific to the causal organism *F. lycopersici*, and not known to be produced by any other species of *Fusarium*.

The *in vivo* symptoms reported earlier (Satyanarayana and Kalyanasundaram, 1952; Kalyanasundaram, 1954) could be brought about by the pure toxin fusaric acid, a 5*n*-Butylpyridincarbonic acid, with the empirical formula $C_{10}H_{13}O_2N$ and molecu ar weight of 179, isolated from *F. lycopersici*, *F. vasinfectum*, *F. hetero porum* and *Gibberella fujikuroi* (Gäumann *et al.*, 1952). The spectrum of action of fusaric acid is as unspecific as the spectrum of the causal organisms which form this toxin (Gäumann *et al.*, 1952; Kalyanasundaram, 1953). This toxin can cause damage to host plants of the families *Gramineæ*, *Papilionaceæ*, *Solanaceæ* and *Malvaceæ*. It is biologically interesting to note that the toxins of pathogenic fungi can kil] different host plants, which the organisms are unable to attack under field conditions of pathogenicity. But, within this framework, considerable quantitative and qualitative differences are noticed. Cotton plants are hundred times more sensitive to fusaric acid than maize, twenty times more so than paddy and fifteen times more sensitive than tomato. This differing sensitivity of these hosts viewed in the light of differences in the synthesising capacity of the pure toxin by the three species of *Fusarium* under *in vitro* conditions—*F. moniliforme* (*Gibberella fujikuroi*) produces the highest concentration of fusaric acid in synthetic culture media followed by *F. lycopersici* and *F. vasinfectum*—naturally poses the question, " Is this a case of physiological specialisation in the genus *Fusarium* to parasitic adaptation ?"

Another factor affecting the biological activity of fusaric acid seems to be the pH of the substrate. A quantity of toxin which in the acid region (pH 4·3) caused stem necrosis damaged only the leaves in the alkaline region (pH 7·2). To cause stem necrosis in the alkaline range, the quantity and the concentration of the pure toxin have to be increased. This behaviour of the toxin seems to be due to the dissociation of the toxin in the alkaline ranges.

The production of antibiotics is a character of most of the soil inhabiting fungi. That *F. vasinfectum* belongs to this group was indicated by Subramanian (1950). Fusaric acid, one of the toxins of *F. vasinfectum*, also possesses antibiotic potency (Gäumann *et al.*, 1952). This was further established in this laboratory as could be detected by the bioassay method (Kalyanasundaram, 1955). Following this method this toxin could be detected in the culture filtrates of *F. vasinfectum* and *F. moniliforme*, even in one week old cultures. The early appearance of this toxin was mainly conditioned by the C/N ratio of the medium (Kalyanasundaram, unpublished). It is

necessary to refer here to the contention of Scheffer and Walker (1953) that lycomarasmin, one of the toxins of *F. lycopersici*, is produced in culture media only after two months as a product of lysis, and in almost all cases of wilt under *in vivo* conditions the plants hardly lived for 14 days after infection, and that within this period lycomarasmin could not be produced inside the infected plants. But it must be borne in mind that lycomarasmin is not the only toxin produced by *F. lycopersici* and the second toxin, fusaric acid, could be detected in culture media even as early as four days (Kalyanasundaram, unpublished) using the bioassay technique. Hence it is logical to assume that fusaric acid could be formed within the host in a short period of 14 days, within which period, however, lycomarasmin could not possibly be formed. Once the plant tissue is affected by the early formed fusaric acid, conditions may become conducive for the production of the lytic product, lycomarasmin. It is needless to stress once again that the phytotoxin lycomarasmin is highly specific to the causal organism *F. lycopersici*, unlike fusaric acid which is produced by many pathogenic species of *Fusarium*.

With the knowledge that phytotoxins produced by plant pathogenic fungi have also antibiotic property, we have to take into consideration the possible role of these antibiotics in the rhizosphere and root surface of host plants, especially in the light of recent evidence of gliotoxin production by *Trichoderma viride* in unsterilised soil (Wright, 1952) and stability of antibiotics in different types of soil (Jefferys, 1952).

The stimulation of the microflora in the rhizosphere region seems to be mainly due to the rich organic source of food by way of root secretions and sloughed-off materials. In fact, very recent work of Katznelson, Rouatt and Payne (1954) has shown the presence of amino acids in root secretions. Recent work of Kalyanasundaram (unpublished) has indicated that the pathogen, *F. vasinfectum*, produced considerable amounts of the toxin fusaric acid in sterilised soil amended with oats or green leaf choppings. It was demonstrated earlier by Agnihothrudu (1954) that *F. vasinfectum* was present in the rhizosphere and root surface of susceptible cotton plants far in advance of infection and symptom appearance. Naturally it leads us to hypothesise, that this fungus may produce considerable amounts of this toxin in the rhizosphere and root surface of susceptible host plants using the phenomenally rich food in those regions.

Whether or not this toxin which may be produced in the rhizosphere and root surface, can be taken up by the plant vascular system, as phytotoxins are known to be taken up by the plants from the soil (Brian, Wright, Stubbs and Way, 1951), it stands to reason to assume that the amount of

toxin produced can at least play a vital part in determining the resistance and susceptibility of the hosts as it is not clear whether toxin production is a prerequisite to infection or infection precedes toxin production, or both are simultaneous acts.

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