SOIL CONDITIONS AND ROOT DISEASES

XIV. Host-Parasite Response to *Fusarium* Wilt*

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INTRODUCTION

MOST plant wilt toxins are non-specific to a wide variety of host plants against which the pathogens themselves show specificity (Gäumann, 1951). The present study was undertaken to know the effect of toxins derived from the fungus *Fusarium vasinfectum* Atk., on many host plants and also to understand the nature of disease resistance in the resistant varieties of cotton.

MATERIALS AND METHODS

Toxins used.—Culture of *F. vasinfectum* was grown in Richard's medium at room temperature (28–30° C.) for 28 days. After this period the culture filtrate was dialysed (Kalyanasundaram and Lakshminarayanan, 1953) and was used as the source of toxins.

Cotton varieties used.—Resistant: Cambodia 2 and Madras Uganda (*Gossypium hirsutum*), Susceptible: Karunganni 2 and Malvi 9 (*Gossypium arboreum*).

Artificial infection.—Both resistant and susceptible varieties of cotton were grown in sterilised soil contained in earthenware pots inoculated with the pathogen as outlined before (Kalyanasundaram, 1954). Uninoculated pots served as control.

Chemical analyses of host tissue.—Ascorbic acid, total carbohydrates and free reducing sugars were estimated both ' per gram ' basis and ' per plant ' basis at intervals of five days upto the 40th day after germination.

Ascorbic acid was estimated as already detailed (Kalyanasundaram, 1952). For estimation of total carbohydrates and free reducing sugars, samples were prepared as outlined by Dunlap (1930). The method of Hagedorn and Jensen (1923) was used to estimate sugars in these samples. Acid hydrolysable carbohydrates and reducing sugars have been expressed as

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percentage dextrose of residual dry weight (Mason and Maskell, 1924). Results of only one resistant and one susceptible variety have been presented for the sake of brevity.

EXPERIMENTAL

Experiments with toxins of F. vasinfectum.—The effect of toxins of *F. vasinfectum* on cut shoots of susceptible and resistant varieties of cotton plants are presented in Table I.

TABLE I

Showing the effect of different concentrations of dialysed culture filtrate of F. vasinfectum *on cut shoots of susceptible cotton (K 2), resistant cotton (CO 2) and red gram*

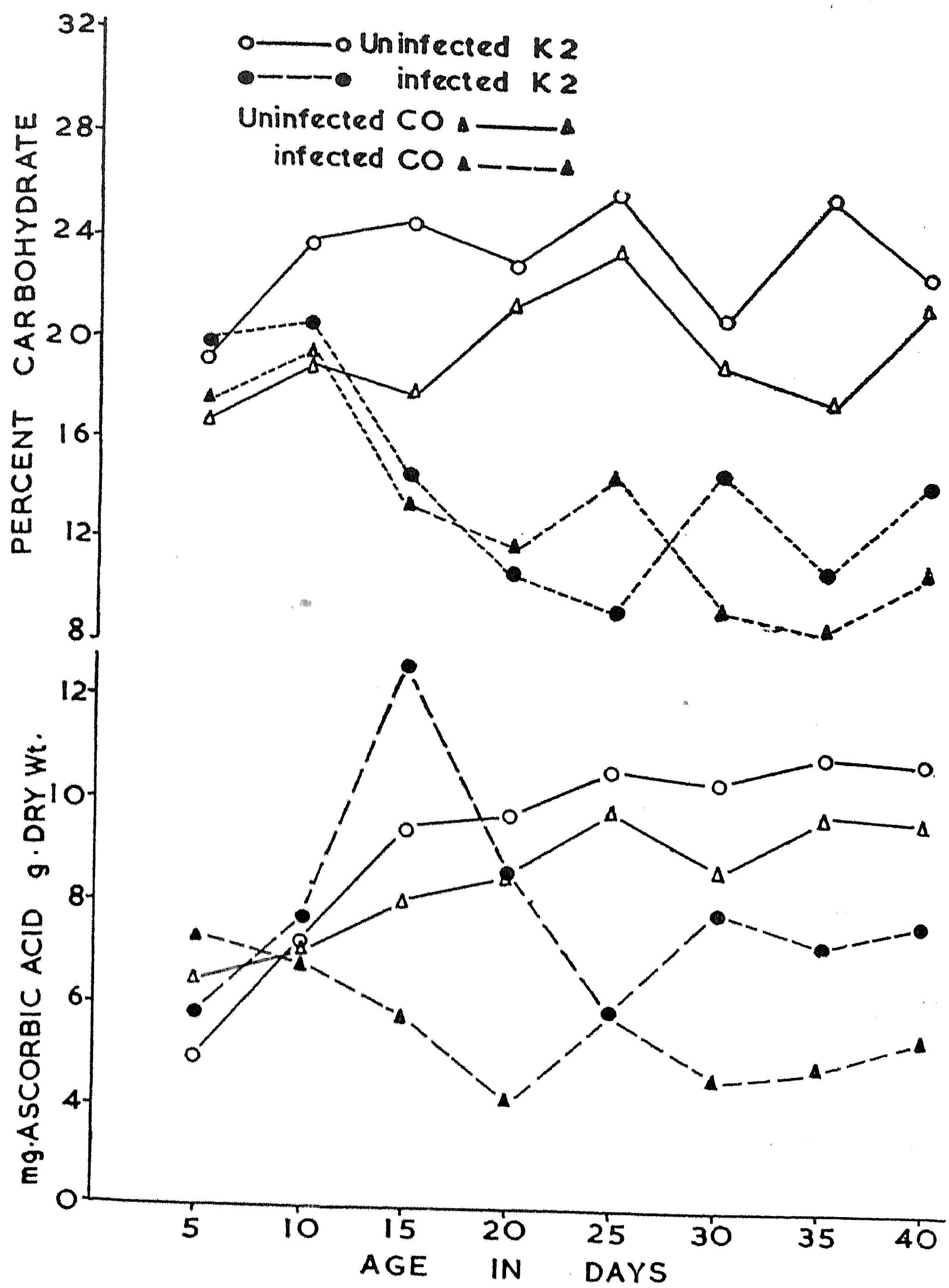
Time after being kept in the toxin in hours	100%			50%			25%			5%		
	K2	CO2	red gram	K2	CO2	red gram	K2	CO2	red gram	K2	CO2	red gram
24	..	..	..	..	..	..	..	..	..	..	..	..
36	..	x	..	..	..	..	..	..	..	..	..	..
48	x	xx	x	x	x	..	..	..	..	..	..	..
60	xx	xxx	xxx	x	x	..	..	..	..	..	..	..
72	xxx	xxx	xxxx	xx	xxx	x	..	..	x	..	..	..
96	xxx	xxxx	xxxx	xxx	xxx	xxx	..	x	xx	..	..	..

x Early symptoms.
xx Fairly advanced symptom.
xxx Advanced symptom.
xxxx Wilted.

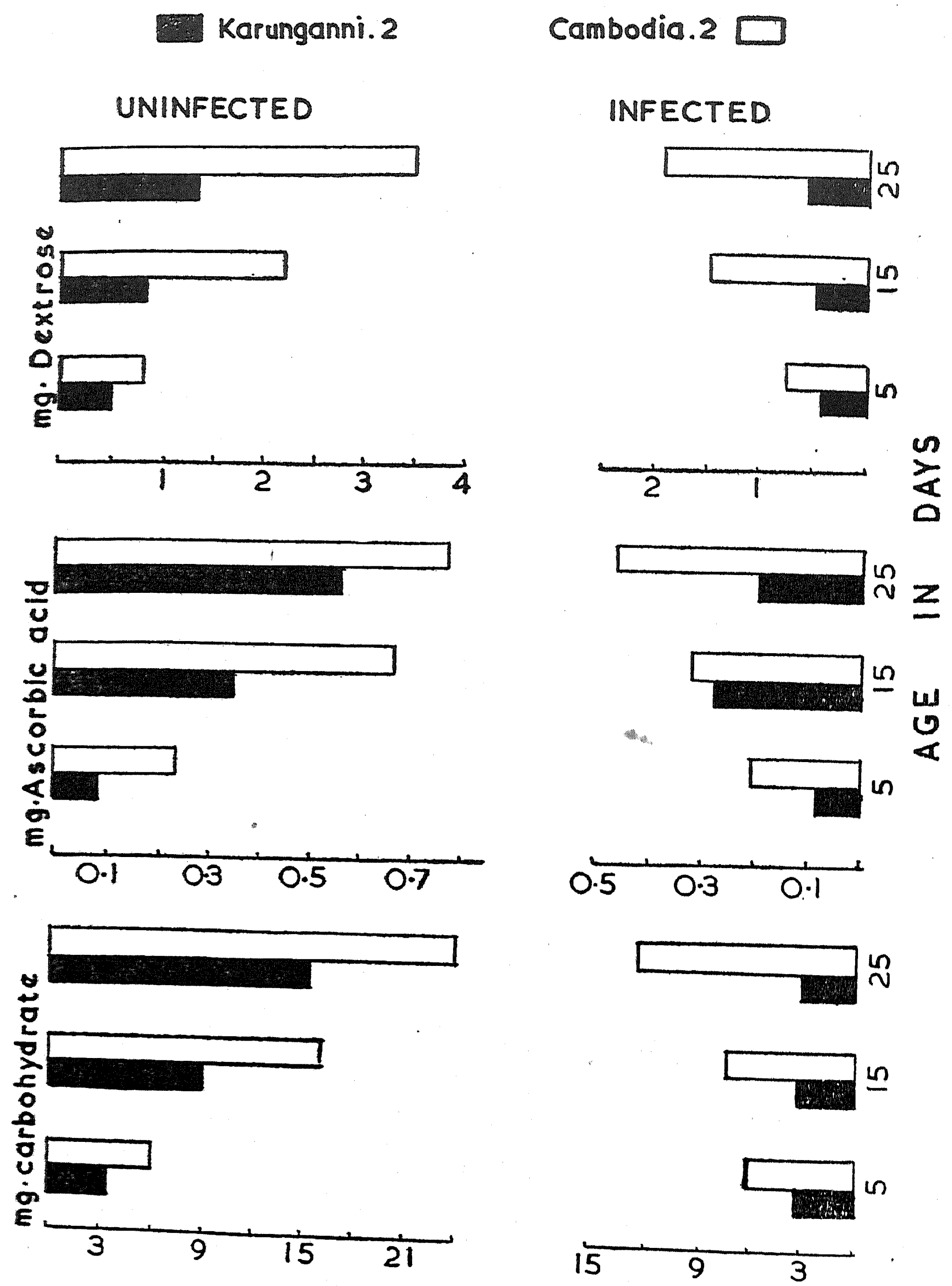
Using the dialysed culture filtrate of *F. vasinfectum*, it was possible to produce the symptoms of stem necrosis and 'vein-clearing' analogous to that obtained with pure fusaric acid (Plate X, Figs. 1, 2 and 3). It will be seen that the naturally resistant variety under field conditions was more susceptible to the toxins than the field susceptible variety.

On cut shoots of red gram (*Cajanus cajan*) toxins of *F. vasinfectum* caused considerable damage (Table I). It is evident that at higher concentrations stem necrosis occurred (Plate X, Fig. 4) while at lower concentrations there was typical 'vein-clearing' and necrosis of interveinal tissues (Plate X, Fig. 5).

Experiments on host metabolism.—Results of ascorbic acid, carbohydrates and free reducing sugar contents of cotton varieties infected by *F. vasinfectum* are presented in Text-Figs. 1 and 2 and Table II.



TEXT-FIG. 1. Showing the ascorbic acid and the carbohydrate content of leaf tissue of resistant [Cambodia (CO 2)] and susceptible [Karunganni (K 2)] varieties of cotton following *F. vasinfectum* infection.



TEXT-FIG. 2. Showing the absolute amounts of carbohydrate, ascorbic acid and reducing sugars in resistant (Cambodia 2) and susceptible (Karunganni 2) varieties of cotton infected and uninfected by *F. vasinfectum*.

Ascorbic acid.—Synthesis of ascorbic acid 'per gram' of leaf tissue was lower in plants that have received a fungal inoculum in the soil, when compared with plants in the uninoculated soil, irrespective of the appearance

TABLE II

Showing the percentage reducing sugar in the leaf tissue of a resistant and a susceptible variety of cotton grown in inoculated (artificially with F. vasinfectum) *and uninoculated soils*

(Result expressed as % residual dry weight)

Age in days	Karunganni 2		Cambodia 2	
	Uninfected	Infected	Uninfected	Infected
5	2·55±0·25	3·13±0·21	2·18±0·16	2·71±0·14
10	1·58±0·17	2·09±0·17	1·86 ±0·14	2·13±0·61
15	2·16±0·42	2·01±0·10	2·38±0·13	2·75±0·77
20	2·51±0·10	2·14±0·10	2·64±0·15	2·27±0·81
25	2·52±0·11	2·10±0·07	3·09±0·14	2·06±0·10
30	2·62±0·13	3·66±0·14	4·30±0·26	3·14±0·11
35	2·40±0·11	3·50±0·15	4·25±0·34	2·80±0·26
40	2·90±0·01	3·90±0·02	4·40±0·11	3·00±0·11

of the disease or their belonging to the susceptible or resistant varieties (Text-Fig. 1). However, the resistant varieties had significantly more ascorbic acid than the susceptible varieties on comparing those of the uninoculated series (Text-Fig. 2).

Carbohydrates.—The percentage carbohydrate contents of all the four varieties growing in the inoculated soil was lower than those in the uninoculated, irrespective of the appearance of the disease or their belonging to susceptible or resistant varieties (Text-Fig. 1). However, the resistant varieties had more carbohydrate than the susceptible varieties on comparing those growing in the uninoculated soil (Text-Fig. 2).

Reducing sugars.—Unlike ascorbic acid and carbohydrates, percentage reducing sugars in the tissues of the leaves of susceptible plants in the infected soil increased after the 25th day whereas in the resistant varieties there was a decrease after the 20th day (Table II), when compared with their respective controls. However, the total reducing sugars in the shoots of resistant plants was higher than that of susceptible plants irrespective of growing in infected or uninfected soil (Text-Fig. 2).

The results of plating roots indicated that all the varieties showed infection by the pathogen *F. vasinfectum* on the 10th day. However, only the susceptible varieties developed disease symptoms (Table III).

TABLE III

Showing the percentage of plants, showing the causal organism within their roots and also the disease (wilt index) in the four varieties of cotton artificially infected by F. vasinfectum

Age in Days	Karunganni		Malvi 9		Cambodia		Madras Uganda	
	% plants infected	Wilt index	% plants infected	Wilt index	% plants infected	Wilt index	% plants infected	Wilt index
5	..	0·0	40	0·0	..	..	..	..
10	33	1·5	75	7·5	70	..	70	..
15	36	4·0	50	13·5	24	..	25	..
20	46	6·5	54	27·0	34	..	10	..
25	60	23·5	52	39·0	22	..	20	..
30	60	32·0	60	46·0	41	..	30	..
35	48	38·0	51	50·0	25	..	27	..
40	29	54·0	46	62·0	37	..	28	..

Two salient points emerge from the results of this experiment. There was a derangement of ascorbic acid and carbohydrate metabolisms in the leaves of all the varieties growing in the infected soil, irrespective of the variety or their developing the disease. The absolute quantities of ascorbic acid, carbohydrates and reducing sugars 'per plant' were significantly higher in the resistant than in the susceptible varieties.

DISCUSSION

Susceptible cotton plants (*Gossypium arboreum*) infected by *F. vasinfectum* produce characteristic symptoms of 'vein-clearing' on the leaves and necrosis of the stems and petioles (Kalyanasundaram, 1954). In the present work, using dialysed culture filtrate of this pathogen it was possible to produce similar symptoms on cut shoots of different host plants. It has since been established that this symptom picture is caused by fusaric acid, one of the phytotoxins produced *in vitro* by this pathogen (Gäumann, Naef-Roth and Kobel, 1952). The resistant varieties (*G. hirsutum*) in spite of being infected by this pathogen do not manifest the visual disease symptoms (Table III). Nevertheless, the toxins of this pathogen cause more damage to cut shoots of these resistant hosts than on the susceptible (Table I). It is of importance to note that the toxins of *F. vasinfectum* damage cut shoots of red gram plants (Table I), though the fungus by itself is unable to infect them. It is obvious from these results that there is no specificity of toxin action despite the fact that there is specificity of host infected by this fungus.

A study of the host metabolism of resistant and susceptible cotton plants infected by *F. vasinfectum* indicated derangement of ascorbic acid, carbohydrates and reducing sugar metabolisms. Both resistant and susceptible plants when infected, showed marked decrease in ascorbic acid and carbohydrate syntheses as against their respective controls (Text-Fig. 1). While in the susceptible plants infection is followed by deranged host metabolism culminating in the disease itself, in the resistant plants, infection is followed by a parallel derangement of host metabolism, there being no disease production. In the synthesis of dextrose, which is the primary energy-yielding product, there is no parallelism in the behaviour between infected plants of the susceptible and resistant varieties (Table II) there being a marked increase in the reducing sugar content in the susceptible infected plants as against a decrease in the resistant infected plants.

Although there is an apparent similarity in the derangement of ascorbic acid and carbohydrate contents of resistant and susceptible infected plants (Text-Fig. 1), a comparison of healthy plants of the resistant and susceptible varieties as far as these above functions are concerned, clearly demonstrates that the absolute amounts of energy-giving substances, *viz.*, ascorbic acid, carbohydrate and dextrose are always higher in the former varieties (Text-Fig. 2).

Since the completion of this work, the author has noticed the very recent findings of Gothoskar, Scheffer, Stahman and Walker (1955), who have postulated that resistance in tomato plants to *Fusarium* wilt, is due to a very labile substance continuously formed at the expense of energy obtained from respiratory process which prevents the spread of the pathogen inside the vascular system. There is every reason to believe that resistance in cotton plants to the disease caused by *F. vasinfectum* may also be a case of metabolic resistance as hypothesised by Gothoskar *et al.* (1955). The energy-liberating substances like dextrose and carbohydrates as well as ascorbic acid which is involved in the oxidation-reduction system of plants are formed in the resistant varieties of cotton to a greater extent than in the susceptible plants (Text-Fig. 2). It is clear from the present work that even in the resistant plants, once they are infected by the pathogen, there is a marked decrease in the reserve food materials indicating an increased utilization of these substances. By virtue of the higher reserve of carbohydrates and ascorbic acid, the resistant cotton plants, despite infection, are in a better position to prevent the generalisation of the pathogen inside the host, probably by forming a toxic substance at the expense of these energy-giving substances present in the host tissues. A study of the enzymatic ractions of these infected resistant plants may well indicate the *modus operandi* behind this altered metabolism responsible for inhibiting the growth of the pathogen and consequent development of the wilt.

SUMMARY

The toxin of *F. vasinfectum*, fusaric acid, has no host specificity despite the fact that there is specificity of hosts infected by this pathogen.

Resistant cotton plants (*Gossypium hirsutum*), in spite of being infected by *F. vasinfectum* do not manifest visual symptoms. A study of their metabolism clearly indicates that resistance is due to the higher reserve of carbohydrates and ascorbic acid over the susceptible plants. It is likely that these energy-yielding substances are favourably utilised in the resistant plants for the formation of a labile toxic substance which inhibits the pathogen inside the vascular system.

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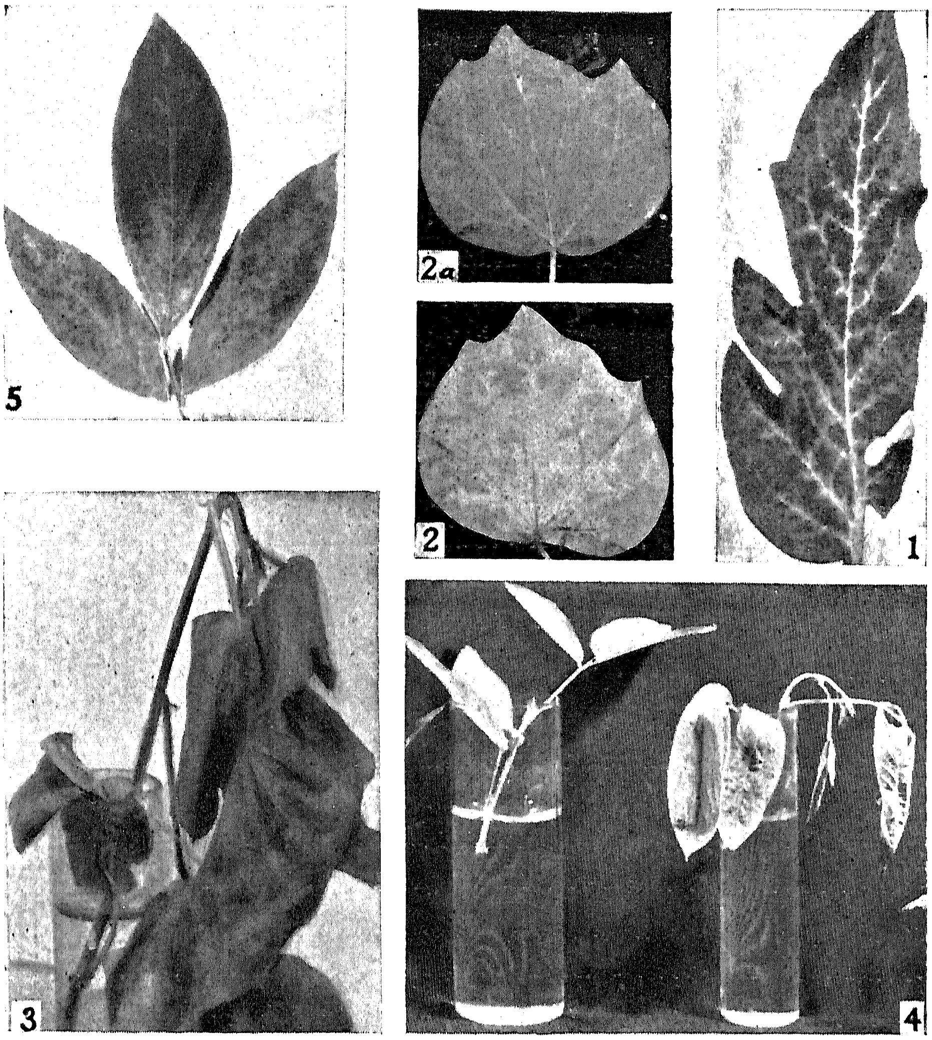
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EXPLANATION OF PLATE X

FIG. 1. Vein clearing in tomato leaf caused by fusaric acid.

FIG. 2. Veinal necrosis in cotton leaves caused by fusaric acid compared with the control (2 *a*).

FIG. 3. Necrosis of the stem and petiole of cut shoots of cotton caused by fusaric acid.

FIG. 4. Necrosis of the petiole of cut shoots of red gram caused by the dialysed culture filtrate of *F. vasinfectum*, on the left is the control.

FIG. 5. Vein clearing in red gram leaf caused by dialysed culture filtrate of *F. vasinfectum*.