TABLE II
Urea adduct formation with wax and dodecane

Temp. °C	Weight of urea in 80 cc of CH_3OH g	Weight of paraffin or dodecane in CCl_4 g	Weight of *n*-paraffin recovered after adduct formation g	First order rate constant × 10^{-4} sec^{-1}	Energy of activation (△E) k.cal/mole
Wax					
60	48	16·3/100 cc	11·9	3·634	8·125
45	36	16·3/100 cc	8·5	2·049	
n-Dodecane					
60	48	3·4/14 cc	1·7	1·926	8·032
45	48	3·4/14 cc	1·1	1·087	

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PREMUNITY IN WILT DISEASE OF COTTON*

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ABSTRACT

Pre-inoculation of cotton plants (*G. arboreum* var. *indicum* L.) with a low level of a virulent strain of the pathogen *Fusarium vasinfectum* Atk. protects these plants against a subsequent inoculation of a high level of the same virulent strain.

INTRODUCTION

DURING an attempt to verify the phenomenon of cross-protection in *Fusarium* wilt of the Indian cotton, *i.e.*, *Gossypium arboreum* var. *indicum* L. (cultivar Karunganni 7) using an avirulent American strain and a virulent Indian strain of the pathogen, an unusual phenomenon was noticed. No protection was observed in the experimental plants, which were pre-inoculated with the avirulent strain followed by a challenge inoculation of the virulent strain. On the contrary, in one of the treatments where the virulent strain itself was used for pre-inoculation, the plants were protected against a subsequent challenge inoculation. As this was considered a very interesting phenomenon, further work was done to confirm this and these results are presented here.

EXPERIMENTAL

An Indian strain (designated I2) virulent on *G. arboreum* var. *indicum* L. and an avirulent American strain (A1) were the pathogens used[1]. Microconidial suspensions of the fungi prepared from twelve day old cultures on potato dextrose agar (PDA) were used for soil inoculation. Plants were raised from seeds in sterilized earthenware pots (10 cm × 5 cm) containing sterilized soil with the desired level of conidia per gram of soil. This is referred to as pre-inoculation. For challenge inoculation, the optimum level of conidia per gram of soil was added and mixed with sterilized soil prepared in fresh batches of sterilized earthenware pots. Plants from the pre-inoculated pots were removed and transplanted carefully to these pots after the roots were thoroughly washed with sterilized water, taking care not to injure the roots (Treatment 1). Plants raised in sterilized soil without pre-inoculation and transplanted to pots containing the challenge inoculation formed the control (Treatment 2). Treatment 3 consisted of plants raised in pre-inoculated soils and transferred to sterilized soil without the challenge inoculation. Treatment 4 represented the conventional pathogenicity trial done in this laboratory[2], namely, plants raised in sterilized soil receiving the optimum level of the inoculum without any further disturbance by way of transplantation.

A series of preliminary experiments confirmed that an inoculum level of 5,000 conidia per gram of soil for pre-inoculation and transplantation after 8–10

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days incubation to soil containing the challenge inoculation gave the best results. Fifty plants were maintained for each of the treatments and the wilt per cent was calculated on the 60th day. The experiments were repeated several times over a period of 3 years under green-house conditions. Data from two such experiments, done in 1976 and 1978, are presented here.

Results

A perusal of the data clearly indicates that pre-inoculation of plants with 5,000 conidia per gram of soil of the virulent strain, followed by high level of inoculum of the same strain after a particular incubation period (Treatment 1) gives a protection of about 60% (Table II) to plants for the period of the experiment when compared to plants that were not pre-inoculated (Treatment 2). To reproduce the same conditions as is obtained in Treatment 1 the pathogenicity test was done as in Treatment 2 in addition to the conventional method (Treatment 4) where the plants raised in inoculated soil were left undisturbed. In Treatment 3 where the plants were in contact with the sub-optimal level of inoculum of the pathogen only for 8–10 days and after which they were transplanted to pathogen-free soil, we do get a low percentage of wilt (Table I).

Discussion

The results clearly demonstrate that pre-inoculation of *G. arboreum* var. *indicum* L. with a low level of inoculum which by itself causes minimal disease (Treatment 3 of Table I) and subsequent transfer after an incubation period to soil containing high level of challenge inoculum of the same virulent pathogen does reduce the incidence of wilt by 60% (Table II). To obviate the criticism that transplantation injury might be responsible for this lowering of wilt incidence seen in Treatment 1, we have compared the results of Treatment 1 with that of Treatment 2, a modified pathogenicity test, instead of the conventional one (Treatment 4). The percentage protection from the disease seen in Treatment 1 when compared with the Treatment 4 is, however, about 65%.

Since we got our first results early in 1976 we have come across references of similar protection reported by Kuc *et al.*[3], and Kochman and Brown[4] in two leaf spot diseases caused by fungi. As far as we are aware, the present report appears to be the first in a systemic infection caused by fungi.

The term 'premunity' may be applied to this form of 'induced immunity' or 'infection immunity' observed in the present study for the reason that the protection is due to an 'existing infection' against 'super infection' by the same virulent strain of the pathogen[5].

Table I

Effect of pre- and challenge inoculations with F. vasinfectum *Atk. on wilt incidence in* G. arboreum *var.* indicum L.

Treatment	Experiment	Pre-inoculation: 5,000 conidia/g of soil	Challenge inoculation: 25,000 conidia/g of soil	% wilt
1	Expt. 1	Yes	Yes	18
	Expt. 2	Yes	Yes	26
2	Expt. 1	none	Yes	60
	Expt. 2	none	Yes	50
3	Expt. 1	Yes	none	10
	Expt. 2	Yes	none	16
		Conventional inoculation: 25,000 conidia/g of soil		
4	Expt. 1	Yes	none	62
	Expt. 2	Yes	none	64

Experiment 1 was conducted between October 1976 and January 1977.

Experiment 2 was conducted between June 1978 and August 1978.

Table II

Data showing the percentage of protection in Fusarium wilt of cotton

Expt.	In Treatment 1 over Treatment 2	Average value	In Treatment 1 over Treatment 4	Average value	In Treatment 1 over the cumulative values of Treatments 2 and 3	Average value
Expt. 1	70	59	70·9	65·15	74·2	67·4
Expt. 2	48		59·4		60·6	

This form of 'induced immunity' or 'infection immunity' has the potentiality to develop into a successful method of plant disease control.

Further studies are in progress for a better understanding of the biochemical changes incited by the pre-inoculant in the host.

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EMBRYOLOGY OF PITTOSPORACEAE—II

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ABSTRACT

The family Pittosporaceae comprises 9 genera which are chiefly confined to Australia (Willis, 1966). They are trees, shrubs or undershrubs with flexuose or straggling branches as *Sollya*. The extipulate leaves exhibit alternate or whorled phyllotaxy. The flowers are usually bisexual, hypogynous, tetracyclic and pentamerous (except the gynoecium which is bicarpellary syncarpous) and are borne in corymbose, umbelliform or paniculate inflorescences (Hutchinson[2]).

THE information on the embryology of Pittosporaceae is meagre. Davis[1] reviewed the earlier literature on the embryology of the family. Subsqeuently only a few taxa of the family have been embryologically investigated (Sheela and Narayana[5]; Narayana and Sundari[3,4] and Sundari and Narayana[6]). The present paper deals with the embryology of *Sollya fusiformis* Hort., and *Sollya heterophylla* Lindl. The materials for the present study were obtained from Australia, through the courtesy of Dr. Hj. Eichler, Mr. R. H. Kuchel and Dr. J. S. Beard.

The anther is tetrasporangiate. The archesporium, differentiated in each of the anther lobes, is a plate of 4-cells (Fig. 1). The archesporial cells divide periclinally producing a primary parietal layer to the outside and a primary sporogenous layer to the inside. The differentiated anther wall comprises the epidermis and three wall layers (Fig. 2). The hypodermal layer develops characteristic banded thickenings and functions as the endothecium. The innermost wall layer functions as the tapetum of the secretory type. The middle layer becomes crushed during the development of the anther.

The tapetal cells are uninucleate to start with. As the microspore mother cells enter meiosis, they become multinucleate and the cytoplasm becomes vacuolate. Nuclear divisions are followed by nulear fusions. The degree of polyploidy in the tapetal cells depends on the number of fusing nuclei (Figs. 3, 4). They are completely absorbed by the time the pollen grains attain maturity.

The cells of the primary sporogenous layer undergo a few mitotic divisions before they function as the microspore mother cells. The microspore mother cells undergo meiosis and form microspore nuclei. Cytokinesis takes place by simultaneous furrowing. Pollen tetrads show tetrahedral arrangement (Fig. 5). In *S. heterophylla*, however, decussate tetrads have also been observed (Fig. 6). Degeneration of one or more or all microspores, while still enclosed in the mother cell wall, has been observed (Figs. 5, 6). The pollen grains are 3-colporate and are 3-celled at the shedding stage (Figs. 7, 8). Accumulation of black granular bodies of unknown nature has been observed in some of the pollen grains (Fig. 8).

The ovule is ana-campylotropous, unitegmic and tenuinucellate. It arises as a small protuberance on the placenta. During growth it undergoes curvature as a result the ovule becomes ana-campylotropous (Fig. 10). The ovules are horizontally placed and best median sections are obtained in transverse sections of the ovaries (Fig. 9). The integument is 12–14 cell thick in *S. fusiformis* and 11–14 in *S. heterophylla*, on the antiraphe side. There is no differentiation of endothelium.

The archesporium in the ovule is hypodermal and single celled. The archesporial cell directly functions as megaspore mother cell without cutting off a parietal cell (Fig. 11). The megaspore mother cell undergoes meiosis giving rise to a tetrad of megaspores which show linear (Fig. 12) or 'T' shaped configuration The chalazal functional megaspore undergoes three