

directly from the synergid cells without fertilization or may be diploid due to syngamy. Scanning the embryological literature on Dioscoreaceae reveals that polyembryony occurs only in *Trichopus zeylanicus*², a monotypic genus which has been raised to the rank of family Trichopodaceae³. The present report is on the occurrence of synergid embryos in *Dioscorea composita*.

The development of the zygotic embryo in *D. composita* will be reported elsewhere. Rarely, one of the synergids, more or less the same size of the egg cell behaves like an egg in developing into an embryo. The zygotic embryo as well as the synergid one develop equally, remain side by side, and present more or less the same architecture. In both the cases, well-developed multicellular, multiseriate suspensor can be observed. The development of the endosperm is as in the monoembryonate seeds.

It could not be ascertained whether the synergid embryo is haploid or diploid. If it is diploid, it should be due to the entry of more than one pollen tube into the same embryo sac which however warrants cytological confirmation.

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BIOLOGICAL CONTROL OF ANTHRACNOSE DISEASE OF JUTE

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THE control of plant diseases by chemicals can be spectacular but this is relatively a short-term measure. Moreover, the accumulation of harmful chemical residues sometimes cause serious problems. Biological methods, on the other hand, can be economic, self-perpetuating and usually free from residual side effects.^{1,2,3}

The jute leaf surface was screened for the isolation of microorganisms antagonistic to the anthracnose fungus *Colletotrichum corchori*. Leaves were washed in sterile distilled water in a mechanical shaker, the

washings concentrated by centrifugation and plated on PDA or NA medium⁴ in Petri dishes following serial dilutions.⁵ The Petri dishes were incubated at 30°C for 48hr-96hr and the microbial colonies developed on the agar plates were transferred aseptically to PDA or NA slants.

Thirty-two strains of twenty fungal species and 15 of 9 bacterial species were isolated from jute leaves throughout the crop season. These organisms were paired⁶ separately with *C. corchori* on PDA medium (pH 5-6). A well-marked space of aversion was evident when *C. corchori* was paired with *Aspergillus nidulans* or *Penicillium oxalicum*, thus indicating an antagonistic reaction. Further tests on the culture filtrate of these organisms, however, revealed that the inhibition of the growth of *C. corchori* was due to a marked change in the pH of these culture filtrates. Among the bacterial isolates, *Bacillus megaterium* (str. B-23) completely inhibited the growth of *C. corchori*. This bacterium, when tested against the other jute phyllosphere fungi by similar dual culture experiments, reduced their growth by 85-90% on PDA medium. Only *Rhizopus stolonifer*, *Aspergillus* spp., and *Alternaria* sp. showed some tolerance to *Bacillus megaterium* B-23. This bacterium was therefore used for biological control of anthracnose disease.

The potentially antagonistic bacterium was tested *in vitro* against *C. corchori* by measuring the percentage germination of conidia and germ tube growth in the cell free culture filtrate of the bacterium. The bacterium was grown for 7 days in Czapek Dox medium⁴ and the bacterial cells removed by centrifugation at 15,000 rpm for 15 mins. Fifty per cent of the supernatant was sterilised by autoclaving for 15 mins at 15 psi. pressure (at 121.6°C) and the rest by a Sintered glass filter (G-5). The filtrate and the autoclaved solutions were tested against *C. corchori*.⁷ There was a 80% reduction in germination and a 66% reduction in germ tube length in the cold sterilised culture filtrate in relation to sterile distilled water control. Autoclaved culture filtrate slightly reduced germination (17%) of the *C. corchori* conidia. It appears that the active principle is partially thermolabile.

To isolate the active principle, equal volumes of the cell-free culture filtrate (unheated) were extracted with four different organic solvents - ethyl acetate, chloroform, hexane and petroleum ether. The solvent fractions and the corresponding aqueous fractions were evaporated under reduced pressure in a rotary film evaporator, the residues dissolved in 1 ml of 0.01M phosphate buffer (pH 6.0) and the aliquots tested⁸ against spores of *C. corchori*. The ethylacetate fraction showed maximum inhibitory activity (94% reduction in germination and 77% reduction in germ tube length) which was evident even after four times dilution. Further dilutions, however, showed

negligible activity.

To test the effect of the bacterium on anthracnose disease intact jute plants were first sprayed with either dilute suspension of the bacterium or its cell-free culture filtrate. After 24 hr, selected plants were inoculated with the spore suspension of *C. corchori* (10×10^6 spores/ml) and incubated for 72 hr for lesion formation. Besides, in order to substantiate the results of this experiment, some treated (either with bacterial suspension or culture filtrate) leaves were detached, inoculated with *C. corchori* and incubated under moist conditions at room temperature (30–32°C). The results of both the experiments are given in table 1.

TABLE 1

In vivo effect of the bacterial suspension and the cell free culture filtrate of B. megaterium on lesion production by C. corchori.

Inoculation	Treatment	*% inhibition of lesion production	*% reduction in lesion spread
On detached leaves**	Bacterial suspension	88	95
	Culture filtrate	75	44
On intact plants #	Bacterial suspension	57	82
	Culture filtrate	21	16

*In relation to distilled water controls.

**Incubated for 48 hr and 96 hr.

Incubated for 72 hr and 120 hr.

Thus, *Bacillus megaterium* (B-23) could be used quite effectively for controlling anthracnose disease of jute caused by *C. corchori* (figure 1). The bacterial suspension appears to be more effective than its cell-free culture filtrate probably because the bacteria can multiply rapidly on the leaf surface before inoculation with the test organism. The antagonistic effect is not due to competition for nutrients alone since the culture filtrate can also reduce disease incidence on the leaf surface to a significant extent. In a similar attempt to control anthracnose of cucumber seedlings, Leben and Daft⁹ have used washed cells of *Pseudomonas* sp. A-180. The bacterium was sprayed 24 hr prior to inoculation with the test fungus which effectively controlled the disease. Swinburne^{10,11} also succeeded in controlling leaf scar of apple (caused by *Nectria galligena*) by prior inoculation with the bacterial suspension of *Bacillus subtilis*.

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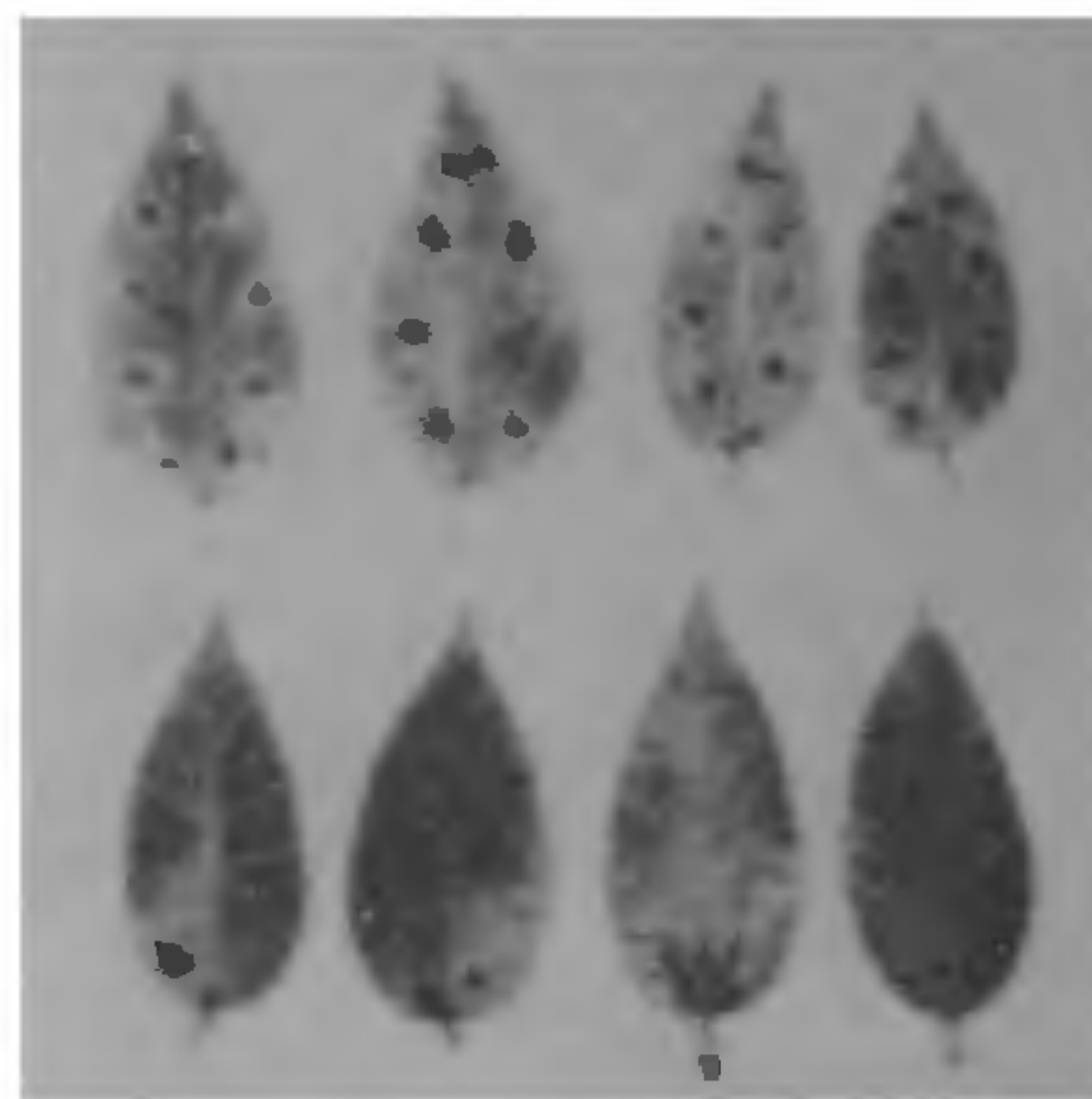


Figure 1. Upper leaves with lesions caused by *C. corchori*, lower leaves treated with bacterial suspension showing a few small lesions.

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SURVIVAL OF RHIZOBIUM JAPONICUM IN CHARCOAL BENTONITE BASED CARRIER

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A MAJOR breakthrough in legume inoculant