Culture deposited at the Indian Type Culture Collection as No. ITCC–2084, Mycology and Plant Pathology Division, IARI, New Delhi.

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WITCH'S BROOM OF COWPEA—A MYCOPLASMAL DISEASE

SINCE 1969, a witch's broom disease of cowpea[5] was observed at various places in northern India. Incidence of the disease varied from season to season. Mostly less than 1% plants were found infected, but in certain seasons (1974 *kharif*) up to 6% infection was recorded in some fields. The diseased plants give typical witch's broom appearance; at later stages trail on the ground, and produce phylloid flowers. Symptomatically, similar disease also occurs in southern India[2]. In this paper histopathology and chemotherapy of the disease are reported.

The culture of cowpea witch's broom disease (CpWB) was maintained on cowpea cv. Pusa Dophasli by grafting. Sap and non-persistent type of transmission by *Aphis craccivora* Koch., were tested by the methods described earlier[4]. For persistent type of transmission. *A. craccivora* were given an acquisition access time of 8 hours to 2 days and inoculation access time of 3 days. Dodder transmission was tested by simultaneously establishing *Cuscuta* sp. on diseased and healthy plants. At least ten cowpea plants were used for each transmission test. For examining the effect of tetracycline antibiotics on disease development, 250 ppm solutions of achromycin (tetracycline hydrochloride), agrimycin 100 (streptomycin + oxytetracycline hydrochloride 1·5%), aureomycin (chlortetracycline hydrochloride) and ledermycin (demethylchlortetracycline hydrochloride) were sprayed on plants twice a week. For electronmicroscopy small pieces (not more than 2 mm wide) of stem and leaves of cowpea cv. Pusa Dophasli, infected with CpWB and of healthy plants were fixed in 2·5% glutaraldehyde in cold for 12 hours, washed thoroughly in cacodylate buffer 0·05 M, post fixed in 1% osmium tetraoxide in cacodylate buffer (0·05 M) pH 7·0 for 2 hours, dehydrated in acetone and embedded in Spurr's low viscosity embedding medium in Beem's capsules. Ultrathin sections were cut with a LKB ultramicrotome UM1 using a diamond knife. Sections were picked on carbon coated grids, post stained with uranyl acetate and lead citrate, and examined in Philips EM 300 Electron Microscope.

CpWB was not transmitted by sap or *A. craccivora*. Graft transmission was more efficient than dodder transmission, as 72% of the test plants were infected by grafting and only 20% by dodder.

Spraying of plants with achromycin and ledermycin, starting six hours after graft inoculation, completely prevented establishment of the disease (Table I).

TABLE I

Effect of antibiotics on development of symptoms of witch's broom in cowpea cv. Pusa Dophasli

	Treatment* of plants after development of symptoms			Treatment of plants immediately after graft inoculation				
Antibiotic (250 ppm)	No. plants used	Remission	Recurrence after days**	No. plants used	% of plants† showing symptoms at different times after grafting			
					19	25	43	73 days
Achromycin	8	Complete	14	12 (5)‡	0	0	0	0
Agrimycin 100	8	Nil	..	13 (10)	10	50	60	60
Aureomycin	8	Partial	7	10 (7)	0	0	29	29
Ledermycin	8	Complete	19	15 (11)	0	0	0	0
Control (Water)	16	Nil	..	26 (16)	19	45	69	69

*15 biweekly sprays given.

** The time taken by the plants to redevelop the symptoms after the last spray.

†% of plants calculated on the basis of number of successful grafts.

‡ Number of plants grafted (number of plants retaining scion up to 5th day).

Aureomycin prevented transmission to a lesser extent; but the plants developed symptoms later than in water sprayed plants. Agrimycin 100 had very little effect on disease development (Table I). When the same chemicals were sprayed on plants after development of symptoms, remission in symptoms was obtained with achromycin, aureomycin and ledermycin, but not with agrimycin (Table I). The symptoms reappeared in plants after stoppage of treatments. The time taken for reappearance of symptoms, however, varied from antibiotic to antibiotic.

Characteristic pleomorphic mycoplasma-like bodies were observed in tubes of phloem of diseased leaves but not in healthy leaves. Size of these bodies varied from 245–737 nm. Each body, bounded by a 12 nm thick limiting membrane, contained fibrillar nuclear material and ribosomes which were smaller than those of the host (Fig. 1). Such bodies were not observed in phloem parenchyma cells.

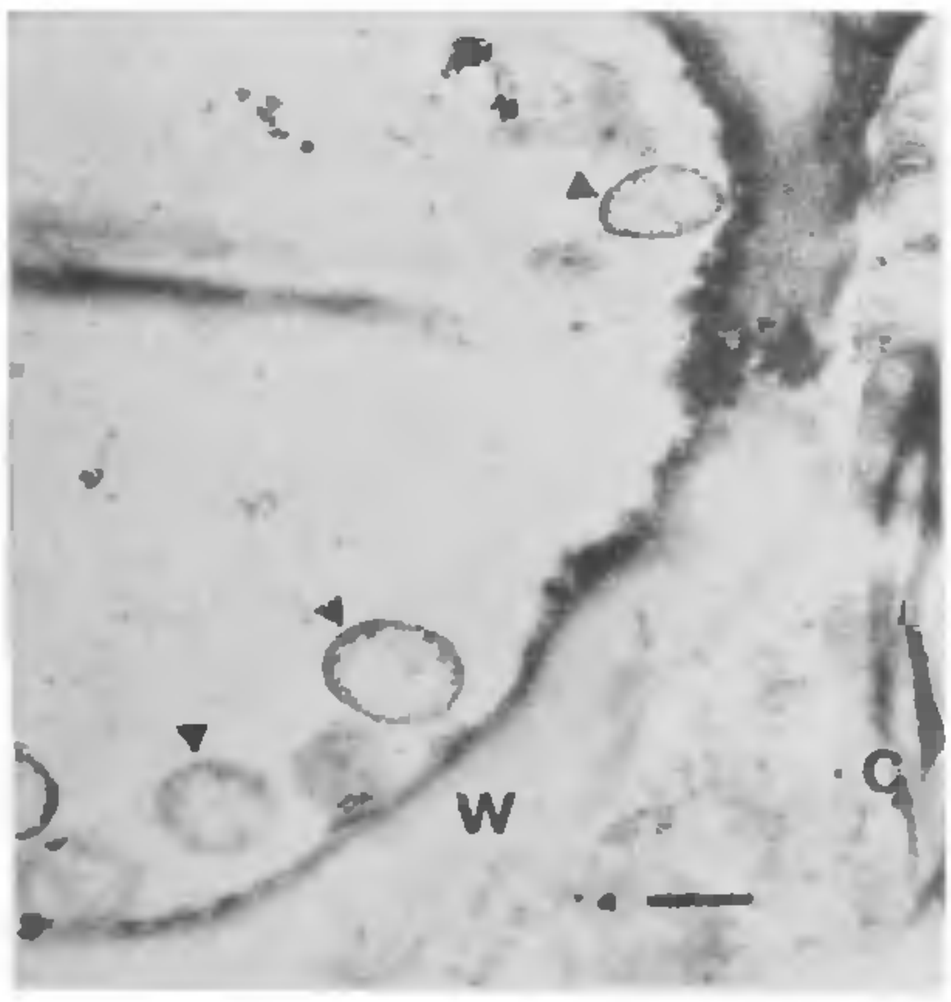

FIG. 1. Mycoplasma-like bodies (arrows) in a sieve tube of infected leaf. Part of a chloroplast in the companion cell is also seen. Bar represents 200 nm; *w*, cell wall; *c*, chloroplast.

Therapeutic effect of tetracycline antibiotics and presence of typical mycoplasma-like bodies in phloem cells suggest mycoplasmal etiology of the disease. Negligible remission of symptoms by treatment with agrimycin 100 indicates inefficacy of tetracyclines at low concentrations. CpWB differs from a witch's broom disease of cowpea in Indonesia which is efficiently transmitted by *Aphis medicaginis*[4], but resembles legume little leaf disease in etology[1].

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PROHIBITINS IN *CATHARANTHUS ROSEUS*

Catharanthus roseus is used in the control of diseases of higher animals. The plant is remarkably free from diseases which naturally suggested to us, the search for prohibitins of phytopathogens[1]. The results are reported here.

Dry root and stem segments, measuring 5–10 mm (25 gm each) were extracted for 15 minutes with 50 ml of boiling water. The boiled tissues were ground and filtered. The tissues were re-extracted in water and the extract filtered.

The extract was centrifuged and the clear supernatant was used, after adjusting the volume to 1 g tissue in 4 ml water. Similarly 70% alcohol extract of the roots was also prepared. Both aqueous and alcohol extracts were assayed against the spore germination of *Helminthosporium oryzae*, *Cladosporium herbarum* and *Curvularis* sp. which was performed in cavity slides by adding 1 drop of the extract. The growth of *H. oryzae* on potato dextrose agar and *Xanthomonas oryzae* on nutrient agar[2] in the presence of extracts was also measured.

Aqueous extracts of root and stem did not affect the mycelial growth of *H. oryzae* and multiplication of *X. oryzae*. Instead, they promoted the growth. In contrast 3 ml of alcohol extract of the root (equivalent to 750 mg tissue) inhibited the linear growth of *H. oryzae* by 50 to 60% but did not inhibit *X. oryzae*.

Aqueous extract of the stem did not inhibit the conidial germination in *H. oryzae*, *C. herbarum* and *Curvularia* sp. although it delayed the germination. But root extract caused 100% inhibition of spore germination of all the test fungi. The alcohol extract