

## Remission of Symptoms of Clover Club Leaf Following Treatment with Penicillin

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### ABSTRACT

Crimson clover plants affected with clover club leaf disease showed remission of symptoms after treatment with either tetracycline hydrochloride or penicillin. These compounds were administered as solutions of 100-1,000  $\mu\text{g/ml}$  in distilled water, in which the potted plants were

soaked for 24 hr at 7-day intervals over a period of 1 month. No permanent cures were effected. The implications of this finding on the suspected mycoplasmal etiology of the disease are discussed.

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*Additional key words:* witches'-broom-aster yellows, Rickettsiae, Chlamydiae.

Crimson clover plants *Trifolium incarnatum* L., on which nymphs of the leafhopper *Agalliopsis novella* (Say) had fed, developed symptoms of a disease which was named "clover club leaf" (CCL) (1). The disease agent, which for many years has been considered as a virus, was shown to be transmitted through the egg of this vector (2), and its multiplication in the insect was also proven (3).

The principal symptoms of the disease in crimson clover are yellowing and clubbing of young leaves, opened leaves often showing uneven expansion of the leaflets with marginal yellowing at the sides of the leaflets, and eventually dwarfing and premature death of the entire plant (Fig. 1-A, B). In *Vinca rosea alba* L., the symptoms which include dwarfing and yellowing of the leaves and a distinctive virescence and reduction of the flowers are equally striking (Fig. 1-C, D).

Since the discovery by Doi et al. (7) of the presence of mycoplasmalike bodies in the phloem of plants infected with witches'-broom-aster yellows type diseases, many workers have produced evidence that mycoplasmalike bodies are associated with a large number of plant diseases of the aster yellows type. Because of the similarity of some symptoms of clover club leaf and those of yellows type diseases, and because of its similar transmission by a leafhopper vector, it was suspected that this disease might also be caused by a mycoplasmalike agent (9).

Since antibiotics of the tetracycline group have been reported to produce remission of symptoms of yellows type diseases, a series of experiments on antibiotic treatments of crimson clover plants was initiated. Concentrations of tetracycline hydrochloride (Achromycin, Lederle Laboratories Division, American Cyanamid Co., Pearl River, N.Y.) ranging from 100-1,000  $\mu\text{g/ml}$  in distilled water were tested. Controls consisted of diseased plants treated with distilled water or with similar concentrations of

penicillin (Penicillin G potassium 1560 units/mg, Lederle Lab., American Cyanamid Co., Pearl River, N.Y.) as well as healthy plants treated with comparable concentrations of the antibiotics. Treatment entailed setting the clover plants which were growing in 2-inch clay pots into plastic beakers containing the solutions for a period of 24 hr at weekly intervals for 1 month.

Remission of symptoms first appeared in plants treated with achromycin (Fig. 1-G). However, plants treated with penicillin, although slower to show alleviation of symptoms, eventually appeared healthier than those treated with achromycin; the penicillin-treated plants (Fig. 1-H) were greener and taller than were plants treated with achromycin at equivalent concentrations. That the achromycin was itself phytotoxic was noticeable in the healthy control plants (Fig. 1-F) which quickly became chlorotic and died within 14 days when treatment involved concentrations of 600  $\mu\text{g/ml}$  and above. Penicillin had no such effect (Fig. 1-E). Infected control plants treated with distilled water showed the normal course of the disease.

Neither antibiotic as used produced a complete cure; recurrence of symptoms was observed in all plants after cessation of the treatments. The length of time which elapsed between cessation of treatment and recurrence of symptoms roughly reflected the concentration of antibiotics used. The penicillin-treated plants in general remained healthy in appearance longer than did the achromycin-treated plants.

The action of penicillin is known to be due to its effect upon bacterial cell wall synthesis. Therefore, that these results were obtained for a disease suspected of belonging to the yellows group, and suspected of having a mycoplasmal etiology was surprising. Moreover, they are in contrast to the results of other workers who have found penicillin to

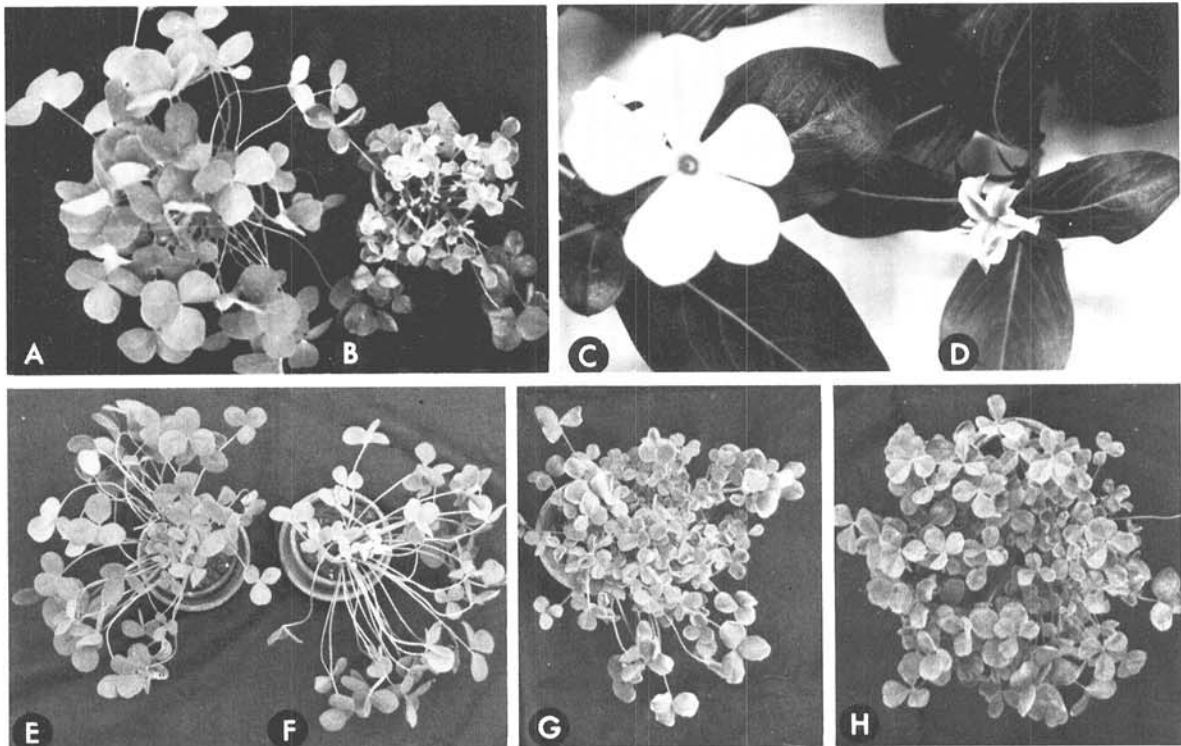


Fig. 1. A) Healthy, untreated crimson clover. B) Untreated clover club leaf (CCL) plant showing clubbing of young leaves and chlorosis at the leaflet margins of expanded leaves. C) Healthy *Vinca* flower. D) Reduction and virescence of flower on CCL *Vinca* plant. E) Healthy crimson clover treated with penicillin at 400  $\mu\text{g}/\text{ml}$  is unaffected by the antibiotic. F) Healthy crimson clover treated with achromycin at 400  $\mu\text{g}/\text{ml}$  shows chlorosis in younger leaves. G) CCL-affected clover treated with achromycin at 200  $\mu\text{g}/\text{ml}$  is also chlorotic but shows remission of club leaf symptoms. H) CCL-affected clover treated with penicillin at 200  $\mu\text{g}/\text{ml}$  shows remission of symptoms, no chlorosis, and is larger than the equivalent achromycin-treated plant.

be ineffective against such diseases (4, 6, 8). However, many investigators have not included penicillin in their treatments. Rather, it appears that the appearance of yellows-witches'-broom symptoms in a leafhopper-transmitted disease susceptible to treatment by a tetracycline antibiotic, and the finding of mycoplasma-like bodies in the phloem of diseased but not of healthy plants, have generally been taken as indicative of a mycoplasma etiology.

The alleviation of the symptoms of clover club leaf disease by penicillin treatment does not eliminate the possibility that it is caused by a mycoplasma-like agent; at least one of the animal mycoplasmas, *Mycoplasma neurolyticum*, Sabin type A, has been shown to be affected by penicillin at low concentrations (10). On the other hand, whereas penicillin treatment is ineffective for most of the animal mycoplasmas, it is effective against microorganisms of other groups such as the Rickettsiae and Chlamydiae, which have also been suggested as possible agents of the yellows-witches'-broom diseases (5, 7). The use of penicillin in antibiotic treatments of this type of disease would appear, therefore, to merit wider consideration, both from the point of view of

practical chemotherapy and of casting more light on the nature of the disease organism.

Further investigations on the effects of antibiotics on clover club leaf disease and electron microscope studies on the tissues of both diseased and healthy plants are now in progress.

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