Summer Death of French Bean: New Hosts of the Pathogen, Vector Relationship, and Evidence against Mycoplasmal Etiology

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ABSTRACT

The French bean summer death (SD) causal agent was transmitted by the leafhopper *Orosius argentatus* to, and recovered from, *Datura stramonium, Beta vulgaris* var. *vulgaris, B. vulgaris* var. *cicla, Callistephus chinensis,* and five susceptible cultivars of French bean. The agent was also transmitted to, and recovered from, the SD-tolerant French bean cultivars Hawkesbury Wonder and Brown Beauty, but not *Phaseolus aureus, P. lathyroides,* or *Solanum nigrum.* Only *D. stramonium, B. vulgaris* var. *vulgaris,* and the susceptible bean cultivars showed disease symptoms; *B. vulgaris* var. *cicla, C. chinensis,* and the tolerant bean cultivars were symptomless carriers. The SD agent was transmitted from *D. stramonium* to *D. stramonium* and tomato (*Lycopersicon esculentum* 'Grosse Lisse'), and from tomato to tomato and *D. stramonium* by grafting.

The minimum latent period of the SD agent in adult leafhoppers was between 24 and 48 hr. Leafhopper infectivity persisted for at least 21 days after access to a diseased plant. Infectivity was not lost during molting of the final instar. Single leafhoppers transmitted both the legume little leaf agent (one of the probable mycoplasmalike agents) and the SD agent.

Viruslike particles or microorganisms were not detected by electron microscopy in SD-diseased plants or infective leafhoppers. Treatment of SD-infected tomato with tetracycline hydrochloride (achromycin) had no apparent effect on graft or leafhopper transmission of the SD agent. These results are interpreted as evidence against possible mycoplasmal etiology of the disease. The causal agent is apparently similar to the beet curly top agent in several respects. Phytopathology 61: 1451-1455.

Additional key words: *Phaseolus vulgaris,* beetroot, silver beet.

Summer death (SD) of French bean (*Phaseolus vulgaris* L.), an economically important disease, was first described in Australia in 1968 (1). The causal agent is unknown. Attempts to transmit it mechanically or by grafting were unsuccessful (1). Subsequently, it was experimentally transmitted from bean to bean by the common brown leafhopper, *Orosius argentatus* Evans (2). Certain cultivars of French bean are affected, whereas others are considered tolerant or resistant (3, 4). Symptoms consist of chlorosis and down-curling of the leaves, vascular necrosis of the stem and root, and rapid death of the plant (4).

This paper reports (i) some new hosts of the SD agent; (ii) graft transmission; (iii) experiments on vector-pathogen relationship; and (iv) the results of electron microscopy and chemotherapy of diseased plants.

MATERIALS AND METHODS.—The SD agent was obtained from a bean plant (cultivar Tendercrop) growing in a Brisbane (Qld.) garden, and initially was maintained in Tendercrop plants by leafhopper transmission. Leafhoppers were obtained from a colony of *O. argentatus* established in November 1968 and maintained on celery (*Apium graveolens* L.). After the discovery that common thorn apple (*Datura stramonium* L.) is susceptible to SD, this species was used as a source of the SD agent, since leafhopper mortality on thorn apple was much lower than on bean.

Young adult leafhoppers were used for transmission, except where nymphs were specifically tested as vectors. Leafhopper infectivity was routinely tested by inoculation of newly emerged seedlings of Spartan Arrow French bean, since SD symptoms in this cultivar were more distinctive than in others (see RESULTS). Unless otherwise stated, all experiments were carried out in an insect-proof glasshouse.

For tetracycline hydrochloride (achromycin) treatment of SD-affected plants, the antibiotic (B.P. grade) was applied as a foliar spray by means of a manual atomizer.

Plant specimens for thin-section electron microscopy were prepared as described previously (8), except that some specimens were fixed in glutaraldehyde and osmium tetroxide (each at room temperature for 2 hr) instead of at 4 C for 20 hr. Leafhoppers were prepared for electron microscopy by the "whole insect" technique of Bowyer & Atherton (9). All sections were cut on an LKB ultramicrotome and mounted on carbon-coated copper grids. They were stained with uranyl acetate and lead citrate, and examined in a Siemens-Elmiskop IA electron microscope.

RESULTS.—*Transmission by O. argentatus.*—Table 1 summarizes the results of the inoculation of various plant species with SD-infective leafhoppers. Bean seedlings were usually inoculated 2 days after germination, and all susceptible cultivars developed symptoms 5 to 7 days later. Symptoms consisted of marked stunting, failure of the first trifoliate leaves to

TABLE 1. Transmission of summer death pathogen from *Phaseolus vulgaris* 'Spartan Arrow' or *Datura stramonium* by *Orosius argentatus* to various species[a]

Test species	Symptoms[b]	Recovery from test plants[c]
Phaseolus vulgaris L.		
'Tendercrop'	+	+
'Spartan Arrow'	+	+
'Redlands Belle'	+	+
'Gallatin 50'	+	+
'Bountiful'	+	+
'Hawkesbury Wonder'	−	+[d]
'Brown Beauty'	−	+[d]
Phaseolus aureus Roxb.	−	−
Phaseolus lathyroides L.	−	−
Datura stramonium L.	+	+
Beta vulgaris L.		
var. *vulgaris* (beetroot)	+	+
var. *cicla* (silver beet)	−	+
Callistephus chinensis (L.) Nees	−	+
Solanum nigrum L.	−	−

[a] After feeding on diseased plants for 4 days, 40 adults were caged on each group of four test plants for 7 days; each test was performed at least twice; as controls, 40 noninfective leafhoppers were caged on parallel groups of plants.

[b] + = definite symptoms; − = no symptoms.

[c] Noninfective adults were fed on test plants for 4 days (after symptom development or 21 days after inoculation); 40 were then tested on Spartan Arrow bean seedlings.

[d] Two of five tests on Hawkesbury Wonder positive, two of four tests on Brown Beauty positive.

expand, pronounced down-curling of the stunted trifoliate leaflets, and sometimes vascular necrosis and wilting of the plant. In Spartan Arrow, severe interveinal chlorosis of both the trifoliate and primary leaves was also characteristic. Plants of other cultivars occasionally developed slight chlorosis of the trifoliate leaves only. Most plants collapsed and died within 2 to 3 weeks, but a few survived for 4 to 5 weeks, and some of these produced stunted, axillary shoots from the primary leaf axils.

Symptoms in thorn apple (Fig. 1) appeared 9-20 (usually 12-14) days after the transfer of infective leafhoppers to the plants. Symptoms consisted of marked down-curling of the youngest leaves, together with pronounced interveinal chlorosis of the mature leaves, and slight stunting of the plant. In contrast to susceptible bean, the disease apparently had no effect on the longevity of thorn apple, and diseased plants produced normal fruit and viable seed from which healthy seedlings were raised. Symptoms in beetroot consisted of marked stunting of the plant, together with puckering and reddening of the leaves. Silver beet, aster, and the tolerant bean cultivars, Hawkesbury Wonder and Brown Beauty, were symptomless carriers of the SD agent. No symptoms developed in *Phaseolus aureus*, *P. lathyroides*, or *Solanum nigrum*, and the SD agent could not be recovered from these species.

Transmission by grafting.—Shoots from SD-diseased thorn apple were grafted by the side cleft technique to thorn apple and tomato (*Lycopersicon esculentum* Mill. 'Grosse Lisse') seedlings. All of 25 grafted thorn apple plants developed symptoms similar to those resulting from leafhopper transmission. Symptoms appeared 9-21 (usually 10-14) days after grafting. When leafhoppers were fed on the grafted plants after symptom development, then tested on Spartan Arrow bean, all test plants developed typical SD symptoms.

Twelve tomato plants were grafted with diseased thorn apple scions. All plants developed a mild interveinal leaf chlorosis and slight down-curling of the leaflets. Six serial grafts from tomato to tomato

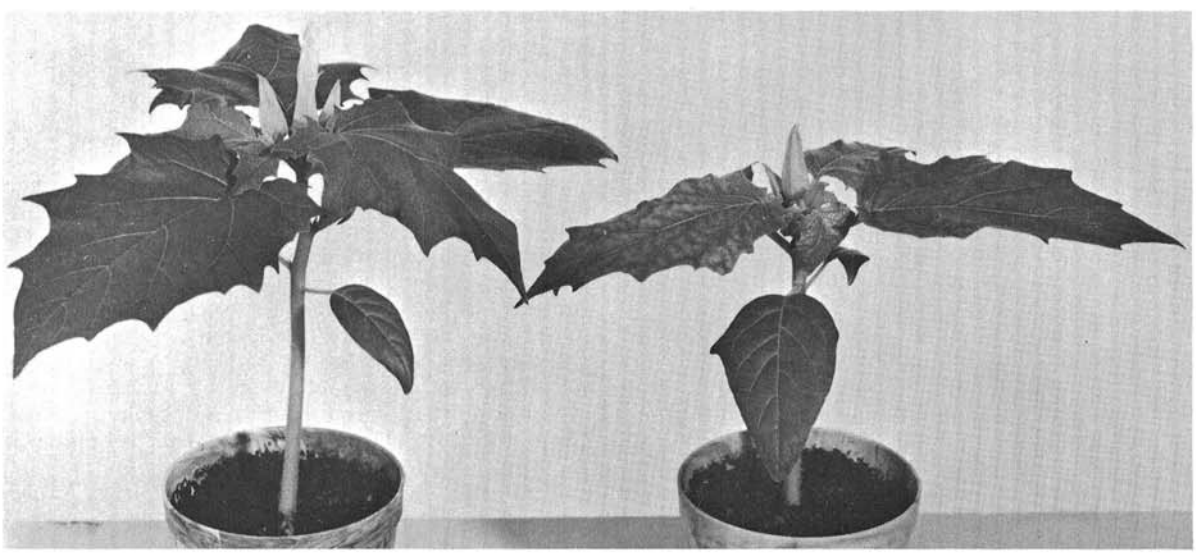

Fig. 1. Symptoms caused by Summer Death pathogen in *Datura stramonium*; plant on right inoculated by a single infective leafhopper (*Orosius argentatus*), and photographed 6 days after appearance of symptoms; note the interveinal chlorosis of the older leaves, down-curling of the young leaves, and stunting of the plant; control plant on left exposed to a noninfective leafhopper.

were performed over a period of 30 weeks, and the last plant was tested by grafting back to thorn apple. Typical SD symptoms developed in the test plants.

Latent period in adult leafhoppers.—Preliminary experiments indicated that the minimum latent period is not greater than 48 hr. In further tests, young adult leafhoppers were given acquisition access periods of 4, 8, or 24 hr on an SD-diseased thorn apple plant. At the end of each of the three periods, a group of 60 insects was transferred to two healthy Spartan Arrow bean seedlings, and subsequently transferred to fresh pairs of bean seedlings at various intervals. The test plants were observed for symptom development. Table 2 summarizes the results of this experiment. The minimum latent period in at least one leafhopper in the group given a 4-hr acquisition feed was between 24 and 32 hr. In the other 2 groups (given 8- and 24-hr acquisition feeds, respectively), the minimum latent period was between 32 and 48 hr.

TABLE 2. Latent period of summer death agent in *Orosius argentatus* as determined by feeding adult leafhoppers on infected *Datura stramonium* for various periods, with subsequent infectivity testing of groups of 60 by sequential inoculations of Spartan Arrow bean

Acquisition period (hr)	Time from beginning of acquisition (hr)	Transmission to test plants[a]
4	6	−
	8	−
	10	−
	24	−
	32	+
	48	+
	72	+
8	10	−
	24	−
	32	−
	48	+
	72	+
24	28	−
	32	−
	48	+
	72	+

[a] − = negative transmission; + = positive transmission.

Persistence in adult leafhoppers.—Young adults were fed on SD-diseased thorn apple for 7 days. Three groups of 50 were then transferred daily to healthy aster seedlings for a period of 21 days. (Preliminary experiments showed that noninfective leafhoppers did not acquire the SD agent during a 24-hr feed on aster seedlings inoculated during the previous 24 hr by SD-infective insects. Therefore, it was assumed that the test leafhoppers did not acquire the agent during each 24-hr feed on aster.) At the end of 21 days, the surviving insects in each group (24, 23, and 20, respectively) were tested for infectivity by transferring them to pairs of healthy Spartan Arrow bean seedlings. All three pairs of test plants developed SD symptoms, showing that infectivity persists in adult leafhoppers for at least 21 days after access to a diseased plant.

Infectivity following molting.— Fifth-instar (final) nymphs were caged on SD-diseased thorn apple for 4 days. Insects which became adults during the acquisition feed were discarded. Remaining nymphs were then caged on healthy thorn apple for 24 hr. During this time, 22 became adults, and they were transferred in two groups of 11 to pairs of healthy Spartan Arrow bean seedlings. Both pairs of test seedlings developed SD symptoms, showing that leafhopper infectivity is retained during molting from final instar to adult.

Transmission of both SD and legume little leaf (LLL) agents by single insects.—A group of young adult leafhoppers was fed on LLL-diseased celery for 28 days, then transferred to SD-diseased thorn apple for 7 days. We then tested 30 insects individually by confining them to single thorn apple seedlings for 10 days. The experiment was carried out in a controlled-environment cabinet with a day temperature of 27 C and a 12-hr photoperiod, with a dark period temperature of 22 C.

Of the 30 leafhoppers tested, 12 transmitted SD only, 2 transmitted LLL only, and 3 transmitted both SD and LLL. In the case of the three vectors of both agents, the test plants developed typical SD symptoms initially (14-19 days after inoculation), followed by typical LLL symptoms 15-18 days later. Thus, 50% of the insects transmitted SD, 16% transmitted LLL, and 10% transmitted both agents. In other experiments, 30-40% of individually tested insects transmitted the SD agent; and 10-20%, the LLL agent.

Electron microscopy of SD-diseased plants and infective leafhoppers.—Samples of diseased bean were prepared for thin sectioning 1, 3, 5, and 10 days after symptom development. Specimens included the midribs of stunted trifoliate leaves and chlorotic primary leaves, fragments of the respective leaf laminae, and segments of necrotic stems and roots. Specimens were taken from six Spartan Arrow and four Redlands Belle plants. Leaf veins and lamina fragments of infected thorn apple, beetroot, and silver beet were also examined. Controls consisted of corresponding material from healthy plants exposed to noninfective leafhoppers.

Final instar nymphs were fed on SD-diseased thorn apple for 7 days, and we tested 10 individually for infectivity by confining them on single Spartan Arrow bean seedlings for 3 days. The whole insects were then processed for electron microscopy.

No microorganisms or viruslike particles were observed in any of the plant or leafhopper specimens examined. Examination of diseased plant material was concentrated on the phloem tissue. The only apparent abnormality was the presence of necrotic tissue in the phloem of diseased bean and beetroot. Four of the leafhoppers tested were vectors. Using the technique described by Bowyer & Atherton (9), we examined the salivary glands and alimentary canals of these insects, but no differences between them and the organs of control insects were detected.

Tetracycline treatment of SD-infected tomato.—Two tomato plants showing the typical

symptoms of mild interveinal chlorosis and leaf curling were sprayed 20 times at 2-day intervals with a 100 μg/ml aqueous solution of tetracycline hydrochloride (achromycin). Two similar plants were sprayed with distilled water as controls. The shoot apex was removed from each plant to stimulate the development of axillary shoots. At the end of the 40-day treatment period, 10 axillary shoots from the treated plants and 10 from the control plants were grafted (side cleft technique) to 10 pairs of healthy thorn apple seedlings growing in 10 pots. One member of each pair was grafted with a shoot from a treated plant; and the other, with a shoot from a control plant. The time intervals between grafting and appearance of SD symptoms in the indicator plants were recorded. In the plants grafted with achromycin-treated scions, SD symptoms developed 12-20 days (mean 14.8) after grafting. In the control series, the range was 12-16 days (mean 13.3). The two sets of results were not significantly different, and it was concluded that achromycin treatment had no significant effect on the time required for symptom development during graft transmission of the SD agent from the treated tomato plants to the indicator thorn apple plants.

Each of the two treated and two control tomato plants was also assayed by means of *O. argentatus*. At the end of the treatment period, groups of adult leafhoppers were fed for 3 days on shoots which had developed after treatment was commenced. Thirty insects from each plant were tested on pairs of healthy Spartan Arrow bean seedlings, all of which developed SD symptoms. These results show that the achromycin treatment did not prevent leafhopper acquisition of the SD agent from the treated plants.

DISCUSSION.—Although this work has provided additional information on the biology of the summer death disease, the nature of the causal agent remains unknown. It has been suggested that a virus or mycoplasmalike agent might be involved (2). Presumably a mycoplasmalike agent was suggested because the same vector transmits legume little leaf and other yellows diseases in Australia (15, 16), and recent evidence indicates that the latter diseases are caused by mycoplasma. This evidence is based on the readily demonstrated presence of mycoplasmalike bodies in diseased plants and infective leafhoppers, their absence from control material, and the marked response by diseased plants to tetracycline therapy (8, 9, 10, *unpublished data*). The results presented here are based on techniques similar to those used for little leaf disease, and do not indicate that mycoplasma is the agent of summer death disease. They do not exclude a possible viral etiology of the disease, but further information on the properties of the agent is required.

The reaction of some varieties of French bean to summer death is similar to their reaction to the beet curly top disease in the USA (4). Curly top has long been considered a virus disease, but the assumed virus has not been purified or seen in diseased plants or leafhopper vectors. We also failed to detect virus particles in SD-diseased plants and leafhopper vectors. Although these are negative results, they may reflect a similarity between the agents of the two diseases. The latent periods of the agents in their respective vectors are also similar. Our results with SD indicate a minimum latent period of 24-48 hr. Bennett & Wallace (7) found that the minimum latent period of the curly top agent in *Circulifer tenellus* was 4 hr, but the number of leafhoppers transmitting in 12-24 hr was much greater than the number requiring less than 12 hr. Our results are based on the use of groups of leafhoppers, and single testing of a large number of insects may reveal a latent period of less than 24 hr for some individuals. Similarly, individual leafhopper testing will be necessary for more precise data on persistence of the SD agent in its vector. But it is evident that the agent is transmitted in a persistent manner, because groups of leafhoppers retained their infectivity for 21 days (the longest time tested) after access to a diseased plant, and because infectivity is not lost during molting. A similar relationship exists between the curly top agent and its vector (7, 13). Most evidence suggests that the curly top agent does not multiply in the vector (6, 7, 13). We have provided no evidence for or against multiplication of the SD agent in *O. argentatus*.

When leafhoppers were given a 28-day feed on a little leaf-diseased plant, followed by a 7-day access to an SD-diseased plant, a few individuals (3 of 30 tested) transmitted both the LLL and SD agents during a 10-day feed on test plants. Frederiksen (12) obtained transmission of both the aster yellows agent and the oat blue dwarf virus by individuals of *Macrosteles fascifrons*. The LLL and aster yellows agents are apparently mycoplasmalike organisms (17).

Two facts suggest that the SD agent is concentrated in the phloem tissue of diseased plants. These are (i) transmission by a leafhopper known to be a phloem-feeder (11); and (ii) the extensive phloem necrosis in diseased bean. The curly top agent appears also to be concentrated in the phloem (5).

The fact that species other than French bean are susceptible to SD-infection might help to explain the maintenance and spread of the disease in the field. However, it is not surprising, because the rapidly lethal effect of the agent in susceptible bean would militate against its survival in such a host. Although only a few species were tested, both symptom-producing and symptomless hosts were readily found, and it is possible that SD, like beet curly top, has a wide host range (14). It is conceivable that thorn apple plays a significant role in the natural spread of SD in bean crops, since it is a common weed, and *O. argentatus* feeds and breeds readily on it. A search might now be made for diseased thorn apple plants in SD-affected bean crops.

The discovery that thorn apple is susceptible to SD-infection has useful experimental application for two reasons. Firstly, it allows the maintenance of the SD agent in plants for long periods. This eliminates the frequent transmissions necessary to maintain the agent in susceptible French bean, as a result of the rapid death of this species. Secondly, when

leafhoppers are fed on thorn apple, the high mortality associated with feeding on French bean is avoided. The high mortality on bean seems to be due to the insects' being physically caught by the epidermal hairs which are abundant on this species.

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