A BACTERIAL DISEASE OF SNAKE BEANS.

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With Plate V.

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INTRODUCTION.

Gardner and Kendrick,[5] in their account of a bacterial spot disease of cow-pea (*Vigna sinensis* (L.) Endl.) caused by *Bacterium vignæ* Gardner and Kendrick, state that a bacterial disease of asparagus bean (*Vigna sesquipedalis* Wight, known also as snake bean and yard-long bean) was observed in Indiana and was probably caused by *B. vignæ*. Burkholder[2] recorded a bacterial disease on the same host and determined the causal organism as a strain of *Phytomonas* (*Bacterium*) *vignæ* var. *leguminophila* Burkholder.

In Australia there is no previous record of the occurrence of a bacterial disease of snake bean, although a bacterial disease was recorded on cow-pea at Glen Innes, N.S.W., in 1932.[7] The causal organism was on the basis of symptoms assumed to be *B. vignæ*.

OCCURRENCE, DISTRIBUTION, AND SYMPTOMS.

In January, 1935, snake beans grown near Mudgee, N.S. Wales, came under notice on account of a condition associated with considerable leaf damage. Microscopic examination of affected leaves showed large numbers of bacteria in the leaf spots. On plates of beef-extract peptone agar prepared from dilutions of macerated tissue in sterile water, numerous bacterial colonies, creamy white in colour, made their appearance. Needle-prick inoculations of the stems of snake bean seedlings with cultures from these colonies resulted in the collapse and death of the seedlings within four days. Re-isolations were made from the stems of infected seedlings about one inch above the point of inoculation. The re-isolated organism was also pathogenic to snake beans.

In the field the disease was evident as reddish-brown spots of varying size on the leaves (Plate V, A). Similar lesions were observed on the petioles, stems, and pods. A narrow zone of yellowish-green leaf tissue frequently occurred around these leaf spots, in the centre of which the tissue later became thin, dry, and brittle, and often fell out. On the underside of the leaf the disease sometimes caused a blackening of the veins or portions of the veins.

Identity of the Causal Organism.

These symptoms agree closely with those described and illustrated by Gardner and Kendrick[5] in the case of the bacterial spot disease of cow-pea caused by *B. vignæ*. Clara[3] in a recent study of some of the green fluorescent bacterial plant pathogens concluded that *B. vignæ* was synonymous with *Bacterium syringæ* (Van Hall) E. F. Smith, the cause of the lilac blight and citrus pit diseases.

For purposes of comparison with the locally isolated organism, the following cultures were obtained:

(i) A culture of *B. vignæ*, from Dr. M. W. Gardner, of the University of California.

(ii) A culture of *B. syringæ*, isolated from lilac, from Dr. W. H. Burkholder, of Cornell University.

(iii) Two cultures of *B. syringæ*, isolated from citrus, from Mr. A. T. Pugsley, Department of Agriculture, Victoria. One of these had been isolated from lemon and the other from orange.

These four cultures were used for the inoculation of young snake bean seedlings and were recovered from the inoculated plants about one inch above the point of inoculation. The original and re-isolated cultures of these organisms were used in morphological, cultural, and host range studies in comparison with the locally isolated snake bean pathogen.

Morphological and Cultural Characteristics.

Morphologically and culturally the various cultures of *B. syringæ*, *B. vignæ*, and the locally isolated organism did not differ significantly from each other except in the fermentation of raffinose. The results obtained by the writer were similar to those recorded by Clara[3] for *B. syringæ* and *B. vignæ* except in the reduction of nitrate. Clara[3] recorded no reduction of nitrate to nitrite, using the α-naphthylamine and dimethyl-α-naphthylamine

tests, but the writer found a very slight production of nitrite within two days at 30° C. in the case of all the organisms studied. This nitrite could be detected by the use of α-naphthylamine and sulphanilic acid but not by the use of Tromsdorff's reagent. Bryan[1] recorded similar results in the study of *B. syringæ* from lilac. Most of the other workers on these organisms used Tromsdorff's reagent and recorded no nitrate reduction.

In these studies perceptible differences were noted in the ability of the various organisms to ferment raffinose. A synthetic peptone-free medium was prepared according to the slightly modified formula of Ayres, Rupp and Johnson in the *Manual of Methods for the Pure Culture Study of Bacteria.* One per cent. of raffinose was added to this basic medium. Brom cresol purple was used as the indicator. The raffinose medium was sterilised by filtration through a Berkefield filter and by heating in the autoclave at 15 pounds pressure for five minutes. After inoculation the tubes were kept at 30° C. The action of the organisms on raffinose is shown in Table I.

TABLE I.

The Action of the Organisms Studied on Raffinose.

Organism Used.	Effect on Medium Sterilised by	
	Filtration.	Heat.
B. syringæ from citrus	+	+
B. syringæ from lilac	−	+
B. vignæ	−	±
Local snake bean organism	−	±

NOTE.—All media were kept at 30° C. after inoculation.
+ =acid reaction (a bright yellow colour) within seven to ten days.
± =acid reaction in some cases within fifteen to twenty days. In other cases, growth, but no acid reaction within twenty days.
− =growth, but no acid reaction within twenty days.

No differences were noted between the various organisms in their action on starch, dextrose, sucrose, maltose, lactose, galactose, arabinose, xylose, lævulose, rhamnose, glycerol, mannitol, dulcitol, salicin, acetic acid, formic acid, succinic acid, lactic acid, and tartaric acid. The results obtained by the writer agreed with those obtained by Clara.[3] In dulcitol, which was not used by Clara, no growth was noted in the case of any of the organisms.

O—April 1, 1936.

Host Range Studies.

Inoculations were made with cultures of the various organisms into stems, shoots, pods, or fruits of a number of different plants (see Table II). The method of inoculation consisted of puncturing the stem, fruit or other plant

TABLE II.

Comparison of Results of Inoculating Various Plants with the Organisms Studied.

Host.		Pathogen.			
				B. syringæ.	
Plant Used.	Part of Plant Inoculated.	Local Snake Bean Organism.	*B. vignæ.* From California.	From Victoria from Citrus.	From Cornell from Lilac.
Snake bean (*Vigna sesquipedalis*) ..	y. st. m. st. p.	++ + +	++ + +	++ + +	++ +
Cow-pea (*V. sinensis*)	y. st.	++	++	+	
Lima bean (*Phaseolus lunatus*)	y. st p.	++ +	++ +	++ +	
French bean (*P. vulgaris*)	y. st. p. m. l.	+ + +−	+ + +−	+ + +−	 +
Scarlet runner bean (*P. multiflorus*) ..	y. st.	+	+	+	
Soy bean (*Soja max.*) ..	y. st.	++	+	+	
Tick bean (*Vicia faba.*)	y. st.	++	+	+	
Lucerne (*Medicago sativa*)	y. sh.	+	+−	+	
Pea (*Pisum sativum*) ..	y. st.	−	−	−	
Lemon (*Citrus limonia*)	m. f. i. f.	− +	− +	+− +	
Orange (*C. sinensis*) ..	m. f.	−	−	+−	
Mandarin (*C. nobilis* var. *deliciosa*)	m. f.	−	−	+−	
Lilac (*Syringa vulgaris*)	y. sh	+−	+−	+	+
Cabbage (*Brassica oleracea* var. *capitata*)	y. st.	−	−	−	
Cauliflower (*B. oleracea* var. *botrytis*) ..	y. st.	−	−	−	

NOTE.—Abbreviations are as follows: y. = young; m. = mature; i. = immature st. = stems; sh. = shoots; p. = pods; f. = fruits; l. = leaves.
++ indicates death of plants in the case of stem inoculations.
+ indicates infection.
+− indicates slight infection.
− indicates no infection.
All inoculations made by needle puncture under comparable conditions.

part with a sharp needle, flame-sterilised before use, and dipped in an agar slope culture so as to cover the point of the needle with a mass of bacteria. In every case adequate control plants or plant parts were pricked with a sterile needle. The Cornell culture of *B. syringæ* from lilac was used for the inoculation of only a few of the plants.

The results of these inoculation studies are summarized in Table II. The results obained with the organisms from lemon and orange from Victoria were very similar and are grouped together.

Discussion.

The only differences noted between the various organisms were in the fermentation of raffinose and their pathogenicity to various hosts. The snake bean organism agreed entirely with *B. vignæ* except in the degree of pathogenicity to tick bean, soy bean, and lucerne. If the citrus pit and lilac blight organisms are both designated *B. syringæ*, Clara[3] is justified in placing the cow-pea organism in the same species. If *B. vignæ* is to continue as a separate species, the citrus pit organism should also be regarded as a distinct species and should be designated *Bacterium citriputeale* Smith. The differences between these organisms is slight, however, and, for the present, the writer is of the opinion that the citrus pit, lilac blight, and cow-pea spot organisms, and the snake bean pathogen should all be designated *Bacterium syringæ* (Van Hall) E. F. Smith.

The Occurrence of a "Rough" Strain of the Locally Isolated Organism.

In the cultural and morphological studies of the snake bean organism, it was observed that, after repeated sub-culturing on agar slopes, the cultures had altered somewhat in appearance. Dilution plates of both beef-extract peptone agar and potato dextrose agar were prepared from these cultures. On these plates, the majority of the colonies had a rough-contoured surface (Plate V, B) raised above the surrounding medium. A few of the colonies, however, were of the original smooth type, smaller in size, with a non-contoured surface, only very slightly raised above the surrounding medium (Plate V, C). Dilution plates prepared from the "rough" colonies

showed all rough colonies, whilst dilution plates prepared from the " smooth " colonies showed all smooth colonies.

At the same time, dilution plates made from three relatively old beef-extract peptone agar slope cultures each showed all smooth colonies. These were sub-cultures of the re-isolated organism five and twelve weeks old respectively, and one from the original isolation which was three weeks old. This suggested that the " rough " organism had made its appearance during the almost daily sub-culturing of the preceding three to five weeks. It seemed that the " rough " type must be either a " rough " mutant of the snake bean pathogen or a contaminant introduced in the sub-culturing operations.

The occurrence of " rough " mutants of bacterial organisms has been recognised for a considerable time. Link[6] has reviewed some of the most important contributions relating to the occurrence of this phenomenon in the case of several bacterial plant pathogens. It is of interest to note that Bryan[1] has recorded a " rough " strain of the lilac blight organism (*B. syringæ*).

Single cell isolations of both the " smooth " and " rough " organisms were made, using the method recommended by Ørskov.[8] Cultures derived from these single cells were used for stem inoculations of snake bean seedlings. The " smooth " strain was more virulent than the " rough ", although the latter was definitely pathogenic and caused wilting and death of the inoculated plants. Dilution plates prepared from the stems of infected seedlings one to two inches above the point of inoculation showed either all rough colonies or all smooth colonies, depending on the nature of the initial culture used for inoculating the plant. Cultures derived from rough and smooth colonies in these plates and the cultures derived from single cells were studied morphologically, culturally, and in their reaction to various plants.

The four cultures agreed entirely in the following characteristics: size, staining reactions (including the Gram stain), action on milk, litmus milk, gelatin, and starch, ammonia production in beef-extract peptone broth, indol production, nitrate reduction, and in the fermentation of 19 sugars, alcohols, and acids.

The " rough " and " smooth " strains differed in motility, the appearance of the growth on agar and in beef-extract peptone broth, and in pathogenicity to various hosts.

The " rough " strain showed only slight motility compared with the active motility of the " smooth " strain. In contrast with the one or two flagella of the " smooth " strain, no flagella were observed in the case of the " rough " strain, although this may possibly have been due to the technique rather than to entire absence of flagella.

The character of " roughness " was much more marked on potato dextrose agar than on beef-extract peptone agar. In beef-extract peptone broth and in most liquid media in which the " smooth " and " rough " strains were grown, a much denser precipitate was produced by the " rough " strain.

The " rough " strain was pathogenic when used for inoculating stems of those leguminous plants listed in Table II to which the " smooth " strain was pathogenic. The virulence was, however, perceptibly less.

After repeated sub-culturing of the " rough " strain derived from a single cell, it was found that the culture lost its homogeneous " rough " nature and became a mixture of " smooth " and " rough " types. No evidence was obtained concerning the factors which influenced the changes from the " rough " to the " smooth " form or *vice versa*.

Transmission, Economic Importance, and Control.

The crop of snake beans in which the disease occurred was grown on land which had not, as far as could be ascertained, grown any snake beans or other species of *Vigna*, or, indeed, any cultivated leguminous crop within the preceding four or five years. Consequently there was good reason for believing that the disease had been introduced with the seed. The seed used had been obtained from a Sydney firm of seed merchants, who stated that practically all their snake bean seed was imported from the United States. Seed harvested from the diseased crop was planted in previously sterilised pots of soil in the glasshouse. On some of the resulting plants, leaf lesions, similar to those observed on plants of the preceding generation, were evident. From these lesions an organism was isolated which proved to be identical with the original isolation. It is considered that this is fairly conclusive evidence that the disease can be seed-borne. It is probable that this is the main method of transmission from one season to the next.

In the crop in which the disease was observed, the leaf damage would probably result in an appreciable reduction in yield. Snake beans are grown only as a garden crop by a relatively small number of growers in this country, but the wide host range of the causal organism suggests that it may become an important disease of other cultivated crops.

Measures suggested by Gardner and Kendrick[5] for the control of the bacterial spot disease of cow-pea and by Tisdale and Williamson[9] for the control of the disease caused by the same organism on lima beans, include the use of disease-free seed as the most important means of combating the disease. Clayton[4] found that the spread of the bacterial spot disease of lima bean was materially reduced by spraying the plants with Bordeaux mixture and other fungicides. However, in the absence of knowledge of resistant types, the use of disease-free seed is suggested as the most effective method of control.

Summary.

A bacterial disease was recorded on snake beans (*Vigna sesquipedalis*) in New South Wales in 1935.

Comparative studies of the causal organism and cultures of the cow-pea spot, the lilac blight, and citrus pit organisms were carried out. Culturally and morphologically the snake bean organism and the other organisms were identical, except in the fermentation of raffinose. Slight differences were recorded in the degree of pathogenicity to various plants. None of the differences were considered sufficient to warrant placing the various organisms in separate species. The locally isolated organism is therefore designated *Bacterium syringæ* (Van Hall) E. F. Smith.

A " rough " strain of the snake bean pathogen arose as a mutant and comparisons were made with the normal " smooth " form.

The disease was shown to be seed-borne.

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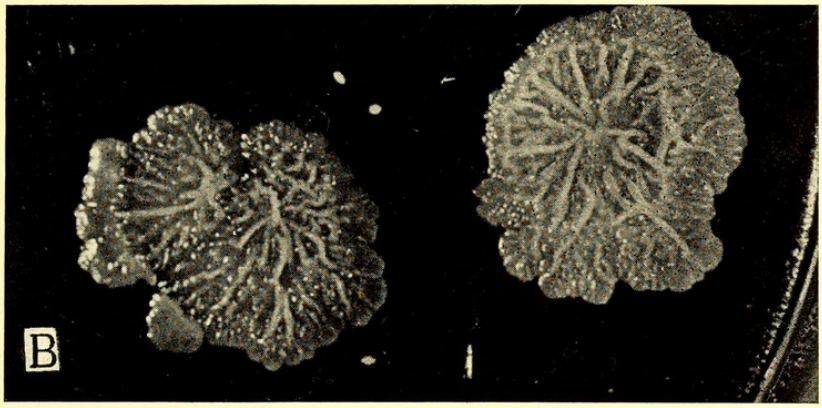
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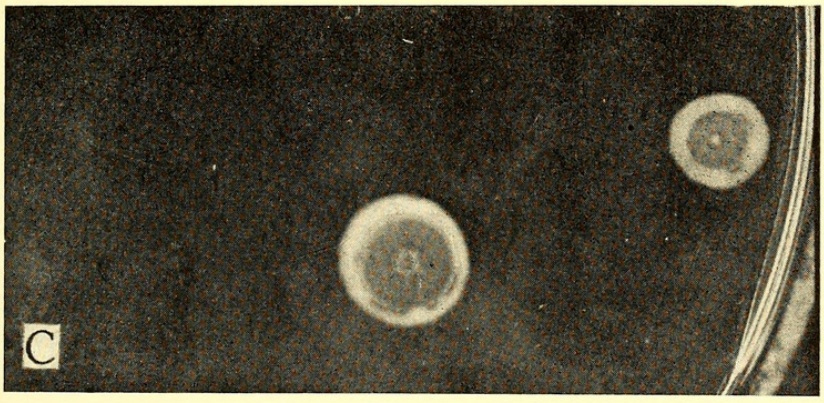
C

Charlotte Elliott, United States Department of Agriculture, for the provision of cultures.

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Explanation of Plate.

A. Natural infection of snake bean leaf. Plants grown in the glasshouse from seed harvested from the originally observed crop.

B. Two weeks old colonies of the "rough" strain of the snake bean pathogen on potato dextrose agar. ($\times 1\frac{1}{2}$.) (Photograph by P. R. Maguire.)

C. Two weeks old colonies of the "smooth" strain of the snake bean pathogen on potato dextrose agar. ($\times 1\frac{1}{2}$.) (Photograph by P. R. Maguire.)