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POD TWIST: A PREVIOUSLY UNRECORDED BACTERIAL DISEASE OF FRENCH BEAN (*PHASEOLUS VULGARIS* L.)

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SUMMARY.

A disease which causes blighting and twisting of the pods of French bean (Phaseolus vulgaris) **is described for the first time.**

Repeated isolations from infected tissues and pure culture inoculation show that the causal organism is a member of the Schizomycetes.

In the field, transmission is effected by the bean thrips (Taeniothrips nigricornis **Schmutz) and by no other apparent means. Glasshouse experiments in which these thrips were shown to be capable of carrying infection to healthy plants are described.**

A pure culture study of the causal organism shows it to be a new species of the genus Pseudomonas. **The organism is described in detail.**

I. INTRODUCTION.

Until 1934 only two bacterial diseases of *Phaseolus vulgaris* L. were recognised in Australia. These were halo blight, caused by *Pseudomonas phaseolicola* (Burkholder) Dowson, and common bacterial blight, caused by *Xanthomonas phaseoli* (Smith) Dowson. In that year, Adam and Pugsley reported the occurrence of a bacterial wilt caused by *Corynebacterium flaccumfaciens* (Hedges) Dowson on French beans in Victoria; and in 1938 Wilson recorded *Pseudomonas syringae* Van Hall as the cause of brown spotting of leaves and pods in New South Wales. With the exception of bacterial wilt, these diseases have been known in Queensland for some years. Other bacterial organisms which have been reported by overseas workers to be pathogenic on beans, but which have not been recorded in Australia, are *Pseudomonas solanacearum* E. F. Smith, *Pseudomonas viridiflava* (Burkholder) Dowson, and *Erwinia lathyri* (Manns and Taubenhaus) Holland.

During the autumn of 1951 it was found that all bean crops being grown at Kingaroy for seed purposes were infected with a bacterial disease, the symptoms of which were apparently different from those of any of the diseases

mentioned above. Its occurrence caused concern because there was not sufficient evidence to establish beyond doubt that it was distinct from common bacterial blight and halo blight, which are both serious problems associated with the production of bean seed. The main difference appeared to be that infection was confined to the young green pods, the older pods and leaves being free from the type of spotting which is so characteristic of these two better-known disorders. Subsequent examination of the causal organism in pure culture in a series of laboratory and glasshouse tests has confirmed its separate identity.

Observations over a number of years have shown that this organism is a widespread and frequently occurring plant pathogen in Queensland. Since 1951 the disease has been observed in all coastal districts between Cairns and Brisbane where beans are grown commercially to any extent, and specimens have been received from home gardens in the Brisbane area, where it is apparently very common at certain times of the year.

There is no suggestion that it is of only recent occurrence. On several occasions the opinion of experienced growers has been sought in an effort to determine the length of time it has been present, and similar information was sought from officers of the Horticulture Branch of the Queensland Department of Agriculture and Stock who have been associated with the crop for many years. In several cases evidence of its presence here for at least 10 years has been obtained. Though the symptoms are distinctive enough to enable easy recognition of its presence in a crop, the absence of adequate evidence to establish its separate identity has no doubt resulted in its acceptance as a form of expression of one of the already recorded bacterial diseases.

In south-eastern Queensland, outbreaks are almost entirely confined to the warm months of January, February and March, but in central and northern coastal areas of the State it often occurs during the winter months. The crops concerned at these times are not of great commercial importance, the greatest part of the annual production coming from plantings in southern districts which mature in cooler weather. Because the disease does not coincide with the main cropping period, it is of only minor economic importance. However, the main cropping times are concerned with green bean production and are determined by consumer demand in the southern States. They do not represent the only times when beans can be grown satisfactorily in these areas. Should the acreage of warm-weather plantings be increased in the future, there is every chance that the disease would prove to be quite serious.

The manner in which the disease affects the plant is such that it is difficult to estimate accurately the losses involved. The destruction of immature pods in the immediate post-flowering stage is the main form of damage. These blighted pods wither and drop to the ground, or hang inconspicuously in the flowering axils of the branches, where they are found only with difficulty. Badly affected plantings may carry only a very light crop of pods which, at the time of harvesting, show little evidence of the true extent of the damage

which has occurred. Some of these pods would be bent and twisted in a manner which is most distinctive and from which the name "pod twist" is derived. These would also represent a loss if the crop were harvested at the green-bean stage.

Whether or not this disease is transmitted from one crop to another through the seed has not been determined. However, because it is unlikely to become serious in the main winter plantings, its presence in a seed crop is not regarded as a matter for concern from the point of view of seed certification.

II. DESCRIPTION OF THE DISEASE.

In the field, infection has been found to occur only on the pods, where rapidly developing watersoaked areas appear. Young pods less than $1\frac{1}{2}$ in. in length, from which the blossom has just fallen, are most likely to show infection. Many of them wither and drop off. Others continue to enlarge, even

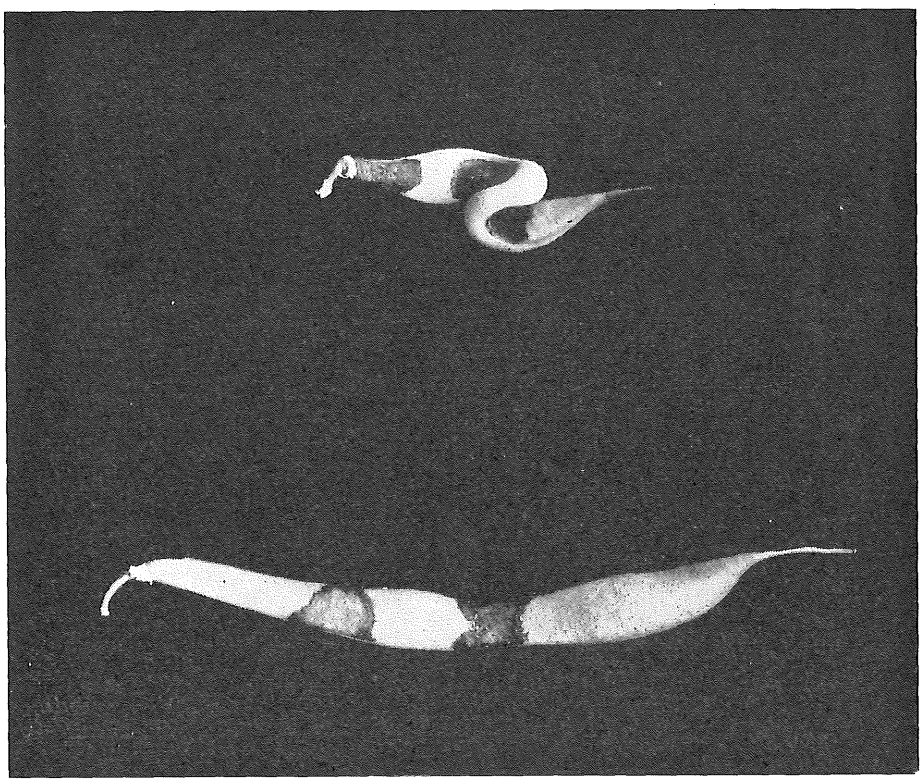

Fig. 1

Pod Twist of Bean. Symptoms produced as a result of artificially inoculating growing pods by needle prick with a pure culture of the causal organism. The plants were grown in the glasshouse.

though a considerable part of their surface may appear to be diseased, and it is then that the most striking symptoms occur. Subsequent development of invaded areas is retarded, and as a result the pod becomes bent and curled in a very characteristic manner. This is illustrated in Figs. 1 and 2. In Fig. 1

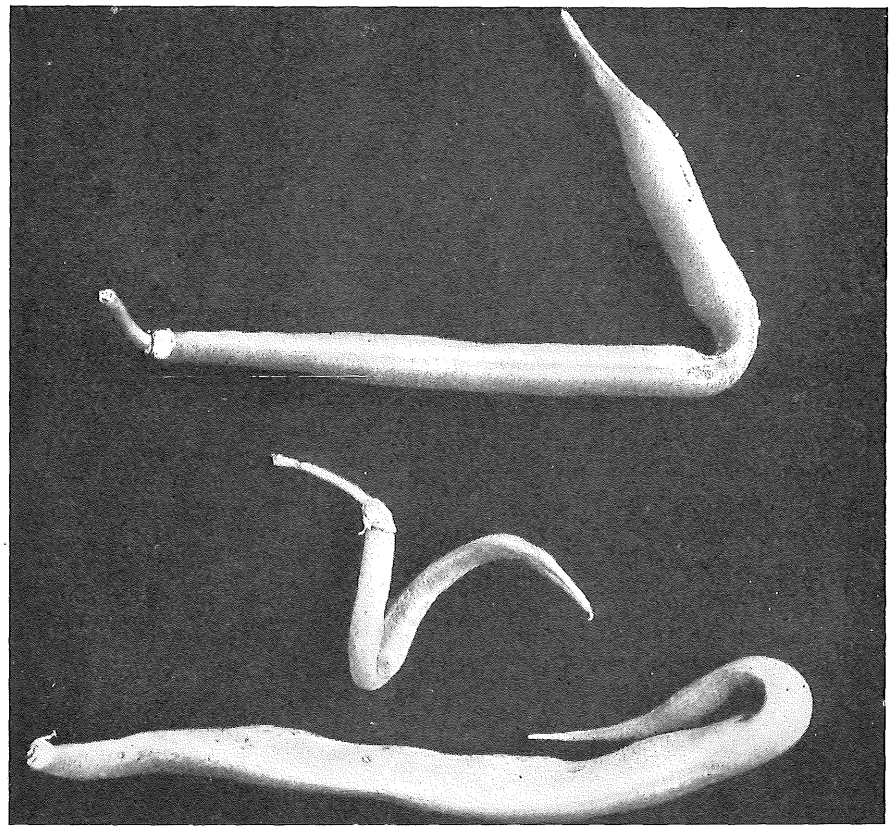

Fig. 2.

Pod Twist of Bean. Natural infection showing twisting about the infected area.

the pod at the top of the photograph was inoculated when about one-third full size and has bent through an angle of 360 degrees. Bending about the infected area is clearly visible. The pod below was inoculated at a slightly more mature stage and did not bend. Fig. 2 illustrates several other types of bending which are commonly encountered. These pods were taken from a field planting.

Infection is usually confined to one side of the pod, where it extends lengthwise, and the invasion of the dorsal suture, which is so common in several other types of bacterial infection, is not a feature of this disease. Shortly after invasion of the pod tissue has become apparent with the enlargement of

the watersoaked area, droplets of translucent white exudate may appear (Fig. 4); later these dry to leave a shiny encrustation over the surface. After a short period of time within the host, the bacteria cease to be active and the invaded area, which does not become necrotic, takes on a more healthy though somewhat darker green appearance.

The disease is most easily found in a crop shortly after a second flush of flowering has occurred and the plants are carrying a small crop of late-set pods. It is the pods formed at this time that are most subject to attack, and infections of this nature occur regularly in seed crops. Serious infection of first-set pods, when it does occur, is most often noticed where succeeding crops are established on adjoining areas, a practice which is often adopted by growers of green beans. Then a large percentage of even the first pick may be so distorted as to be unsaleable. The fact that infection in commercial crops is confined to the pods means that its introduction to a crop takes place after flowering commences. This is consistent with transmission by a flower-frequenting insect.

III. ETIOLOGY.

The causal organism was isolated in the following manner. Small pieces of tissue from young infected pods were macerated with a little distilled water, and from this a series of 10 dilutions (1 in 10) was made. One millilitre of each dilution was mixed with 9 ml. of molten potato dextrose agar (at 45 deg. C.) in petri dishes, which after cooling were placed in an incubator at 27·5 deg. C. After four days many small, slow-growing colonies appeared on the plates, and from these 6–8 were selected and transferred to tubes of beef extract broth containing 1 per cent. peptone, where they were allowed to grow for two or three days before being used for further testing.

The bacteria were present in large numbers at the stage when the exuding droplets formed over the surface of the lesion, and good results were obtained by isolating at this time. However, when isolation from older lesions was attempted, a great predominance of fast-growing cream and yellow colonies of saprophytic bacteria appeared on the dilution plates (Plate 14). Occasionally it was possible to detect the smaller slow-growing colonies of the pathogen on the same plates from three to five days later, but at other times the pathogen appeared to have been completely replaced. In order to determine whether or not it was wholly inactivated, pod inoculations were carried out with macerated tissue suspensions from both young and old infected tissue; it was found that typical symptoms were obtained only if fresh material was used. This is illustrated in Fig. 3.

The isolates were tested for pathogenicity on green bean pods held in large petri dishes on the laboratory bench. Three or four long straight pods were selected and thoroughly washed under the tap. When they had dried on the surface one drop of a broth culture was placed on each and pricked into the tissue with a sterile needle. In this way each pod was inoculated with all

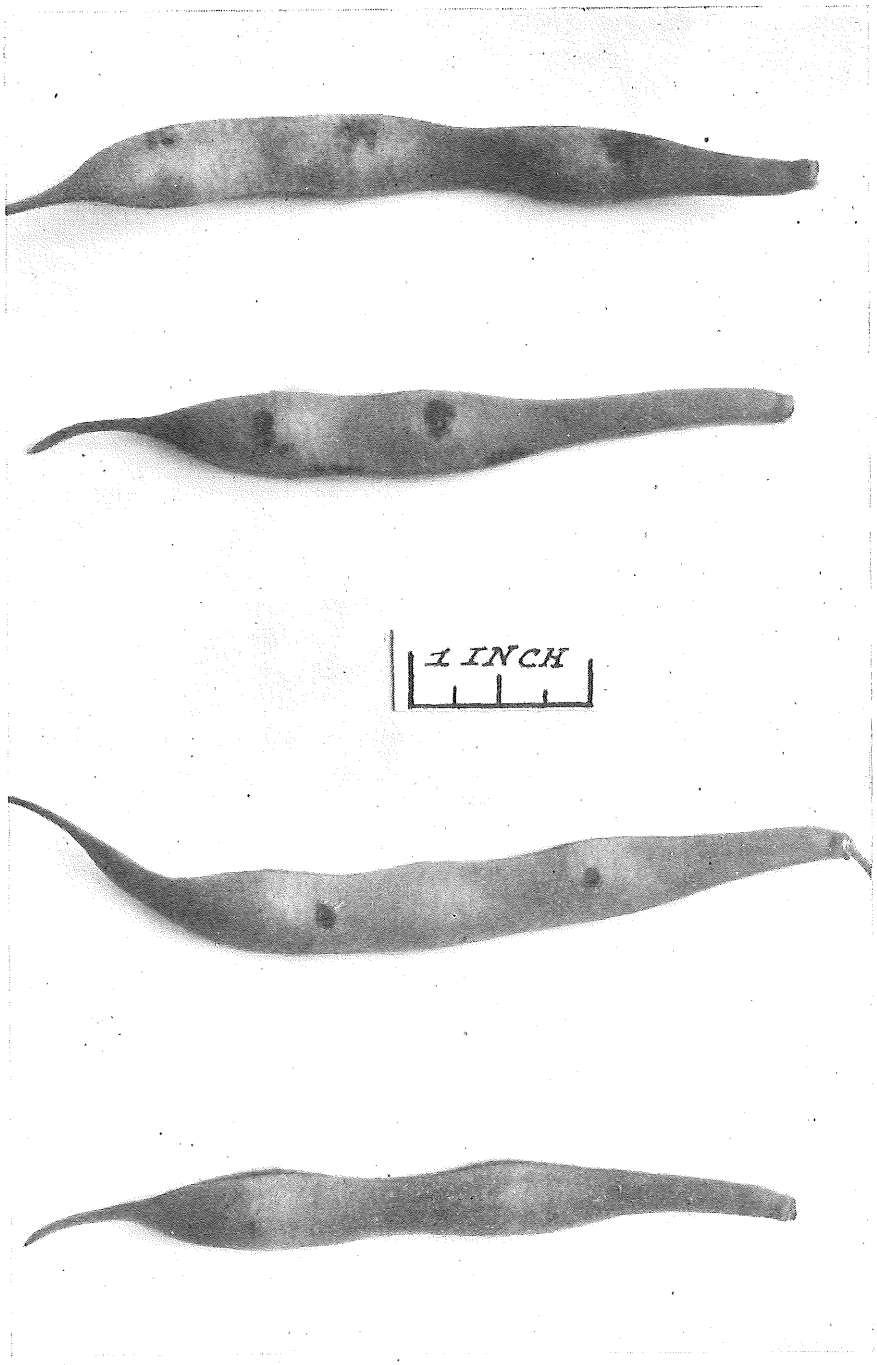

Fig. 3.

Pod Twist of Bean. *Above*: Pods inoculated with a suspension of macerated tissue from young pod lesions. The invasion which is obvious here became much more pronounced in the 24 hours following the taking of this photograph. *Below*: Very restricted invasion, which did not subsequently expand, from inoculation with a suspension prepared from an older lesion (similar to that illustrated in Fig. 1).

of the isolates being tested. Those which were pathogenic showed rapid development, as illustrated in Fig. 4. This method was used as a simple form of replication to guard against the possibility that some pods would prove

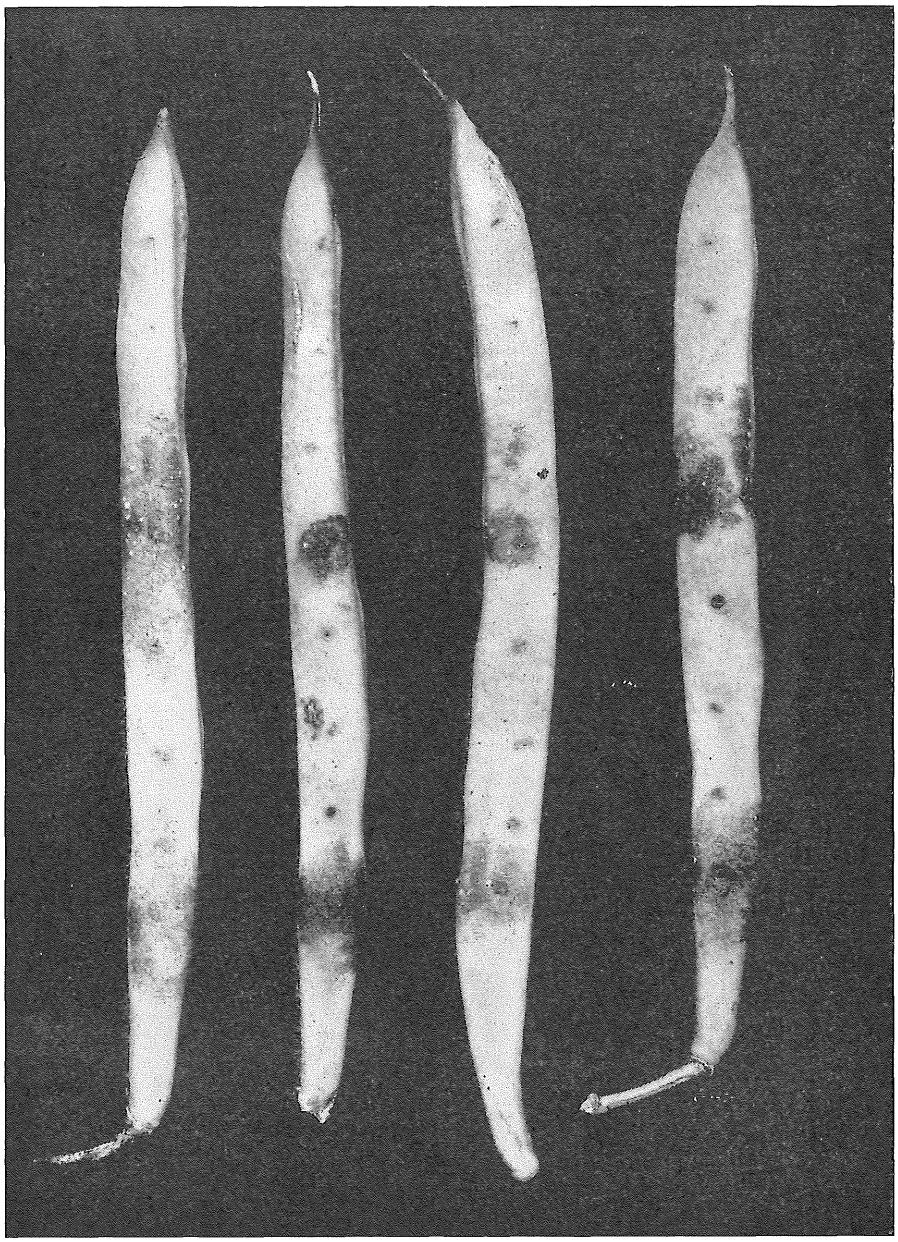

Fig. 4.

Pod Twist of Bean. Illustrating the method used for testing pathogenicity of isolates. Inoculations 4th and 8th from top are positive. The droplets of exudate which appear on the surface of the infected area are visible.

resistant to infection, and to produce a sufficient number of inoculations from which conclusions could be drawn. Attempts to infect both young and older pods simply by smearing a suspension of the organism over their surface were unsuccessful.

In the four years (1951-1955) during which this disease has been under observation, it has been recorded on at least 45 occasions from widespread localities within the State, and casual notice of its presence has been taken at many other times. Isolation was attempted on 17 of these occasions, and pathogenicity of the pure culture isolate was confirmed in 14 cases. Some isolates appeared to be more virulent in the host than others, but all were capable of causing extensive watersoaked lesions when freshly isolated. Isolate 10836 was only weakly pathogenic after six months in culture, whereas 10869 retained its original highly virulent nature after 12 months under such conditions.

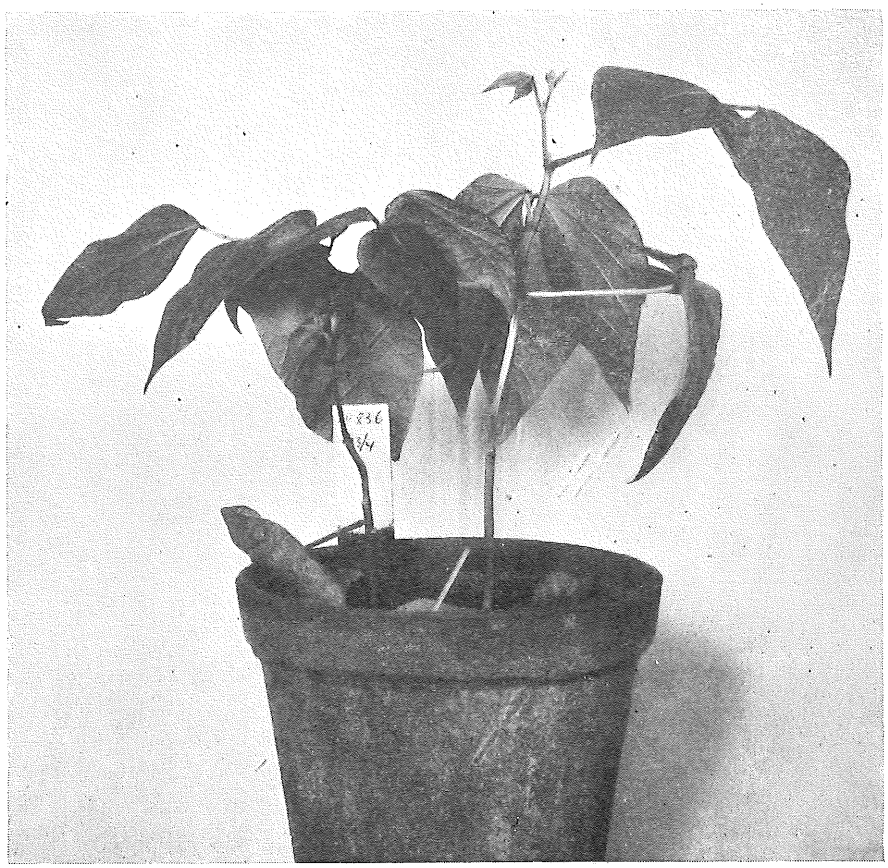

Fig. 5.

Pod Twist of Bean. Plant at left showing a 10-day-old stem infection from a needle prick inoculation at point of bending.

Several attempts were made to infect other parts of the bean plant with the organism isolated. Suspensions similar to those used for inoculating pods were smeared onto both leaf surfaces and onto stems and pricked into leaves, leaf-stalks and stems, and in only one case were these attempts successful. This occurred when a freshly isolated culture was introduced to the region of the stem between the cotyledonary and primary leaf nodes by severely wounding the tissue beforehand with a sterile needle. Subsequently, a necrotic area appeared at the point of inoculation and the stem bent slightly about this section (Fig. 5). The necrosis slowly advanced up one side of the stem, growth became retarded and eventually the growing tip was killed. However, by this time the plant had made considerable lateral regrowth and flowering and fruiting occurred normally on the new growth. Four plants were inoculated in this test and in each case symptoms were similar to those illustrated in Fig. 5. In all tests the plants were placed in a moist chamber for 48 hours after they had been inoculated.

Symptoms similar to those induced by needle prick inoculation into the stem have never been seen in the field by the writer, though many acres of beans infected with the disease have been inspected in the course of examination of seed crops being grown for certification. Neither has any type of leaf spot infection which might be associated with this disease been noticed. From these observations and from the glasshouse tests, it is concluded that the trouble is confined under ordinary circumstances to the green pods, and these are attacked only when some form of pod injury occurs.

IV. HOST RANGE.

A number of species of plants were artificially inoculated with a pure culture of the causal organism.

In attempts to infect these species, the culture used was simultaneously inoculated into fresh bean pods, and failure to infect such species was recorded only in cases where satisfactory infection on bean occurred. Control inoculations with sterile broth or other suspension media were also made. Freshly inoculated plants and fruits were confined for approximately 48 hours in moist chambers to prevent drying out at the point of inoculation, except in some cases when the inoculum was well pricked into the tissues and the need for such treatment was not great.

Lupin.—Maturing green pods were infected by pricking a drop of broth culture into them in the same manner as detached bean pods were inoculated. The inside wall of the pod and the enlarging seeds became extensively watersoaked. No symptoms appeared on stems and leaves.

Phasey Bean (*Phaseolus lathyroides*).—The growing pods of this species develop watersoaked lesions and twisting in the same manner as does *P. vulgaris* when subjected to needle-prick infection.

Apple (*Williams' Favourite and Delicious*).—A drop of broth culture was pricked into the ripe fruit. No symptoms developed.

Cowpea.—A drop of broth culture was pricked into the upper part of the stem of seedling plants and into young pods, with negative results.

Cucumber.—No infection occurred on seedlings inoculated as in the case of cowpea.

Lemon.—Same as for apple.

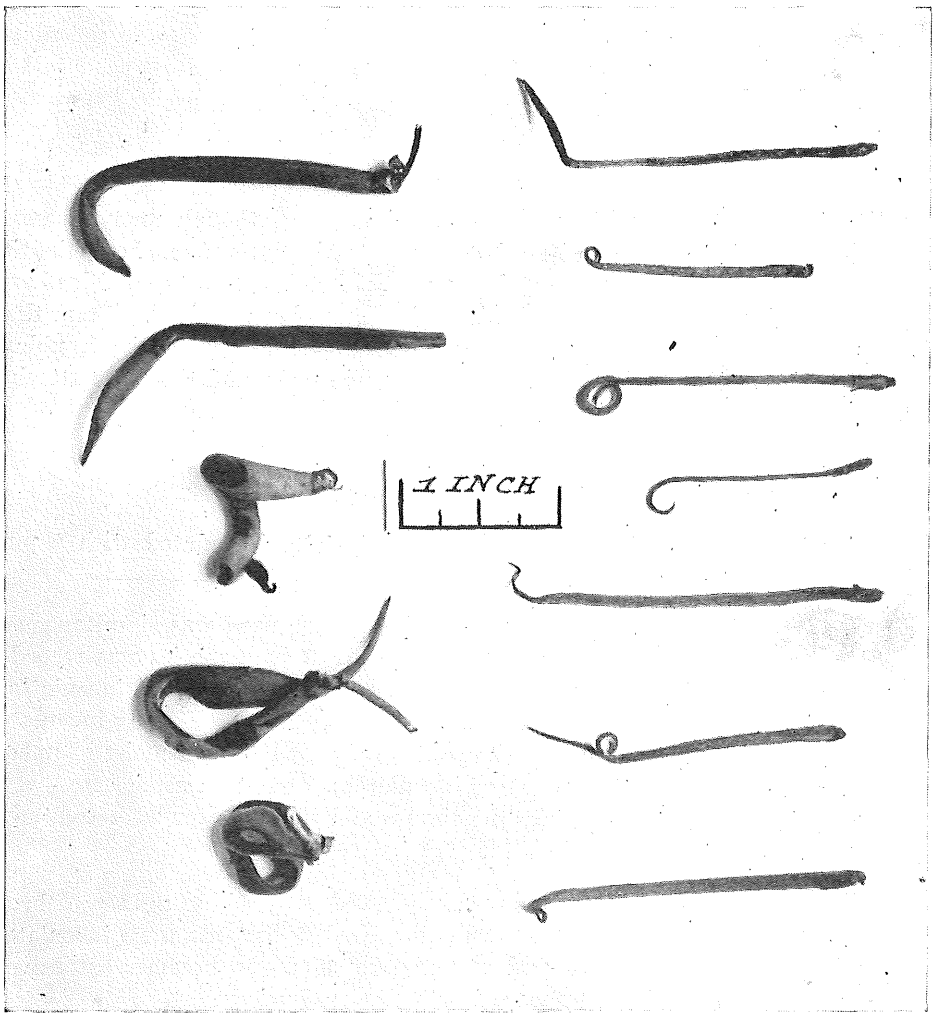

Fig. 6.

Pod Twist of Bean. *Right*: Symptoms exhibited by *Phaseolus lathyroides* pods from which the pod twist organism was isolated. *Left*: Diseased pods taken from a French bean planting in the vicinity of the plants from which the pods on the right were collected.

Soybean.—Broth culture was pricked into the upper stem of seedlings and rubbed onto both sides of the leaves with the fingers. No infection resulted. The varieties used were Nanda and Clemson Non Shatter; 24 plants of each were inoculated.

Sunflower.—No infection developed on inoculated seedlings.

Tomato (var. Simi—this variety is susceptible to Ps. solanacearum).—No infection occurred on inoculated seedlings.

Phaseolus lathyroides L. as an Alternative Host.

This plant has become well established over a wide area of the State since its introduction some 70 years ago, and because of its close relationship to *P. vulgaris* was considered to be a likely alternative host of the pod twist organism.

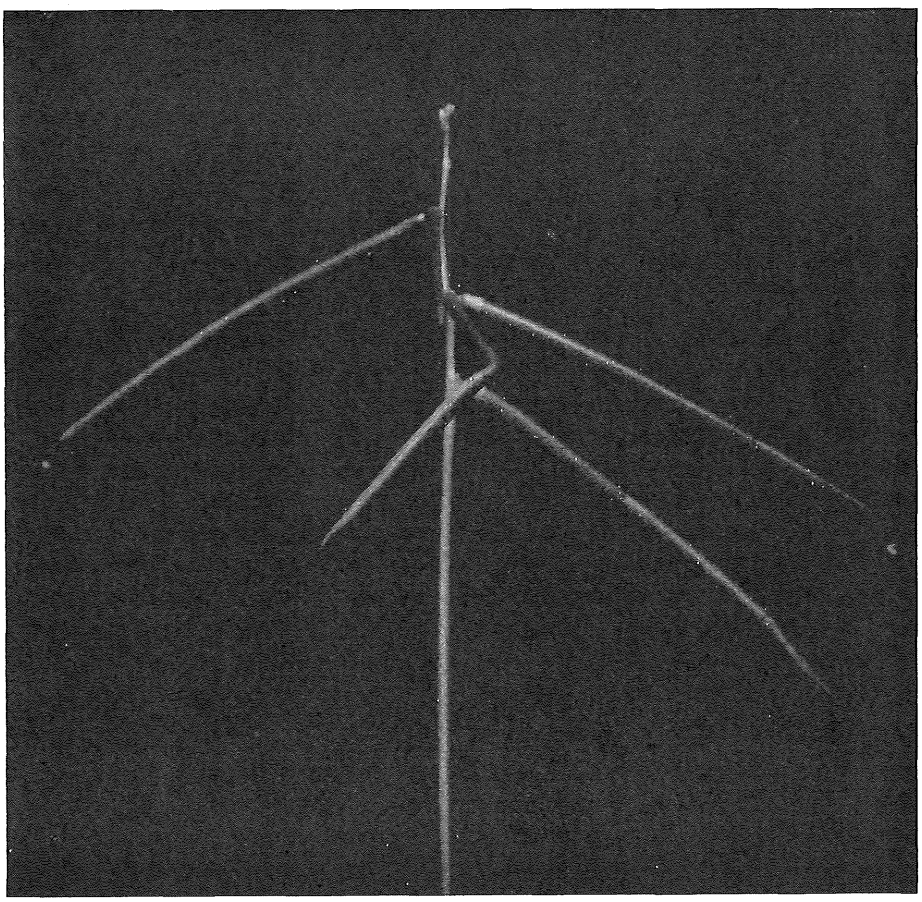

Fig. 7.

Pod Twist of Bean. Symptoms produced on a pod of *Phaseolus lathyroides* following inoculation with a pure culture of the pod twist organism from French bean.

During the autumn of 1954 *P. lathyroides* was found growing in abundance on an area of land beside a crop of beans infected with pod twist, and an examination of the young pods of these plants showed that they too were exhibiting symptoms suggestive of infection with this organism. Pods of both species were collected and photographed for comparison (Fig. 6). There was strong circumstantial evidence in this instance that transmission of the disease from phasey bean had taken place.

Although pod twisting can be found with little difficulty on infected phasey bean plants, most of the damage takes the form of complete invasion of immature pods, which wither and fall to the ground before attaining more than half their normal full size.

Isolation from pods of *P. lathyroides* on this occasion produced typical cultures of the pod twist organism, and pathogenicity of the pure cultures to both *P. lathyroides* and *P. vulgaris* was established in glasshouse inoculation experiments. Isolates from *P. vulgaris* were also pathogenic to *P. lathyroides* (Fig. 7).

In subsequent years there has been further abundant evidence of the pod twist disease occurring naturally on *P. lathyroides* in widespread localities.

V. DISSEMINATION.

One of the most notable features of this disease is the rapidity with which infection may spread through a planting in the absence of factors which are responsible for the dissemination of other bacterial diseases of bean.

A close examination of the progress of the disease was made in a planting of three acres at Redcliffe. This crop received heavy rainfall over the period of planting, which was carried out in three stages at fortnightly intervals. After that time no more rain fell and conditions became sufficiently dry to retard growth as the flowering period was reached. The crop was inspected when the first planting was maturing pods and no disease was detected. On a second inspection carried out one week later a single infected immature pod was observed in the latest planting. In the time which elapsed between then and when the crop was finally examined in the following week, practically every plant which was bearing immature pods had become affected. In some cases, the small pods had withered and fallen, so many plants in the youngest section of the crop bore no pods at all.

Since infection had been found from this and previous observations to be confined to the pods, it must have been introduced to the area at a time when pods were being produced, and not prior to flowering. This pointed to the existence of a very efficient mechanism for dissemination, a mechanism different from that operating in respect of other bacterial pathogens of bean. When lesions first appeared the size of the young infected pods indicated that infection must have occurred within the flower, for the incubation period of the disease is not less than the time taken for the pods to attain the size they did.

Indications of Thrips Transmission.

Many pods in the crop just described were damaged by thrips and close examination showed these insects to be present in the flowers in large numbers. A quantity of the infected flowers was collected and taken to the glasshouse, where the thrips were liberated on flowering bean plants. The 10 plants which were used were covered with insect-proof bags for three days after the thrips had been introduced. Seven days later an examination of the pods which had been formed during the time of feeding was made and four were found to show symptoms of the disease. The pathogen was isolated from these and subjected to a number of laboratory tests, which confirmed its identity with other isolates.

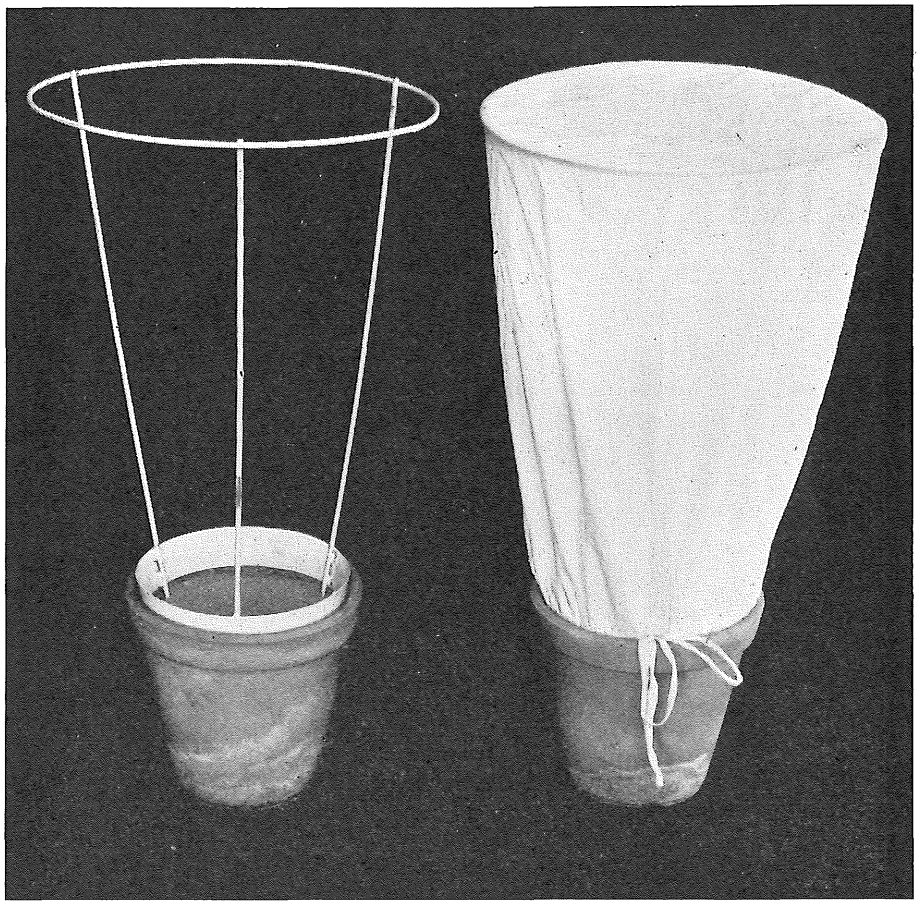

Fig. 8.

An Insect-proof Cage for Confining Thrips to Bean Plants. The frames, which were made to fit 8-in. pots, were supported by legs penetrating 4 in. into the soil. After being placed in position (at left) sand was added to both the inside and the outside of the flat iron collar until a satisfactory seal was obtained. The covering material was a good quality lawn.

Other evidence pointed towards thrips being the vectors of the disease. Firstly, these insects were present in every crop in which the trouble has been detected. Moreover, thrips and pod twist were both found to occur during the summer and early autumn months, and peak infection periods corresponded very closely to the times when the thrips population was very high. Secondly, their feeding habits are such that they are present in the crop only during the flowering period, which is in most cases very brief, so they must possess the high mobility required of a vector capable of transmitting the disease in the rapid manner described. These insects feed almost exclusively in the flowers.

Confirmation of Thrips Transmission.

It was difficult to obtain for transmission experiments colonies of disease-free or non-infective thrips which might have enabled greater development of this phase of the work. Even though thrips occurred in abundance throughout the warm months of the year, they were nearly always

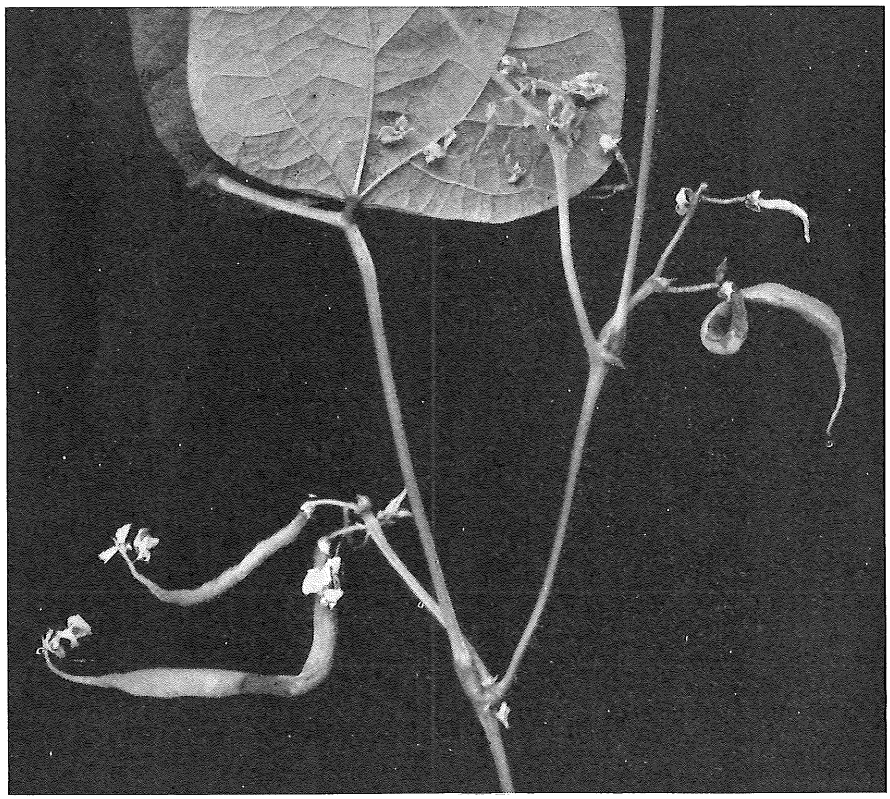

Fig. 9.

Pod Twist of Bean. Symptoms produced by confining infective thrips to bean plants during the flowering period. This particular stem bore six diseased immature pods, two of which had begun to enlarge and take on the typical twisted appearance. The uppermost truss was completely blighted.

associated with diseased plants and facilities were not available for breeding these insects under controlled conditions. However, several more transmission experiments were carried out with field collections of thrips and these are briefly discussed here.

The procedure adopted was essentially the same in each case. Flowers were gathered from plants on which thrips occurred, the amount of pod twist present varying from nil to abundant in the different experiments. The thrips were collected in glass tubes in the laboratory and later liberated on covered flowering plants in the glasshouse. For this purpose cages of the type illustrated in Fig. 8 were used. After 8–13 days the covers were removed and results assessed. These are given in Table 1. In all but one of these experiments the causal organism was isolated from the diseased pods and its pathogenicity confirmed.

The symptoms produced on glasshouse plants by infective and non-infective thrips are illustrated in Figs. 9 and 10. These symptoms are the same as those encountered in the field.

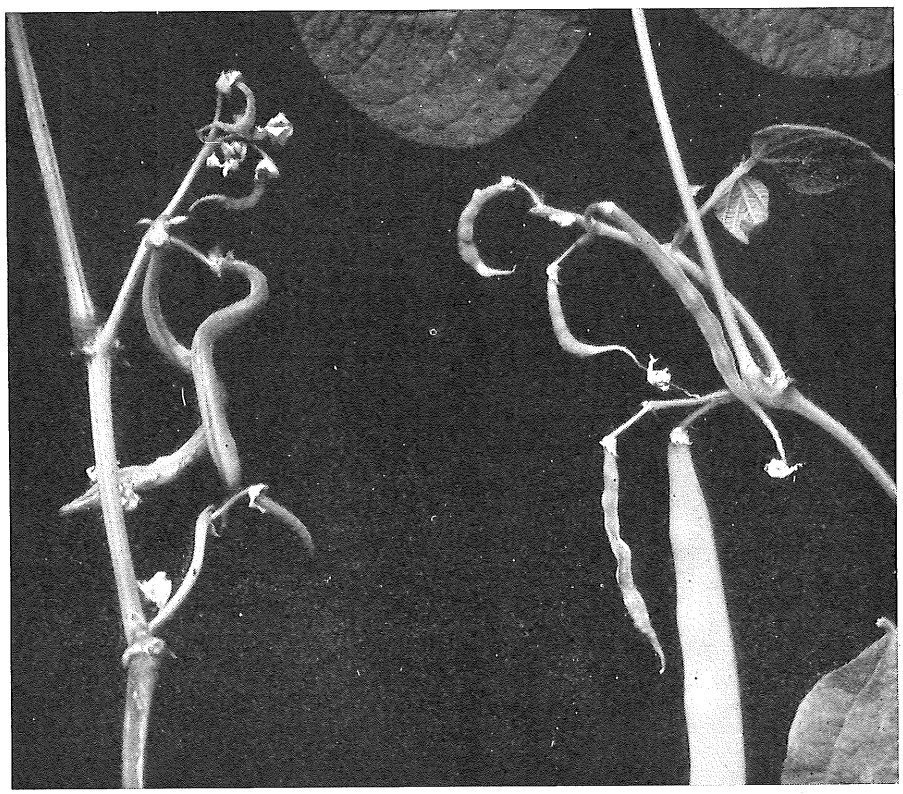

Fig. 10.

Thrips Injury. Symptoms produced by confining non-infective thrips to bean plants during the flowering period. The lumpiness and distortion of the young pods shown is typical of thrips damage as it occurs in the field in the absence of the pod twist disease.

The manner in which the pathogen is carried and introduced to the tissues of the host plant will form the subject of future work.

Table 1.

CONDITION OF PODS AS A RESULT OF ENCLOSING FIELD COLLECTIONS OF THRIPS ON FRENCH BEAN PLANTS.

Source and Treatment of Thrips.	Number of Pots.	Number of Thrips Per Pot.	Healthy and Undamaged.	Thrips-Damaged, not Diseased.	Thrips-Damaged, Diseased.
(1.) Kingaroy—					
French bean moderately diseased	5	40	..	..	6
(2.) Brisbane—					
French bean badly diseased	6	50	13	58	27
(3.) Brisbane—					
French bean very lightly diseased ..	6	30	23	63	5
(4.) Brisbane—					
Phasey bean badly diseased—					
(*a*) Thrips exposed to slope culture of pod twist *Pseudomonas* for 4 hours	6	20	54	29	7
(*b*) Thrips exposed to sterile slope ..	6	20	42	27	9
(5.) Brisbane—					
Phasey bean apparently disease-free—					
(*a*) Thrips fed on diseased French bean pods in tubes for 4 hours ..	5	20	16	19	5
(*b*) Straight from phasey bean flowers ..	1	20	3	4	0

Buchanan (1932) demonstrated in controlled feeding experiments in the greenhouse that *Heliothrips femoralis* Reut. was able to transmit a bacterial blight of bean. This was probably the first occasion on which thrips were shown to act as vectors of a bacterial plant pathogen, but their part in spreading the disease under field conditions was considered by Leach (1940) to be of little importance. On the other hand, in the case of pod twist, the disease appears to be wholly dependent upon the thrips for its transmission.

That thrips should be able to act as a means of spreading bacterial diseases is not surprising, as their migratory habits and method of feeding, wherein the surface cells of the host are punctured by a rasping action of the mouthparts, seem well suited to the purpose. It is therefore strange that there appears to be no recorded instance, other than the one just mentioned, of these insects being associated with the spread of a bacterial disease.

Identification of Bean Thrips.

When thrips were being collected for transmission studies, the insect population of the bean flowers was sampled on a number of occasions so that specimens could be submitted for identification. These were mounted and

identified by Mr. W. A. Smith (Entomologist, Queensland Department of Agriculture and Stock) and then forwarded to the Commonwealth Institute of Entomology, London, where Dr. G. D. Morison confirmed the identifications. These, together with the host species, locality and time of collection, are presented in Table 2. A number of permanent slides used in the identification have been placed in the reference collection of the Entomology Section of this Department.

Taeniothrips nigricornis Schmutz was the only species found to be commonly present and was the one used in the transmission tests. (This species in the adult stage is a large thrips, black and very active, which can be readily distinguished from other species occurring in the same crop in comparatively small numbers.)

Observations of the thrips population in bean flowers at many times of the year and in various parts of the State indicate that a close association between *T. nigricornis* and *Phaseolus vulgaris* exists (Officers of the Department of Agriculture and Stock 1951).

Table 2.

IDENTIFICATION OF THE THRIPS SPECIES INHABITING THE FLOWERS OF TWO SPECIES OF PHASEOLUS.

Host.	Date of Collection.	Locality.	Species Present.	Number.
P. lathyroides (phasey bean)	2–4–54	Kingaroy ..	*Taeniothrips nigricornis* Schmutz	27
			Thrips tabaci Lind.	1
P. vulgaris	7–4–54	Brisbane ..	*Taeniothrips nigricornis* ..	33
			Frankliniella sp.	1
P. vulgaris	9–4–54	Thornlands ..	*Taeniothrips nigricornis* ..	24
			Haplothrips gowdeyi Franklin	1
			Pseudanaphothrips achaetus Bagn.	1
			Thrips tabaci	1
P. vulgaris	6–5–54	Rochedale ..	*Taeniothrips nigricornis* ..	44
			Thrips tabaci	1

Phaseolus lathyroides as a Source of Primary Infection.

The existence of *P. lathyroides* as an alternative host has already been mentioned.

In the earlier stages of this investigation, when thrips were first suspected as being vectors of the trouble, the flowers of many summer legumes were examined and it was found that *Taeniothrips nigricornis* occurred in abundance in the flowers of this species (Table 2). Furthermore, in the case where

transmission of the disease from phasey bean to nearby bean plantings was suspected, it was noticed that the flowers of the two species were infested to the same extent.

During the autumn of 1956 a close examination of phasey bean plants growing profusely along roadsides and on vacant land in and around Brisbane revealed that pod twist infection of this species was general, and any collection of thrips taken from these plants could be assumed to contain infective individuals. Thrips from infected *P. lathyroides* have been shown in glasshouse experiments to be capable of carrying the disease to French bean (Table 1).

Phaseolus lathyroides flowers for a much longer period than does *P. vulgaris* and would maintain a thrips population for a correspondingly longer time and provide a continuous source of infection where it occurs. Individual flower spikes of the former may produce blossoms right throughout the summer and it is common to see the dry seed pods which have long since shed their seed attached to the still flowering stems, and in some cases bearing unmistakable evidence of having been infected with the pod twisting disease.

That infection from the widely spread phasey bean accounts for much of the damage caused to beans is strongly suggested by the occurrence of what is often severe infection in bean plantings in localities well isolated from other bean-growing districts. In such cases it seems certain that both the disease and the vector have become well established in the area by the time the plantings are made.

In conformity with this idea the greater severity of infection on late-formed pods and in later successional plantings, as mentioned earlier, could be explained on the basis of an increased thrips population resulting from migration. Where the influx of insects to the area as a whole is continuous, the latest formed flowers are likely to be subjected to a more concentrated attack. Further support is lent to this theory by the fact that the insects apparently confine their activities to the flowers, and do not return to the pods as they subsequently become diseased.

VI. IDENTITY OF THE CAUSAL ORGANISM.

This description is based on 10 isolates obtained from different sources; the number used in individual tests appears in brackets immediately following the name of the test. Slight differences between some of these isolates occurred, but for the most part they behaved similarly. For example, variability in colony appearance, temperature relations, rate of acid production from some sugar solutions and pathogenicity was noticed (and without doubt many other differences of a similar degree occur) but in every case the character described was clearly demonstrated.

Methods Employed.

The criteria adopted in an attempt to identify the organism were its cultural characteristics, morphology, biochemical reactions, parasitism (under natural and induced conditions), host range, mode of transmission and seasonal occurrence. The tests carried out were those more often associated with identification of plant pathogens, and comparisons with other species which attack beans were given particular attention. In this respect the organisms *Pseudomonas phaseolicola, Ps. syringae* and *Xanthomonas phaseoli* were often included. *Ps. solanacearum* was also used in some cases.

Investigations were carried out along the lines suggested in the "Manual of Methods for Pure Culture Study of Bacteria," and the data presented could be transferred to the Standard Descriptive Chart if desired.

Some difficulty was experienced in maintaining viable cultures on solid media, but this was not the case with fluid media. The organism made poor growth in some tests and often the usual duration of these had to be extended or the medium altered to ensure adequate testing. Where insufficient growth occurred, a note to this effect is made. When a negative result was recorded, such as in the case of hydrogen sulphide production, the procedure adopted is indicated.

Whenever possible a number of tests were carried out together, using the same broth culture to inoculate each, after considerable care had been taken to ensure its purity. After inoculations had been made the same tube was

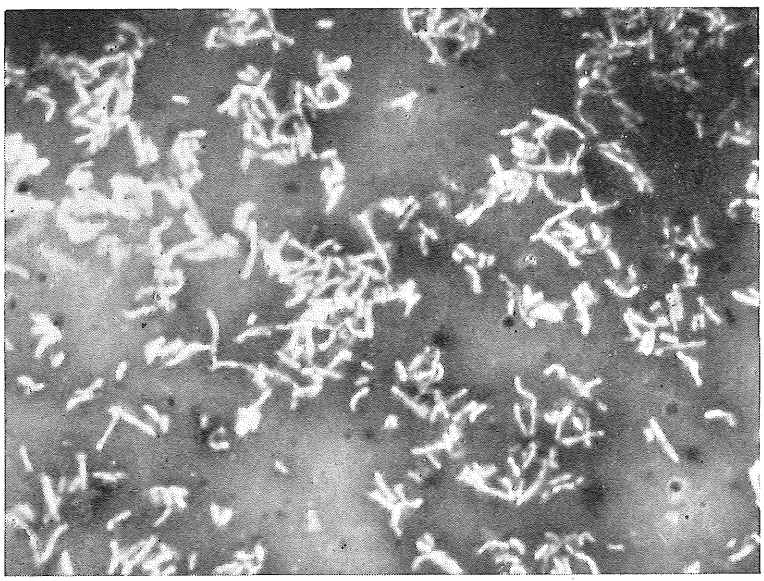

Fig. 11.

Pod Twist *Pseudomonas*. Negative stain (X 1500). From a 7-day-old culture on potato dextrose agar.

streaked over four to six plates of potato dextrose agar to confirm freedom from contamination and also inoculated into fresh bean pods to confirm pathogenicity. All culture media was held for several days and carefully examined for sterility prior to use and all tests were replicated. In the case of carbohydrate fermentation, four tubes were inoculated on each occasion with each isolate and for other tests the number used varied from four to six. Many tests were repeated a number of times in order to confirm results. Where necessary, inoculated and uninoculated controls were included.

Morphology.

Cell description.—(3). Medium P.D.A.;* temp. 27·5 deg. C.; time 24 hours. Rods, singly and in pairs, 0·5–0·75 x 1·4–2·0μ (negative stain), with rounded ends (Fig. 11). Gram negative.

Motility.—(3). Medium P.D.A.; temp. 27·5 deg. C.; time 24 hours. Motile by 1-2 polar flagella (Fig. 12). Motility observed by diffusing organisms out of freshly-infected tissue and tube cultures into water, and viewing in a hanging drop under the phase contrast microscope.

Spores.—(3). Medium P.D.A.; temp. 30 deg. C.; time 5–8 days. Absent. Both agar slope cultures and saline water suspensions heated at 80 deg. C. for 10 min. and then streaked out onto P.D.A.

Cultural Characteristics.

Solid media.—(10). Medium M.I.A.; temp. 27·5 deg. C.; time 5 days. Circular, 1·0–1·5 mm. dia., convex, amorphous, smooth and glistening surface, entire edge, greyish white, translucent, butyrous, easy to emulsify, odour absent. Colonies similar, but smaller, on M.E.A.

Fluid media.—(10). Medium M.I.B.; temp. 27·5 deg. C.; time 24 hours. slight turbidity and slight sediment, no surface growth, odour absent, no pigment. Similar result on M.E.B.

Agar stroke.—(10). Medium P.D.A.; temp. 27·5 deg. C.; time 5 days. Growth moderate, filiform, glistening, not fluorescent, odour absent, butyrous consistency, medium unchanged.

Steamed potato slant.—(3). Temp. 30 deg. C.; time 14 days. Growth moderate, greyish white and glistening at first, later becoming flesh-coloured.

Gelatin stab.—(8). Temp. 20 deg. C.; time 8 days. No liquefaction. Slight growth. 12 per cent. plain gelatin used.

Temperature relations.—(3). Medium P.D.A.; pH 7·0; optimum 29–30 deg. C.; range 14–37 deg. C. Agar stroke cultures were grown in a multi-temperature incubator, with a range of 4–40 deg. C., which was divided into 20 compartments each showing approximately 2 deg. C.

* For description of culture media, see appendix.

difference. They were examined after 48 and 96 hours and growth assessed visually. Only a few colonies developed at 37 deg. C. Two strains showed no growth above 34 deg. C. Subculturing after 14 days in the range showed that all cultures held above 24 deg. C. had died out.

Thermal death time.—Suspensions were killed by exposure for 10 min. to a temperature of 40 deg. C., but not at 35 deg. C. Saline water suspensions from P.D.A. slopes were tested in thin-walled ½-in. test tubes, about 1 ml. being placed in the pre-heated tube on each occasion.

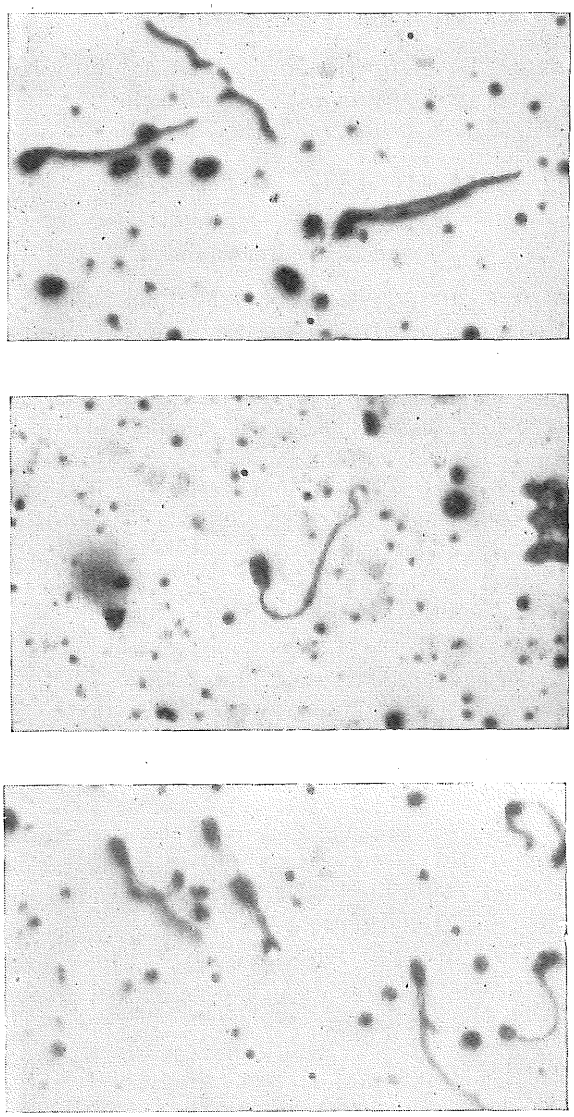

Fig. 12.

Pod Twist *Pseudomonas*. Flagella stain. Leifson's method. (X 1500).

Resistance to drying.—(1). Able to withstand drying for at least 15 days. Small glass rods, dipped in a 48-hour-old broth culture, were allowed to dry in petri dishes at 30 deg. C. They were removed and dropped into broth at 3-day intervals.

Fluorescence.—(10). Was observed in yeast extract synthetic basal medium as a ring in tube cultures where galactose or glycerol was added in 1·0 per cent. concentration. It has also appeared consistently, though slightly, on P.D.A. Occasional P.D.A. cultures have shown a marked fluorescent halo in ordinary light. No growth occurred in Sullivan's solution (in which *Ps. solanacearum* grew well).

Additional data.—Best growth on a solid medium occurred on P.D.A. Viable and pathogenic after 10 months' storage in B.E.B. at 8 deg. C. Pathogenic after 20 months' culture on artificial media. No growth in broth plus excess calcium carbonate.

Biochemical Reactions.

Carbohydrate fermentation.—(8). (*a*) Medium basal (with 1 per cent. peptone); temp. 30 deg. C. Acid from dextrose, mannose, sucrose and galactose in 24 hours, and slightly from salicin in 10–20 days, using bromthymol blue indicator. Acid from dextrose, mannose and sucrose in five days and slightly from salicin in 15–20 days, using bromcresol purple indicator. No acid was produced from maltose, lactose, starch, glycerol, mannitol, sorbitol or aesculin when they were incorporated in this medium. Control tubes were inoculated to test for the presence of fermentable carbohydrate in the peptone used, but none was detected. No gas formation.

(*b*) Yeast extract synthetic basal medium; temp. 30 deg. C. (no growth in this basal medium without yeast extract). Acid from dextrose and sucrose in 48 hours and from galactose, glycerol and salicin in 15–20 days, using bromthymol blue indicator. Acid from dextrose and sucrose in 5 days using bromcresol purple indicator. No acid from arabinose, lactose, maltose, mannose, mannitol, sorbitol, aesculin or starch. No gas formation.

The carbohydrates were incorporated in the basal media at a 1 per cent. concentration (except in the case of starch and aesculin, which were used at strengths of 0·2 and 0·1 per cent. respectively). They were prepared as 10 per cent. stock solutions and sterilized by steaming for one hour on each of three successive days, and added in 1 ml. quantities with a pipette to 9 ml. of basal medium, two or three days prior to inoculation. All reagents were of the finest bacteriological grades available.

A notable feature here was that acid was produced readily from mannose in the peptone medium but not in the synthetic medium. A similar effect occurred in the case of galactose.

Starch hydrolysis.—(5). (*a*) Medium M.E.B. plus 0·2 per cent. soluble starch; temp. 30 deg. C. No hydrolysis. Cultures were tested at 2, 4, 7 and 10-day intervals, as described in the "Manual of Methods for Pure Culture Study of Bacteria," Vol. 49, p. 19.

(*b*) Medium, P.D.A.; temp. 30 deg. C.; time 14 days. Slight hydrolysis. When plates were flooded with Lugol's iodine a reddish brown, slightly clearer zone 2–3 mm. wide against the surrounding blue was evident around the colonies.

Methyl red test.—(3). Medium glucose phosphate peptone water; temp. 27·5 deg. C.; time 5 days. Negative. The organism made poor growth in this medium (in which *Ps. syringae* grew well.) A few drops of methyl red were added to the 5-day-old culture. No red colour developed.

Voges-Proskauer test.—(3). Medium glucose phosphate peptone water; temp. 27·5 deg. C.; time 3 days. Negative. One millilitre of 10 per cent. KOH solution was added to the 3-day-old cultures, which were then incubated overnight. No colour developed.

Indole.—(8). Medium trytophane broth; temp. 27·5 deg. C.; time 14 days. Negative. Moderate growth occurred on this medium (on which *Ps. syringae* grew well). A few millilitres of Erlich-Bohme solution was layered on the surface of cultures at various ages from 2 to 14 days.

Hydrogen sulphide.—(5). (*a*) Medium M.E.A. plus ·05 per cent. lead acetate; temp. 27·5 deg. C.; time 3 weeks. Not produced. Stab inoculation. A small amount of growth 2–3 mm. across occurred at the surface, with little growth visible along the line of the puncture.

(*b*) Medium M.E.B.; temp. 30 deg. C.; time 3 weeks. Not produced. No darkening of lead acetate papers.

Litmus milk.—(4). Temp. 30 deg. C.; time 6 weeks. No change. *Ps. solanacearum, Ps. syringae* and *Ps. phaseolicola* were used as checks. All gave characteristic reactions.

Ammonia from broth test.—(3). Medium M.E.B.; temp. 30 deg. C.; time 4–10 days. Negative. No precipitate with Nessler's reagent after 4, 7 and 10 days. Method from Dowson (1949, p. 45).

(*Ps. solanacearum* also failed to show ammonia production by this method, which must therefore be regarded as being of doubtful value. The test was repeated using "Difco" peptone, with the same result).

Action on nitrates.—(7). (*a*) Medium nitrate peptone water; temp. 30 deg. C. Not reduced. To two drops of starch iodine solution one drop of dilute HCl (1 in 6) and one loopful of culture were added. No colour developed. This was carried out at daily intervals up to 10 days.

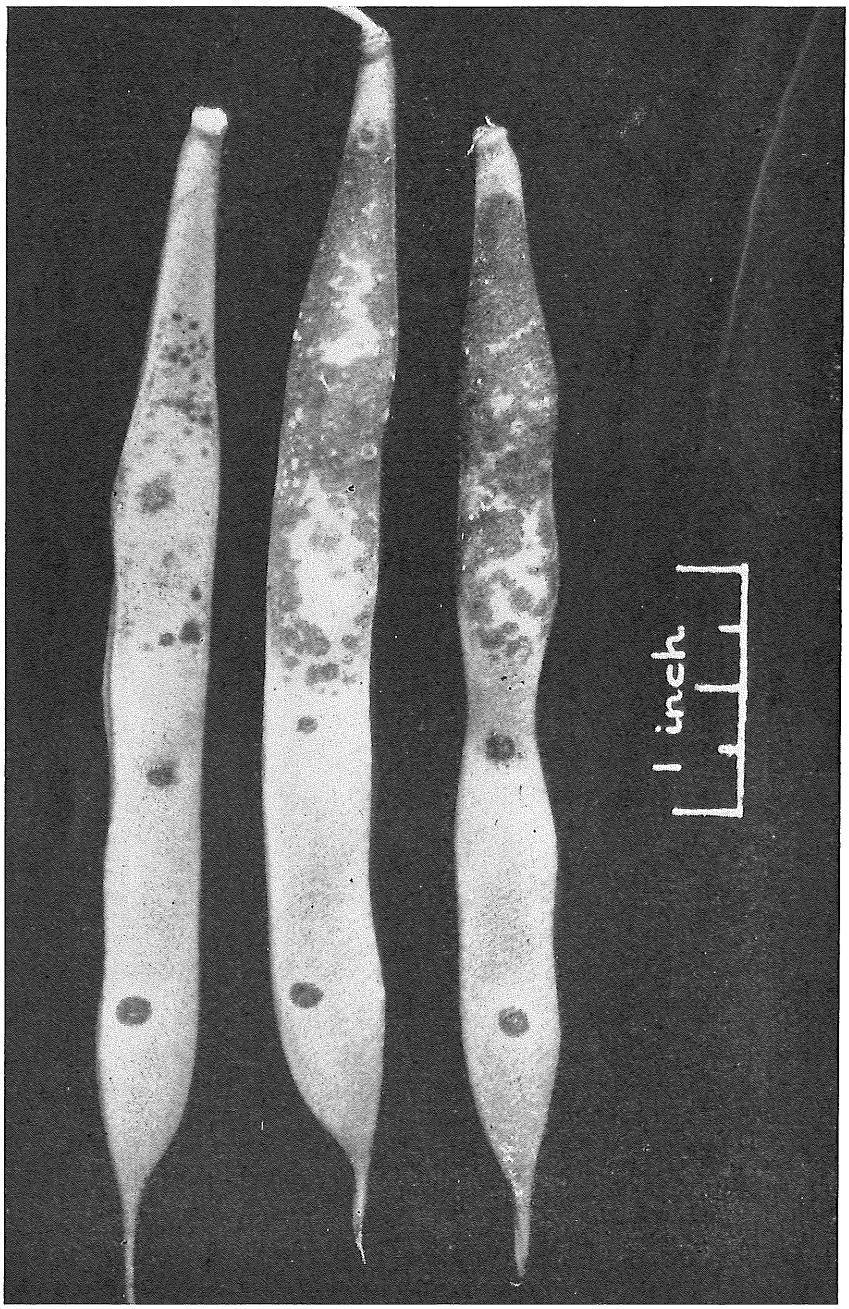

Fig. 13.

Symptoms Produced on *Phaseolus vulgaris* Pods by Prick Inoculation With Three Organisms *Ps. phaseolicola* (below), *X. phaseoli* (centre) and pod twist *Pseudomonas* (above).

(*b*) Yeast extract synthetic basal medium plus 0·2 per cent. potassium nitrate. Not reduced. The same procedure was adopted. The presence of nitrate after 10 days was confirmed with diphenylamine. (*Ps. solanacearum* completely reduced the nitrate in this medium.)

Liquefaction of gelatin.—(4). (*a*) Medium P.D.A. plus 0·4 per cent. gelatin; temp. 30 deg. C.; time 6 days. Negative. The method used was Smith's (1946) modification of a procedure described by Frazier.

(*b*) Medium 12 per cent. plain gelatin; temp. 30 deg. C.; time 6 weeks. Negative. The tubes were inoculated with 0·5 ml. of a suspension of the organism and incubated in a sealed plastic container to prevent drying out.

Comparison with Some Other Bacterial Pathogens of Bean.

In the biochemical reactions, a close similarity exists between the organism being described and *Ps. phaseolicola* and *Ps. syringae*. So far as carbohydrate fermentation is concerned, distinctions between *Ps. phaseolicola* and the test species occur in the case of arabinose, salicin and glycerol. The arabinose, mannitol and glycerol reactions distinguish it from *Ps. syringae*. A significant difference lies in the ability to attack gelatin. *Ps. syringae* does it readily, *Ps. phaseolicola* rather slowly, and the test organism not at all. Both these species induce an alkaline reaction in litmus milk, but the pod twist organism causes no change. The most obvious distinction to be found here, however, is the manner in which each of them attacks the host, particularly with regard to symptom expression and mode of transmission. On this basis

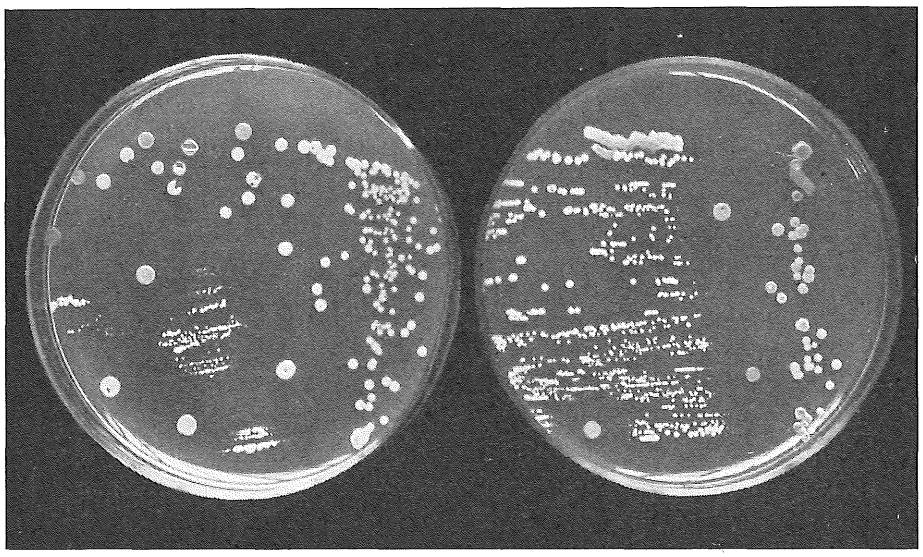

Fig. 14.

Replacement of the Pod Twist Pathogen by a Saprophytic Bacterium. Potato dextrose agar isolation plates depict a partial suppression of the pathogen (small colonies) by a secondary invader. Isolations from aging pod twist material often indicate complete suppression.

Table 3.

A Comparison of the Pod Twist *Pseudomonas* with Three Other Species of *Pseudomonas* Recorded on Bean[1].

Character.	*Ps. syringae.*	*Ps. phaseolicola.*	*Ps. viridiflava.*	Pod Twist *Pseudomonas.*
Morphology	Rods 0·75–1·5 x 1·5–3·0 μ. Motile, 2-polar flagella. Single, in pairs or short chains.	Rods 0·75–1·5 x 1·5–3·75 μ. (Clara 1934). Motile by a single polar flagellum. Single, or in pairs or chains.	Rods 0·75–1·5 x 1·5–3·15 μ. Motile, 1–2 polar flagella. Single or in pairs.	Rods 0·5–0·75 x 1·4–2·0 μ. Motile, 1–2 polar flagella. Single or in pairs.
Optimum temperature ..	28–30 deg.C.	20–23 deg.C.		29–32 deg.C.
Fermentation—				
Arabinose	A	A	A	—
Maltose	—	—	—	—
Mannose	A	A	A	A
Dextrose	A	A	A	A
Lactose	—	—	—	—
Sucrose	A	A	—	A
Mannitol	A	—	A	—
Galactose	A	A	A	A
Salicin	—	—	—	A (Slight)
Glycerol	A	A	A	A
Gelatin	Liquefied	Slowly liquefied	Liquefied	Not liquefied
Litmus milk	Alkaline in 2–3 days	Alkaline in 2–3 days	Alkaline and clears	No change
Starch	Not hydrolyzed	Not hydrolyzed	Not hydrolyzed	Slight hydrolysis
Symptoms—				
On beans in the field ..	Brown spots on leaves and pods at all stages. Pod spots sunken and not watersoaked	Small watersoaked spots on leaves, stems and pods, becoming brown later. Leaf spot marked by conspicuous halo	Brown spots on older pods only	Large diffuse watersoaked lesions on twisted young pods. Older pods showing characteristic bending and twisting. No symptoms on leaves and stems.

Pure culture inoculation of bean pods	Sunken brown spots, circular, up to 8 mm. dia.	Watersoaked spots, circular, 2–5 mm. dia.	Sunken reddish brown spots 3–8 mm. dia.	Large diffuse watersoaked lesions 3–5 cm. long
Host range—				
Ripe lemon fruit ..	Susceptible	Not susceptible		Not susceptible
Lupin pods		Not susceptible	Not susceptible	Susceptible
Transmission	By splashing and probably by seed	By seed and splashing of water	By seed	By thrips which feed in flowers of beans and other legumes
Seasonal occurrence ..	In cool, wet weather in south-eastern Queensland	Serious in cool, wet weather in south-eastern Queensland	Not recorded in Queensland	Serious only in warm weather — December to March— in south-eastern Queensland, when thrips populations are high

[1] From Bergey's Manual (Sixth edition), Dowson (1949), and observations by the writer.

they are so distinct that it would seem impossible to confuse them. In the field, definite pod spotting, which is so characteristic of halo blight, common bacterial blight and brown spot, is absent. In the laboratory, the lesions produced by inoculation of mature green pods with a pure culture of the organism are from three to four times larger than those caused by *Ps. syringae*, which in turn are larger than those caused by *Ps. phaseolicola* and *X. phaseoli* (Fig. 13). The predominance of infections involving newly-formed pods, the marked twisting of older pods, and the absence of leaf, stem and systemic infections are features by which it may easily be recognised. A general comparison of the pod twist organism with three other related species pathogenic on beans is given in Table 3.

Taxonomy and Nomenclature.

The causal agent of pod twist belongs to the group of rod-shaped, polar flagellate, gram negative, non-spore-forming organisms, heterotrophic and fluorescent in culture, and giving white or creamish growth on meat

Table 4.

BRIEF COMPARISON OF THE POD TWIST *Pseudomonas* WITH SOME OTHER SPECIES WHICH IT SOMEWHAT RESEMBLES.

Species.	Points of Difference.	
	Character.	Pod Twist *Pseudomonas*.
Ps. glycinea	Litmus milk turns blue and separation occurs	No change
	Starch not hydrolysed	Slight hydrolysis
	Opt. temp. 24–26 deg. C. ..	Opt. temp. 29–32 deg. C.
	Pathogenic on soybean	Non-pathogenic
Ps. savastoni	Litmus milk becomes alkaline ..	No change
	Starch hydrolyzed	Slight hydrolysis
	Colour of potato cylinders deep olive-buff (Brown 1932)	Growth flesh coloured
	Opt. temp. 24–25 deg. C. ..	Opt. temp. 29–32 deg. C.
	Causes hypertrophy on susceptible hosts	No hypertrophy
Ps. tonelliana	Litmus milk becomes alkaline ..	No change
	Starch not hydrolyzed	Slight hydrolysis
	White growth on potato cylinders (Adam & Pugsley 1934)	Flesh coloured
	Thermal death point 51 deg. C.	Not above 40 deg. C.
	Causes hypertrophy on susceptible hosts	No hypertrophy
Ps. melophthora	No fluorescent pigment produced	Fluorescent on some media
	Agar colonies pink	Colonies never pink
	Starch not hydrolyzed	Slight hydrolysis
	Opt. temp. 21–25 deg. C. ..	Opt. temp. 29–32 deg. C.
	Pathogenic on apple fruit ..	Not pathogenic

infusion and starch agars, which constitute the genus *Pseudomonas*. It complies with the description of the genus given by Migula, as published in Bergey's Manual of Determinative Bacteriology (1948). There is also good agreement with the amended definition offered by Dowson (1949).

A perusal of the literature has failed to provide any indication of a relationship with other species of *Pseudomonas* which is close enough to identify the organism, nor does it appear to be a strain of a previously described species.

According to the keys provided in Bergey's Manual it falls very close to the *glycinea–savastoni–tonelliana* group. A similarity to the non-fluorescent *Ps. melophthora* is also shown. None of these organisms have been recorded in Queensland, although their host species—soybean, olive, oleander and apple respectively—have been in cultivation for many years. Some differences which exist between the pod twist organism and these species are given in Table 4. Unless otherwise shown, the information is drawn from Bergey's Manual (Sixth Edition).

On the evidence available it seems necessary to conclude that the pod twist organism is as yet unnamed, and the binomial *Pseudomonas flectens* is accordingly now proposed. The epithet *flectens* refers to the characteristic bending of the pods of *Phaseolus lathyroides* and *P. vulgaris* when they are infected with this organism. Type cultures have been forwarded to the American Type Culture Collection, Washington, D.C. and to the National Collection of Type Cultures, London.

Brief Description.

Pseudomonas flectens sp. nov.

Gram negative, rod-shaped aerobe, 1·7 x 0·6μ (mean), motile with one or two flagella. Five-day-old colonies on meat infusion agar are circular, convex, amorphous, smooth and glistening, with an entire edge, greyish white, translucent, butyrous and easy to emulsify. Odour is absent. Colonies are smaller on meat extract—peptone agar, and larger on potato dextrose agar. A fluorescent pigment is produced on potato dextrose agar, and in yeast extract synthetic basal medium when galactose or glycerol is added in 1·0 per cent. concentration. Broth slightly turbid with a slight sediment, no surface growth and no odour. Gelatin not liquefied. Litmus milk remains unchanged. Optimum growth temperature 29–32 deg. C., growth range 14–37 deg. C. Nitrate not reduced. No hydrogen sulphide or indole produced. Slight starch hydrolysis. Acid produced from dextrose, sucrose, mannose, galactose, glycerol and very slightly from salacin, but not from arabinose, lactose, maltose, mannitol, sorbital, aesculin or starch. Pathogenic on young pods of *Phaseolus vulgaris* L. and *P. lathyroides* L. in Queensland, causing a characteristic pod twisting. Transmitted by the bean thrips (*Taeniothrips nigricornis* Schmutz).

VII. ACKNOWLEDGEMENTS.

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APPENDIX.

Description of Culture Media.

Basal medium:

"Difco" Peptone	10 gm.
NaCl	5 gm.
Tap water	1 litre

pH adjusted to 7·4 and sterilized in autoclave at 15 lb. for 20 min. Indicator (bromthymol blue or bromcresol purple) added before sterilizing.

Gelatin media:

(*a*) P.D.A. plus 0·4 per cent. gelatin. The gelatin was added to molten P.D.A. and the whole sterilized at 12 lb. for 15 min.

(*b*) 12 per cent. gelatin in tap water, pH adjusted to 7·4 before autoclaving at 12 lb. for 15 min.

Glucose phosphate peptone water:

Peptone	5 gm.
K_2HPO_4	5 gm.
Distilled water	1 litre

Steamed until dissolved, filtered hot and reaction adjusted to 7·4 when cool; then 5 gm. glucose added and autoclaved at 10 lb. for 10 min.

Meat extract agar (*M.E.A.*):

Meat extract broth plus 17 gm. agar.

Meat extract broth (*M.E.B.*):

Peptone	10 gm.
NaCl	5 gm.
Meat extract	5 gm.
Distilled water	1 litre

pH adjusted to 7·4 before sterilizing at 15 lb. for 20 min.

Meat infusion agar (*M.I.A.*):

Meat infusion broth plus 20 gm. agar.

Meat infusion broth (*M.I.B.*):

Bullock's heart	500 gm.
Peptone	10 gm.
NaCl	5 gm.
Tap water	1 litre

After steaming for 1½ hours and filtering through cotton-wool, 35 ml. N/1 NaOH was added. The solution was then autoclaved and filtered again through filter paper, pH adjusted to 7·4, and autoclaved again.

Nitrate peptone water:

"Difco" peptone	10 gm.
KNO_3	2 gm.
Distilled water	1 litre

Warmed to dissolve, pH adjusted to 7·4, filtered through filter paper and tubed. Sterilized by steaming for one hour on three successive days.

Potato dextrose agar (*P.D.A.*):

Potato	200 gm.
Dextrose	20 gm.
Agar	20 gm.
Tap water	1 litre

Potatoes chopped finely and steamed for 40 min. in 1 litre of water; filtered through cotton-wool and diluted back to 1 litre. Dextrose and agar added, followed by steaming again for 40 min.; filtered through cotton-wool and autoclaved in tubes at 15 lb. for 20 min. (No adjustment of pH, which is usually about 7·0).

Sullivan's solution:

Asparagin	10 gm.
$MgSO_4$ (anhydrous) ..	1 gm.
K_2HPO_4 (anhydrous) ..	1 gm.
Distilled water	1 litre

Tubed in 5-8 ml. lots and autoclaved at 15 lb. for 20 min.

Synthetic basal medium:

$(NH_4)H_2PO_4$	1·0 gm.
KCl	0·2 gm.
$MgSO_4.7H_2O$	0·2 gm.
Distilled water	1 litre

The pH was adjusted to 7·0 before sterilizing in the autoclave at 20 lb. for 15 min.

Synthetic basal medium plus yeast extract:

Synthetic basal medium plus 0·5 per cent. "Difco" bacto yeast extract.

Tryptophane broth:

"Difco" bacto-tryptone ..	10 gm.
NaCl	5 gm.
Tap water	1 litre

Preparation same as for basal medium.

Yeast extract synthetic basal medium:

Synthetic basal medium plus 0·5 per cent. "Difco" bacto yeast extract.