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DIVISION OF PLANT INDUSTRY BULLETIN No. 482

"HALO-LESS" HALO BLIGHT OF FRENCH BEAN IN QUEENSLAND

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SUMMARY

Cultural and comparative pathogenicity studies showed that the organism responsible for bacterial blight symptoms of French bean in North Queensland closely resembles *Pseudomonas phaseolicola.*

I. INTRODUCTION

Four bacterial blight diseases of French bean (*Phaseolus vulgaris* L.) are well known in Queensland. These are caused by the organisms *Pseudomonas flectens* Johnson, 1956 (pod twist) (Johnson 1956), *P. phaseolicola* (Burkholder, 1926) Dowson, 1943 (halo blight), *P. syringae* van Hall, 1902 (brown spot), and *Xanthomonas phaseoli* (Erwin Smith, 1897) Dowson, 1939 (common blight).

Blight-free seed production is an important industry in the Burdekin and Atherton Tableland areas of North Queensland. One of the objectives of a joint State Department of Primary Industries and seed industry "Approved Seed Scheme" which operates in the area is that only seed free of seed-transmissible blight diseases will be marketed. Since it has been recently shown by Harrison and Freeman (1965) that *P. syringae* is readily seed-borne, *P. flectens* is the only one of the four pathogens considered to fall outside this category. It is important therefore that the symptoms of halo blight, common blight and brown spot may be readily distinguished from those caused by *P. flectens,* physiological upsets, insect injuries and other injuries which bear some resemblance to them.

Until recently neither halo blight nor common blight had been recorded in commercial seed crops in these areas. In the winter and early spring of 1966, two areas of beans near Atherton were found by State Department inspectors to include plants which showed pod symptoms identical with those caused by

halo blight, but no characteristic leaf symptom was in evidence. Leaves on some plants bore tiny dark brown spots, especially towards the margins, and older leaves were yellowing and wilting (Figure 1).

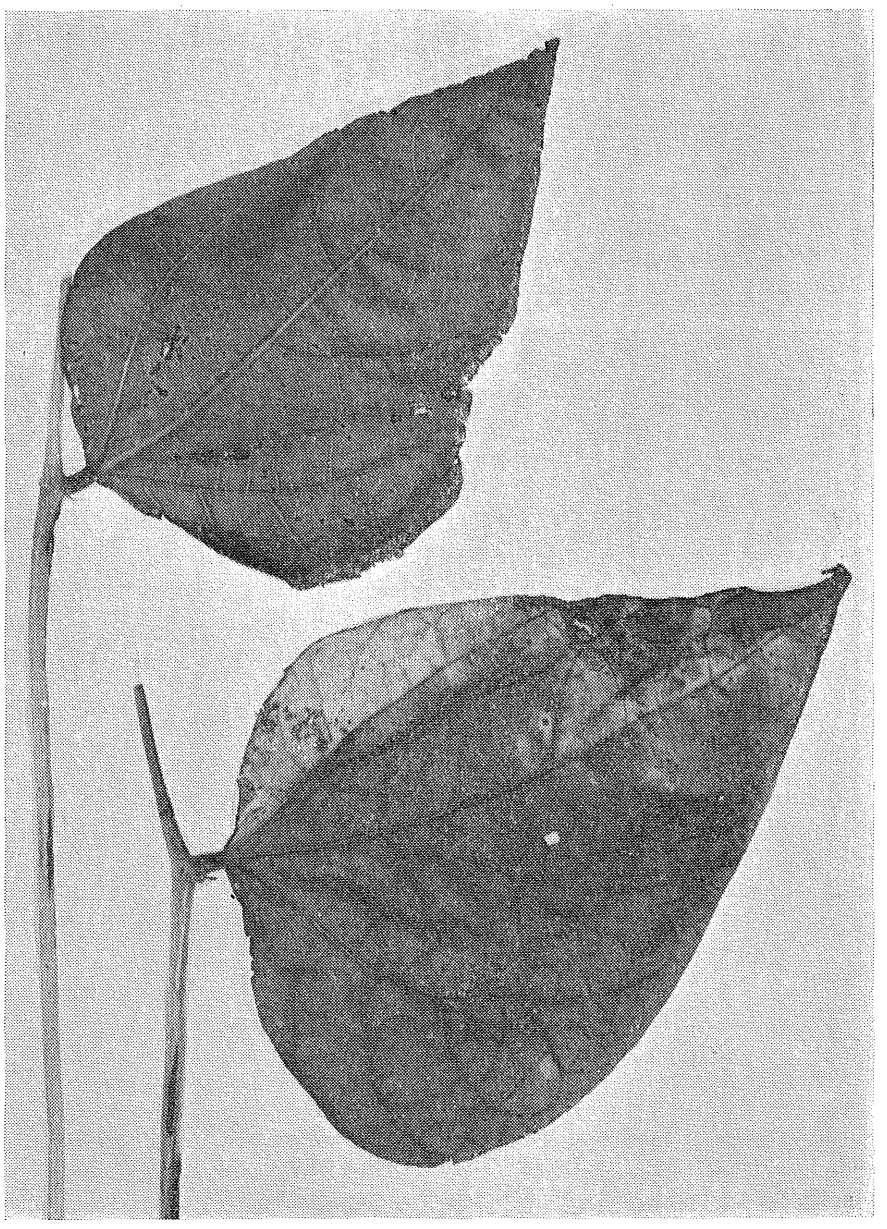

Fig. 1.—Leaflet and leaf stalk symptoms of natural infection with "halo-less" halo blight. The older leaflet below shows characteristic yellowing and withering which follows vascular invasion by the pathogen.

Reference to these occurrences and to the subsequent widespread detection of the disease in North Queensland seed crops was made in 1967 (Anon. 1967, p. 14).

Studies of the organism responsible for the disease were undertaken.

II. MATERIALS AND METHODS

The following media and methods were used during the investigation:

Potato dextrose agar (P.D.A.): Potato 200 g, dextrose 20 g, granular agar 20 g, tap water 1 l. pH 6·9.

Beef extract broth: "Oxoid Lab. Lemco" 0·5%, NaCl 0·5%, "Difco" bacto-peptone 1%, tap water. pH 7·2.

Sucrose peptone broth: Sucrose 20 g, "Difco" bacto-peptone 5 g, K_2HPO_4 0·5 g, $MgSO_4 \cdot 7H_2O$ 0·25 g, distilled water 1 l. pH 7·2 –7·4.

Gelatin stabs: "Baltimore Biological Laboratory" nutrient gelatin 12·8 g, distilled water 100 ml.

Flagella stain: Rhodes' modification of Fontana's silver-plating method (Rhodes 1958).

Size determination: Nigrosin relief: 4 g nigrosin, 10 drops formalin, distilled water 100 ml.

Carbohydrate base medium: Carbon compounds were incorporated into the synthetic medium of Ayres, Rupp, and Johnson (Society of American Bacteriologists 1957). Bromo-thymol blue was the indicator used. Durham tubes were included in the tubes for the detection of any gas production.

III. ISOLATIONS AND PATHOGENICITY

When isolations were made from the original disease samples, it was at once obvious from colony characteristics that the pathogen was not *X. phaseoli*. Suspensions were made from the small cream colonies which appeared on the isolation plates, and these were used to inoculate bean seedlings in the glasshouse. After 3 days numerous small watersoaked spots about 0·5 mm in diameter appeared on young leaves of each of the varieties Epicure, Pinto and Redlands Greenleaf. These tiny spots failed to enlarge further and became darker and eventually very similar to those present on the original samples. The pathogen was reisolated.

IV. THE PATHOGEN

For a more detailed examination, fresh isolates were obtained from two diseased seed crops on the Atherton Tableland situated approximately 40 miles apart. In each case the variety concerned was Tendercrop, and it is likely that the two plantings were derived from the same mother seed stock. Neither was

registered in the approval scheme. Macerated infected pod tissue was streaked out onto potato dextrose agar plates and readily yielded numerous colonies similar to those of *P. phaseolicola.* For comparative work, isolates from other bean plants naturally infected with brown spot and typical halo blight were obtained at the same time.

After 6 days, colonies of *P. syringae* on potato dextrose agar (P.D.A.) were larger than those of the test organism and *P. phaseolicola,* which were themselves similar in all respects. In beef extract broth all three organisms produced in 3 days a turbid growth with a light, easily broken pellicle. Growth of all three was more rapid in sucrose peptone broth (S.P.B.) and after 10 days the halo blight organism produced a pronounced yellow-green fluorescent pigment (viewed under ultraviolet light); this pigment was produced only very slightly by the test organism. Only *P. syringae* caused liquefaction of gelatin (stabs). Both *P. phaseolicola* and the test cultures produced moderate growth on steamed potato slants, which became slightly brown after 3 days. Similar growth by *P. syringae* remained light cream in colour.

Triplicated carbohydrate fermentation tests on both test isolates and *P. phaseolicola* showed acid without gas after 3 weeks at 27°C from glucose, L-arabinose, xylose, mannose, fructose, sucrose and glycerol. No acid was produced in rhamnose, lactose, maltose and salicin. This is consistent with reactions listed for *P. phaseolicola* in Bergey's Manual of Determinative Bacteriology, Seventh Edition (Breed, Murray, and Smith 1957). *P. phaseolicola* did not produce acid from mannitol but the two test cultures did. In this reaction the test organism was inconsistent with Bergey's description for *P. phaseolicola.*

After 10 weeks in culture, *P. phaseolicola* began to produce large wrinkled colonies on P.D.A. (up to 1 cm diam. in 7 days) whereas the test organism had retained its original smaller, smooth, circular and entire colony type. Microscopic examination showed the test organism to be Gram-negative, to possess polar flagella and to conform in size with the accepted range for *P. phaseolicola.*

V. COMPARATIVE PATHOGENICITY STUDIES

Bean pods at the green harvest stage were inoculated in the laboratory with pure culture water suspensions of the test organism, *P. phaseolicola* and *P. syringae* by placing a drop of suspension on the pod and gently scratching the surface within the drop. After 7 days, the test organism and *P. phaseolicola* had each produced typical grease spots on all varieties used—Pinto, St. Andrews and Tendercrop. *P. syringae* produced only brown sunken spots which were quite different. This evidence, together with that from cultural tests, was sufficient to show that the pathogen under investigation was not a form of *P. syringae.*

When 10-day-old bean seedlings were inoculated in the glasshouse, distinctly different leaf symptoms were produced by the test organism and *P. phaseolicola.* Spots caused by the latter were surrounded by characteristic lemon colored haloes, whereas those caused by the test organism were not.

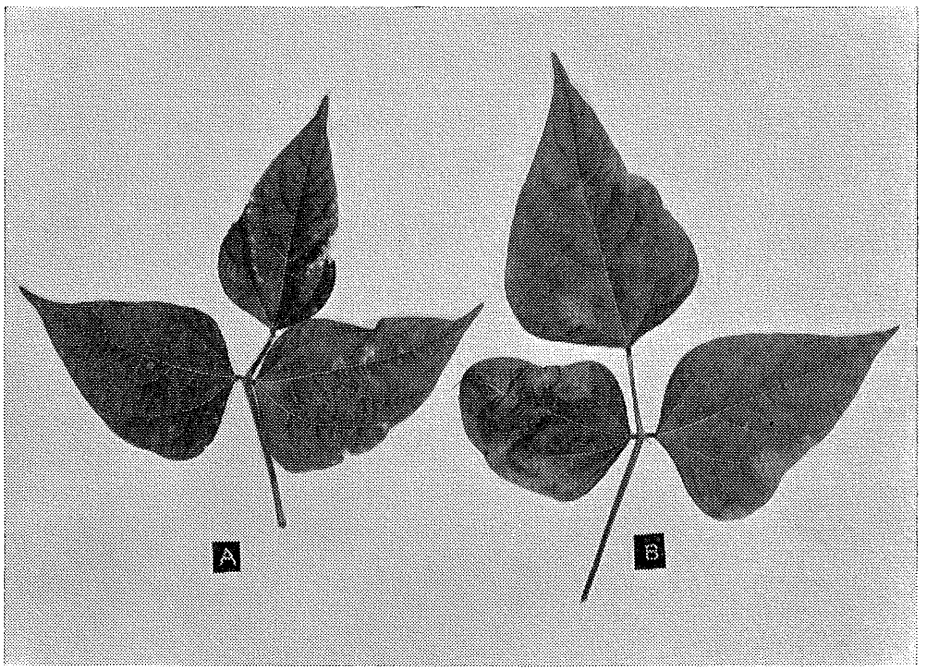

Fig. 2.—Bean leaves artificially inoculated with pure cultures of the "halo-less" (A) and normal (B) forms of halo blight, after 10 days.

When fully developed after 10 days, these halo-less lesions were 0·5 mm or less in diameter and could be seen with the naked eye only if examined very closely. Glasshouse temperatures did not exceed 24°C. These symptoms are illustrated in Figures 2 and 3. Lowering the incubation temperature to 16°F in a growth chamber did not result in halo production. Erosion of the leaf margin, as seen in Figures 1-3, was commonly observed both in glasshouse inoculations and in field-infected material. Needle-prick inoculation of bean stems with the test organism resulted in elongated watersoaked lesions which became reddish-brown with age, and the infection was observed to move up the vascular elements, causing successive leaves to wither (Figure 4). Subsequently, curls of exudate (Figure 5) appeared on the upper main stem, and these, when fresh, proved to be a useful source of inoculum for both pathogenicity tests and isolation plates. These are common features of halo blight.

VI. VARIETY SUSCEPTIBILITY STUDIES

During the course of this work, comparative symptoms caused by the test organism, *P. phaseolicola* and *P. syringae* on varieties of known reaction to halo blight were examined in several glasshouse trials. Those used were Brown Beauty and Tendercrop (susceptible) and Epicure and Pinto (field resistant). Seedlings at the stage where the second trifoliate leaf was half expanded were inoculated

by applying water suspensions to each of the trifoliate leaves (both half and fully expanded) with a glass spatula under light pressure. After 24 hours' incubation in a moist chamber the test plants were removed to the glasshouse bench, where they were examined after 8 and 15 days for disease development. Results are summarized in Table 1.

Both *P. phaseolicola* and the test organism finally became systemic in the varieties Brown Beauty and Tendercrop (by migrating from the leaves into the stems) but not in the varieties Epicure and Pinto. This indicates a close physiological relationship between the two organisms. Further, it was observed that the halo symptom induced by *P. phaseolicola* was associated with a more severe type of systemic disease. Another interesting observation was the high resistance of Pinto to brown spot.

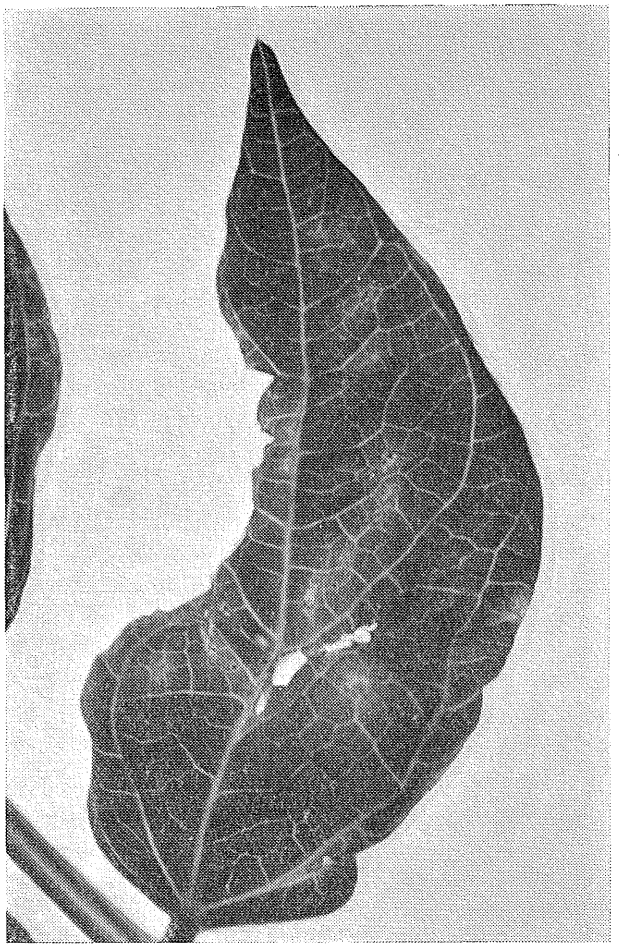

Fig. 3.—Close-up view of 10-day-old leaf spots caused by "halo-less" halo blight. Leaf margin erosion is characteristic (see Fig. 1, top leaflet).

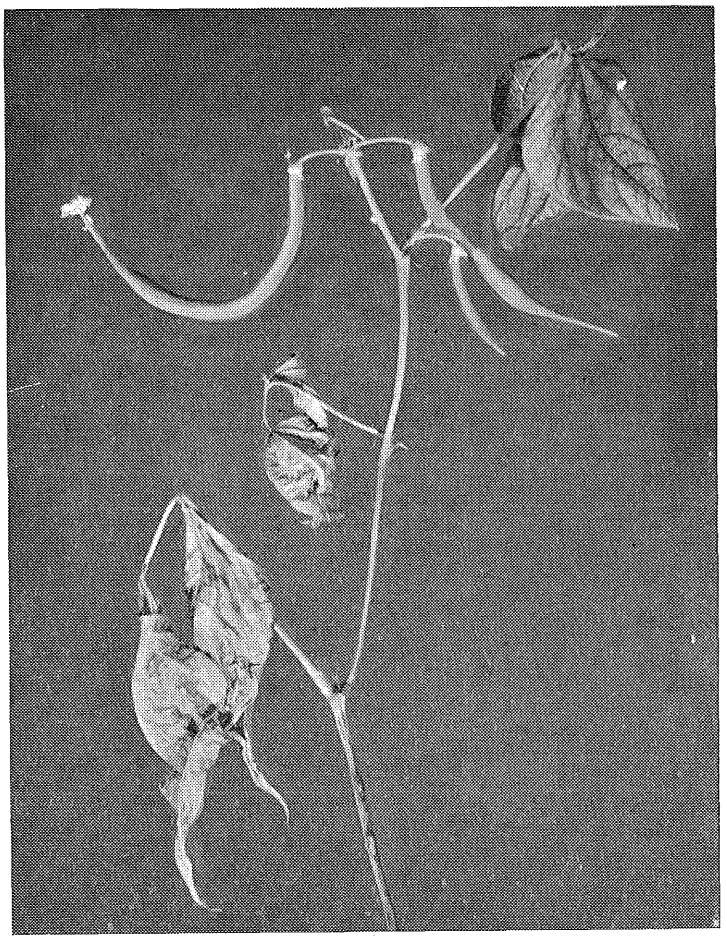

Fig. 4.—Premature withering of bean leaves following artificial inoculation with a pure culture of the "halo-less" halo blight organism at a position low on the stem.

VII. DISCUSSION

This study has demonstrated that the organism responsible for bacterial blight symptoms in North Queensland bean seed crops in 1966 resembles *P. phaseolicola* sufficiently to be classed as a particular strain of this pathogen.

There are a number of references in the literature to isolates of *P. phaseolicola* which could not be induced to cause haloes on bean leaves. Such a strain was first documented by Jensen and Livingston (1944), and others were isolated by N. Hubbeling in Wageningen and W. D. F. Hagborg in Winnipeg (Randolph and Stahmann 1966). Patel and Walker (1965) worked with similar forms. Those studied by Jensen and Livingston did not differ significantly from their halo-inducing strains in growth characteristics on artificial media, or in fermentation tests. This was not strictly so in the present case. Our isolates readily became

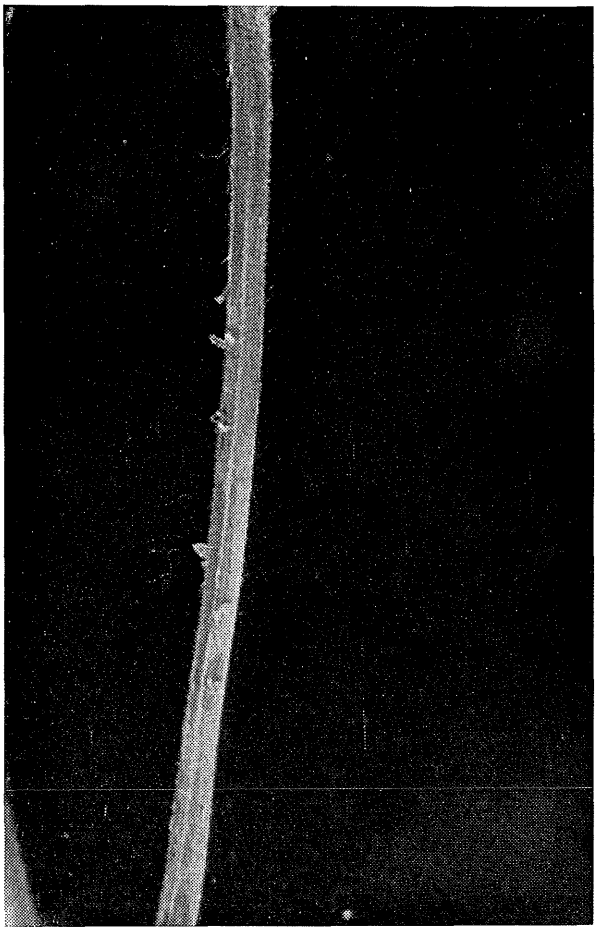

Fig. 5.—Exudate "curls" in close-up view—translucent structures which disintegrate on contact with water and yield almost pure cultures of *P. phaseolicola* when streaked out on nutrient agar.

systemic in susceptible bean varieties, which was not the case with Jensen and Livingston's isolates. Patel and Walker did not report a systemic phase. Here, then, is further evidence of the variability of *P. phaseolicola.*

Despite references in the literature to non-halo-inducing strains, no description of a widely occurring disease wholly incited by such a strain has come to the writer's notice. Since the present study was undertaken further occurrences have been noted. The varieties Red Kidney, Redlands Autumncrop and Redlands Greenleaf have been affected in various localities throughout the State. Tendercrop beans grown for processing at Bundaberg, and raised from seed produced in the United States, were infected, suggested that the pathogen was imported on this occasion with the seed. Red kidney beans grown in 1967 from seed produced in the previous season on the same farm were infected despite very severe

TABLE 1

SYMPTOMS PRODUCED BY THREE PSEUDOMONADS ON FOUR VARIETIES OF *Phaseolus vulgaris*
Five plants. Leaf spot symptoms at 8 days. Systemic symptoms recorded after 15 days

Variety	Known Halo Blight Reaction	*P. syringae*	*P. phaseolicola*	Test *Pseudomonas*
Brown Beauty	Susceptible	Typical spotting and distortion of young leaves. Slight spotting of older leaves	Typical severe halo blight symptoms. Leaf spots to 5 mm in diam. Later becoming systemic, causing wilting	Pin-prick size translucent spots with bright margin, leaf edge erosion and distortion on young leaves. Tiny dark spots on older leaves. Later becoming systemic, causing wilting
Tendercrop	Susceptible	Severe spotting of young leaves and larger grey-brown blotches on older leaves	Typical severe halo blight symptoms. Leaf spots up to 5 mm in diam. with some distortion. Later becoming systemic, causing wilting	Small translucent leaf spots, some to 1 mm in diam. Later becoming systemic, causing wilting
Epicure	Field resistant*	Typical spotting and distortion on young leaves	Severe halo blight symptoms on leaves. Not systemic	Pin-prick size translucent spots with bright margin, quickly turning brown. Distortion of younger leaves. Not systemic
Pinto	Field resistant**	No infection apparent	Small dark spots with typical halo symptoms. One plant developed larger leaf spots to 5 mm in diam. Not systemic	Pin-prick size translucent spots with bright margin on one plant only. Other plants developed no symptoms. Not systemic

* Information supplied by Rumseys Seeds Pty. Ltd., 331 Church Street, Parramatta, New South Wales. See also Noble (Pittman 1938).

** Some strains are listed by Patel and Walker (1965) as resistant (to race 1). Zaumeyer and Thomas (1957, p. 84) and Reid (1945) recorded Pinto as resistant to halo blight. Our line of Pinto was a mixture of genotypes with respect to leaf spot development, but all plants resisted systemic infection.

rogueing of the lightly infected seed crop. What appears to be the same kind of organism has recently been obtained from pure stands of *Glycine javanica* L., a plant now widely grown both for seed production and grazing. These isolates produced symptoms similar to those described here when transferred to bean plants in the glasshouse.

Spasmodic occurrences of what now must be regarded as the same disease have been observed on many occasions in recent years in southern Queensland, and until the present investigations were looked upon as something of a curiosity. Generally speaking, the disease is much less damaging then that caused by halo-inducing strains and pod spotting is not always present.

It is not difficult to conjecture how such strains of halo blight have become prevalent. Since most commercial seed crops are subjected to some form of visual inspection for the presence of blights, it is likely that the more obvious halo-inducing forms have been selectively eliminated.

In the light of the present widespread occurrence of this disease in Queensland, it is believed that a sufficiently good reason exists for distinguishing it from typical halo blight. Therefore it is proposed that the name "halo-less" halo blight, as was used by Jensen and Livingston (1944), be given to it. This will serve to identify for plant pathologists and field inspectors this not so obvious, though still undesirable form of halo blight.

VIII. ACKNOWLEDGEMENT

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