PATHOGENIC VARIATION IN *XANTHOMONAS ALBILINEANS* (ASHBY) DOWSON, THE CAUSAL AGENT OF LEAF-SCALD DISEASE OF SUGAR CANE

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Abstract

The comparative pathogenicity of four isolates of *X. albilineans* was examined on 13 varieties of sugar cane. The isolates could be differentiated on the basis of their aggressiveness on seven varieties. The significance of varying aggressiveness of isolates in the epiphytology of the disease, and in the screening of varieties, is discussed.

I. INTRODUCTION

Leaf-scald disease has been of considerable economic importance to the Australian sugar industry since at least 1900. The most efficient means of control is by the use of sugar cane varieties (*Saccharum* spp. hybrids) resistant to the disease. The decline of leaf scald as a major disease in northern Queensland in the 1960s has been correlated with a large increase in the proportion of resistant varieties grown (Egan 1971). The disease is present in epiphytotic proportions in restricted areas of the Mackay district of central Queensland, where the susceptible variety, Q.63, is widely cultivated.

In Queensland, all promising new seedlings are tested for their resistance to leaf-scald disease in special plots well isolated from commercial cane, either at Myola in the far north of the State, or at Eight Mile Plains in the south. The resistance ratings given to varieties in these trials, and their behaviour in the field should they be released as commercial canes, may be influenced by variation in *Xanthomonas albilineans*, the causal agent of leaf-scald disease.

Certain varieties of sugar cane show varying degrees of susceptibility to leaf-scald disease in different countries (Spence 1957). In Mauritius, the breakdown of the resistance of certain varieties has been attributed to the introduction or evolution of a new strain of the pathogen (Antoine and Perombelon 1965). Ricaud and Paulo (1971) compared eight Mauritian isolates and found differences in pathogenicity among them but no evidence of any variety–isolate specificity.

The work reported here is concerned with the comparative pathogenicity of four isolates of *X. albilineans* on 13 sugar cane varieties.

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The following terms are used according to the definitions proposed by Van der Plank (1968):

Pathogenicity—ability to cause disease in a particular host species,
Virulence—ability to cause disease in particular varieties of a certain host species,
Aggressiveness—a measure of disease intensity on a susceptible host.

II. Materials and Methods

(*a*) *Origin of Isolates*

The sources of the isolates of *X. albilineans* were:

No.	Variety	Origin
1	Q.63	Pleystowe, central district
2	Q.71	Tinana, southern district
3	Q.44	Babinda, northern district
4	Q.71	Yandina, southern district

Isolates were obtained from infected stalks, using the standard isolation procedures adopted in Queensland (Persley 1972). They were propagated on Wilbrink's agar (Dowson 1957) from single colonies.

They were similarly pale yellow and translucent in appearance on Wilbrink's agar medium, forming circular, low convex, smooth, glistening colonies. They were very slow-growing on first isolation, individual colonies being 0·5–1 mm in diameter after 10 days growth at 28°C.

(*b*) *Preparation of Inoculum*

Cultures 48 hr old were suspended in sterile distilled water to give a turbidity equivalent to tube 2 of the barium sulphate opacity standards of Brown (1920) (approximately 6×10^8 cells/ml).

(*c*) *Inoculation Methods*

The shoots were decapitated between the third and fourth dewlap and the inoculum applied with a 2-cm paint brush. This is essentially the method described by Koike (1965), except that no protective aluminium foil caps were used. Knives were sterilized between plots with 0·1% benzalkonium chloride solution.

(*d*) *Design of Trial*

The pathogenicity of the isolates was compared by simultaneously inoculating them into 4-month-old plant cane of each of 13 varieties and observing the disease development over 11 months. The varieties tested were N.Co.310, Pindar, P.O.J.2878, Q.44, Q.57, Q.58, Q.63, Q.66, Q.71, Q.78, Q.81, Q.87, and Trojan. There were three replicates and 20 stalks were inoculated per variety per replicate. The varieties and isolates made up a split-block design. Inoculum preparations were split plots within each whole plot.

(*e*) *Disease Assessment*

The scale devised for the 3-month count on leaves directly infected at decapitation ranged from 1, few streaks; 2, many streaks; through 3 and 4, few and many streaks with chlorosis; to 5 and 6, few and many streaks with necrosis of tissue; systemic developments were recorded as: 7, streaks; 8, streaks and chlorosis; and 9, streaks and necrosis. The systemic infection at 6 and 11 months was recorded on the basis of 1 for few streaks, 2 for many, 3 and 4 for few and many streaks with necrosis, 5 for chlorosis, 6 for chlorosis and necrosis, 7 for wilting, 8 for wilting and chlorosis, and 9 for death of the plant.

The results of the disease assessment at each time interval were statistically analysed. In considering the relative behaviour of varieties and isolates, two means were taken into account. These were:

Disease index—the average disease rating of a particular isolate on a variety at a given time.

Index of aggressiveness—the average disease rating over all varieties tested for a particular isolate at a given time.

III. Results and Discussion

The four isolates showed considerable variation in aggressiveness on several varieties. The agressiveness of each isolate, expressed in terms of an index, is given in Table 1. The details of the reaction of each isolate on individual varieties at the three time intervals are given in Table 2.

Table 1

INDICES OF AGGRESSIVENESS OF ISOLATES*

Isolate No.	Time after inoculation (months): 3	6	11
1	3·22	0·09	0·27
2	3·51	1·09	3·81
3	3·16	0·17	0·24
4	4·93	1·31	3·41
L.S.D.			
5% level	0·30	0·14	0·71
1% level	0·39	0·19	0·94

* Assessed as described in Section II(*e*).

The results indicate that the population of *X. albilineans* in Queensland is not a homogeneous one and that isolates differing in aggressiveness do exist. There were no instances of a variety being resistant to all but one isolate, nor of any isolate giving a significantly stronger or weaker reaction on a variety than that expected on the basis of its overall aggressiveness.

The existence of isolates of different aggressiveness has implications in the epiphytology of the disease and in screening varieties for resistance. It may explain the sometimes poor correlation between the behaviour of a variety in resistance trials and in the fields after its release as a commercial variety. Such anomalies have been noted in Queensland (Hughes *et al.* 1965), Hawaii (Anon. 1953), and Mauritius (Ricaud 1969). In these instances it may be that the aggressiveness of the isolates against which the varieties were screened was different from that of the dominant isolate(s) in the natural population.

Of particular interest in this regard is Q.57. This variety has shown some susceptibility in resistance trials in Queensland on several occasions but no leaf scald has been found on it in the field, although it has been widely cultivated for many years in northern Queensland, where the disease is endemic (Egan 1968). In the present work, Q.57 was susceptible to isolates of high aggressiveness, but resistant

TABLE 2

DISEASE INDICES* OF ISOLATES AT 3, 6, AND 11 MONTHS AFTER INOCULATION

Variety	Time after inoculation (months)	Isolate No.: 1	2	3	4
Pindar	3	1·87	1·97	1·93	3·96
	6	0	0·15	0	0·27
	11	0	0	0	0
N.Co.310	3	2·19	1·74	1·56	3·35
	6	0·04	0·22	0·07	0·43
	11	0	0	0	0
Q.58	3	2·57	0·83	3·33	2·66
	6	0·04	0·02	0·02	0·03
	11	0	0	0	0
Q.81	3	3·23	4·26	4·09	5·55
	6	0	0·31	0·05	0·71
	11	0	0·11	0·02	0·12
Trojan	3	4·61	4·68	4·49	5·27
	6	0·04	0·37	0·06	0·92
	11	0·05	0·67	0	0·22
P.O.J.2878	3	0·93	0·56	2·05	3·06
	6	0	0	0	0·03
	11	0	0	0	1·23
Q.78	3	4·66	4·58	4·83	5·01
	6	0·16	0·61	0·10	0·20
	11	0	0·55	0	0·32
Q.57	3	4·64	4·73	3·44	5·25
	6	0·09	1·12	0·14	1·33
	11	0	3·22	0	1·82
Q.63	3	3·47	4·51	2·13	5·78
	6	0·04	0·87	0·20	1·56
	11	0	4·63	0	5·05
Q.71	3	2·68	4·72	2·25	5·38
	6	0·09	0·58	0·10	0·55
	11	0	6·65	0·02	5·40
Q.87	3	2·23	3·55	2·08	5·57
	6	0·05	1·28	0·07	0·97
	11	0	7·53	0·12	6·32
Q.66	3	4·00	3·69	3·65	5·61
	6	0·38	3·07	0·41	3·37
	11	0·38	7·04	0·38	5·78
Q.44	3	4·81	5·85	5·30	7·70
	6	0·29	5·60	0·95	6·71
	11	2·22	7·75	1·86	7·89

* Assessed as described in Section II(*e*). Least significant differences at the 5% level: 1·07 (3 months), 0·51 (6 months), and 1·59 (11 months); at the 1% level: 1·42 (3 months), 0·67 (6 months), and 2·09 (11 months).

to those of low aggressiveness. The dominant isolate(s) in the areas where Q.57 is grown may be of low aggressiveness. If this were so, it would account for the field resistance of the variety. Perhaps in support of this hypothesis is the observation that both the isolates of high aggressiveness came from southern districts, while those of low aggressiveness came from northern and central districts. However, a comprehensive survey is required before any conclusions may be drawn about the geographical localization of isolates of different aggressiveness.

The "breakdown" in the resistance of a variety may be due to a change in the composition of the population of the pathogen, resulting in the dominance of a more aggressive isolate. Ricaud and Paulo (1971) consider that such behaviour is responsible for the present epiphytotic of leaf scald in Mauritius, in which several varieties previously considered to be resistant have developed the disease.

The population of a plant pathogen is considered to be in a dynamic state. However, if neither the medium for growth nor the environment is changed there will be little variation in the frequency of the components (Watson 1970). This is not the case with sugar cane, nor with many other crop plants in which the cultivation of resistant varieties, and the change in the varietal composition in an area with time, impose pressures on the pathogen to adapt to a new situation.

It seems preferable to use isolates of high aggressiveness when screening varieties for resistance. Screening against isolates of low aggressiveness could result in the wide-scale propagation of varieties which are susceptible to isolates of higher aggressiveness. Should the latter isolates become dominant in the natural population, the so-called resistant varieties will develop the disease.

Another observation which has relevance to resistance trials is the influence of time of counting on the rating of a variety on a disease scale. It was found impossible to distinguish between resistant and susceptible varieties at 3 months after inoculation, when only the symptoms on the cut directly inoculated leaves were apparent. This is in agreement with the work of Egan (1968). He suggested that a final rating may be given on the basis of systemic symptoms 12–15 weeks after inoculation.

The development of disease over 11 months was not the same in all varieties. Some, such as Q.71 and Q.87, showed a noticeably late development as evidenced by a sharp rise in the final count.

A count at least several months after inoculation by the technique used here is necessary to differentiate between those varieties which initially show mild systemic symptoms but recover, e.g. Trojan, and those which similarly show mild symptoms at an early stage, but later have much chlorosis and death, e.g. Q.87. The former is a potential carrier of the disease, while the latter could cause considerable loss in commercial plantings. This procedure has been adopted in Queensland in recent years.

The varieties Q.44, Q.57, Q.63, Q.66, Q.71, and Q.87 show some promise for use in differentiating isolates. In general, varieties rated as susceptible or intermediate in reaction are more likely to be of value in differentiating isolates on the basis of aggressiveness than those of high resistance, since the most easily discerned differences among the Queensland isolates are in their ability to cause mild or severe systemic symptoms in the host.

Varieties rated as resistant in Queensland may be useful in detecting pathological specialization in *X. albilineans*. The resistance of some varieties in one country and

their susceptibility in another is circumstantial evidence that such specialization occurs. However, the direct comparison of isolates from different countries is required to establish if this is the case.

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V. References

Anon. (1953).—*A. Rep. Hawaiian Sug. Plrs' Ass. Exp. Stn* 1953, p. 23.

Antoine, R., and Perombelon, M. (1965).—Leaf scald. *Rep. Maurit. Sug. Ind. Res. Inst.* 1964, pp. 56–8.

Brown, H. C. (1920).—Further observations on standardization of bacterial suspensions. *Indian J. med. Res.* **7,** 238–50.

Dowson, W. J. (1957).—"Plant Diseases due to Bacteria." 2nd Edn. (Cambridge University Press.)

Egan, B. T. (1968).—Evaluation of the aluminium cap method for leaf-scald disease resistance testing in Queensland. Proc. 13th int. Congr. Soc. Sug. Cane Technol. pp. 1153–8.

Egan, B. T. (1971).—The decline of leaf scald as a major disease in northern Queensland. Proc. 38th Conf. Qd Soc. Sug. Cane Technol. pp. 157–61.

Hughes, C. G., Steindl, D. R. L., and Egan, B. T. (1965).—Leaf scald. *A. Rep. Bur. Sug. Exp. Stns Qd* **65,** 70–1.

Koike, H. (1965).—The Al-cap method for testing sugar-cane varieties against leaf scald disease. *Phytopathology* **55,** 317–19.

Persley, G. J. (1972).—Isolation methods for the causal agent of leaf-scald disease. *Sug.-cane Pathologists Newsl.* No. 8, p. 24.

Ricaud, C. (1969).—Leaf scald. *Rep. Maurit. Sug. Ind. Res. Inst.* 1968, pp. 55–7.

Ricaud, C., and Paulo, M. (1971).—Leaf scald. *Rep. Maurit. Sug. Ind. Res. Inst.* 1970, pp. 87–92.

Spence, J. A. (1957).—The incidence of leaf scald disease of sugar cane in the West Indies. Proc. 57th Meet. Br. W. Indies Sug. Technol. pp. 140–6.

Van der Plank, J. E. (1968).—"Disease Resistance in Plants." p. 16. (Academic Press: New York.)

Watson, I. A. (1970).—Changes in virulence and population shifts in plant pathogens. *A. Rev. Phytopath.* **8,** 209–30.