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Bacterial blotch of melons caused by strains of *Acidovorax avenae* subsp. c*itrulli*

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Summary. The watermelon fruit blotch organism (*Acidovorax avenae* subsp. *citrulli*) was confirmed as the cause of a bacterial disease of rockmelon seedlings in January 1996. Further outbreaks occurred in commercial nurseries during 1996–98. An associated field disease was not observed in rockmelon and honeydew crops until May 1998 when wet conditions led to severe leaf spotting and fruit infection in many crops in the Burdekin district of North Queensland. Isolates of *A. avenae* subsp. *citrulli* originating from these outbreaks were considerably more pathogenic to rockmelon plants than isolates originating from watermelon crops in South Queensland. They were also less pathogenic to the weed host *Cucumis myriocarpus* and could constitute new strains of the fruit blotch organism. Tests showed the disease was readily seed transmissible from naturally infected rockmelon and honeydew fruit for at least 3 months after seed extraction.

Additional keywords: watermelon, fruit blotch.

Introduction

Melon production is an important (A$40 million) horticultural industry in Queensland. Major production areas are in North Queensland (Ingham, Ayr, Bowen), Central Queensland (Emerald, Bundaberg) and South Queensland (Chinchilla, Gatton). A mix of 3 melon types: watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), rockmelon (*Cucumis melo* L. var. *reticulatus* Ser.) and honeydew (*Cucumis melo* var. *inodorus* Jacq.) is grown in each district but rockmelon and honeydew predominate in North Queensland. Almost all rockmelon and honeydew crops are initiated from nursery-produced seedlings and grown on plastic mulch with trickle irrigation. Similar production methods are used for many watermelon crops although some farms still practise direct sowing with either overhead irrigation or just rainfall.

A fruit blotch disease of watermelon was observed at Bowen in November 1967. Although identified as *Pseudomonas* sp. (BRIP 17775) a colour photograph taken of an affected fruit (Vock 1978) clearly shows the symptoms are similar to the disease now attributed to the bacterium *Acidovorax avenae* subsp. *citrulli (*Schaad *et al.*) Willems *et al*. The disease was again observed in watermelon crops in 1975, 1986 and 1995.

Over the past decade, bacterial fruit blotch has been a disease of major concern in watermelon production areas in the USA (Latin and Hopkins 1995). In many ways, the sporadic nature of outbreaks since Crall and Schenck (1969) described the disease in experimental plots at Leesburg, Florida, is similar to its occurrence in Queensland crops. A report of fruit blotch severely affecting watermelon crops in the Mariana Islands in 1987 (Wall and Santos 1988) was, however, soon followed by several reports describing an increasing prevalence in USA production areas (Latin and Rane 1990; Somodi *et al.* 1991; Evans and Mulrooney 1991; Jacobs *et al*. 1992; Black *et al.* 1994; Hamm and Spink 1997).

Although other cucurbits are known to be susceptible, extensive field losses have been restricted to watermelon (Latin and Hopkins 1995). Symptoms on rockmelon fruit have been described as water-soaked pits on the fruit surface (Latin 1996) while on honeydew fruit, lesions are circular, 3–10 mm diameter, not extending into the flesh (Isakeit *et al.* 1997).

In January 1996, rockmelon seedlings in a commercial nursery in Bowen showed water-soaked bacterial lesions on cotyledons and leaves. In some cases seedlings died, while in others, affected areas dried to

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become light brown leaf spots. Symptoms were similar to those reported for watermelon seedling infection by *A. avenae* subsp. *citrulli* (Latin and Hopkins 1995). The disease spread rapidly and also affected watermelon seedlings in the same nursery. During 1996–97, although several similar outbreaks occurred in 3 commercial nurseries, there was no resultant field disease.

In February–March 1998, rockmelon seedlings (cvv. Dubloon, Eastern Star) showed symptoms in a Bowen nursery. In May, a bacterial disease was noticed in several rockmelon and honeydew crops in the Ayr–Bowen district. Infected areas on leaves were at first water-soaked, often with a thick white bacterial ooze during showery conditions, then became dark brown and finally dried to light brown, papery dead tissue. Lesions were frequently alongside veins and around margins. Symptoms on young fruitlets included water-soaking over much of the surface and also of internal tissues. Such fruitlets were often aborted. Infection of fruit at a later stage of development resulted in small, depressed infection points surrounded by a water-soaked area which, in rockmelon, later failed to develop netting. Infection extended to the flesh causing a reddish-brown discolouration in both rockmelon and honeydew. Cavities often developed in the affected tissue, particularly under the rind. Affected areas were firm until secondary organisms caused rapid breakdown of internal tissues. Varieties affected included rockmelon cultivars Eastern Star, Dubloon, Hammersley and honeydew cultivars Casper, Honeybabe and Dew Sweet.

In this report we present evidence that strains of *A. avenae* subsp. *citrulli* with increased virulence to rockmelon and honeydew are the cause of the bacterial disease now affecting these crops in North Queensland.

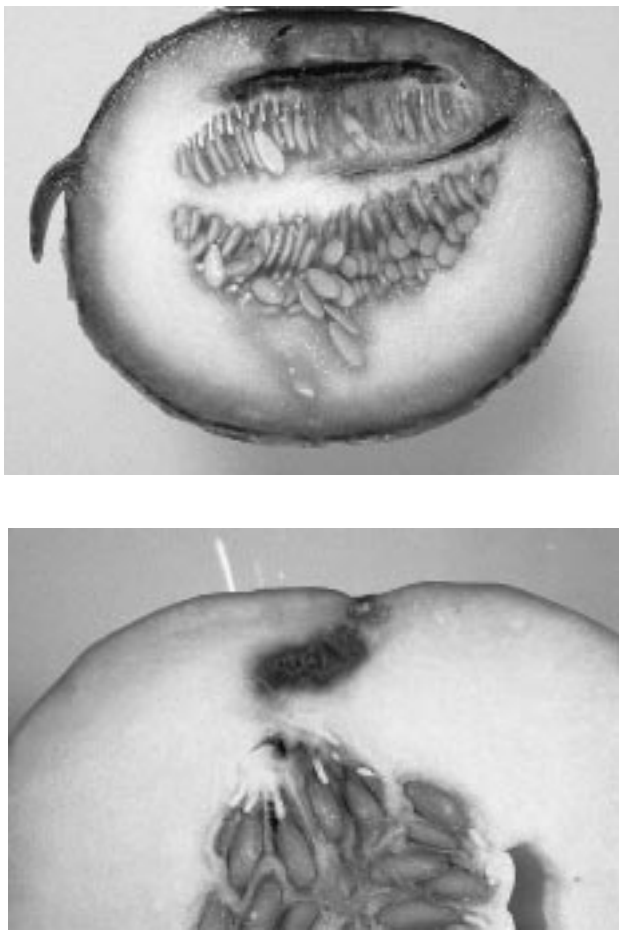

Figure 1. Fruit infection in immature rockmelon (top) and mature honeydew (below).

Materials and methods

Pathogen isolation and identification

Bacterial isolations were made from seedling leaves as well as leaves and fruit (Fig. 1) from field grown plants. Small pieces of surface-sterilised leaf or fruit tissue were macerated in drops of sterile water then streaked on King's medium B agar (KB) (King *et al*. 1954). On this medium, *A. avenae* subsp. *citrulli* produces slow-growing, circular, white colonies which are non-fluorescent under near ultraviolet light. Single colonies with these characteristics were selected and identified using the Biolog system (Biolog, Hayward, CA, USA).

Seed-borne transmission

Rockmelon fruit (cv. Eastern Star) and honeydew fruit (cv. Honeybabe) showing advanced symptoms of fruit blotch, but without secondary breakdown, were collected from fields in the Ayr district in May 1998. Seed was extracted, thoroughly washed in running tap water to remove pulp then dried. After a storage period of 14 weeks, 100 seeds of each were sown on UC mix in seedling flats, covered with 1 cm of coarse vermiculite and wet to capacity. A clear plastic cover was placed over each tray, which was then incubated in a controlled environment cabinet at 25^{o}C. Infection counts were made during the period 6–11 days after sowing as cotyledons expanded. At each inspection, the covers were removed at least 1 h earlier to allow dispersal of natural water soaking on leaves, which can occur under conditions of high humidity. Seedlings with permanently water-soaked lesions were removed and a sample checked to confirm the presence and identity of bacteria.

Strain differentiation

Biolog reactions. The Biolog system of identification of bacteria is based on different bacterial species having characteristic abilities to utilise (oxidise) 95 different carbon sources. The test is carried out in microplates with a different carbon source lining each of 95 wells. Utilisation of a carbon source is indicated by a colour change. During identification of isolates, consistent differences were observed between isolates in

their abilities to utilise particular carbon sources in the Biolog GN MicroPlates. Since this could be a simple way to differentiate strains, we compared 6 isolates collected from South Queensland with 16 collected from nurseries and fields in North Queensland. Isolates in both groups were collected over a 2-year period. Identifications were made using the standard format for Biolog identification of Gram-negative bacteria. Cultures were grown on the Biolog Universal Growth Medium for 24 h, then saline (0.85% NaCl) suspensions containing about 3×10^8 cfu/mL were prepared and dispensed to GN MicroPlates. After incubation for 24 h at 30°C, microwells were examined for colour changes.

Pathogenicity to rockmelon and watermelon. Seedlings of 2 watermelon cultivars (Hercules, Candy Red) and 2 rockmelon cultivars (Hyline, Planters Jumbo) were germinated in vermiculite then transplanted to 12 seedling flats of UC mix. Each flat contained a 5 plant row of each cultivar. When the first true leaves were emerging, 4 replicate flats were inoculated with either a bacterial suspension of isolate 4391 (ex. watermelon, Chinchilla); isolate 4412 (ex. rockmelon, Bowen) or sterile water. Inoculum strength was about 1×10^8 cfu/mL in sterile distilled water with 1 drop of Tween 80 added per 100 mL. Inoculum was derived from 48-h cultures grown on KB. Plants were inoculated to surface wetness with a Preval sprayer and each flat covered for 48 h with a clear plastic cover. None of the plants were deliberately wounded and we did not try to cause the inoculum to infiltrate the leaves. Glasshouse temperatures ranged from 14 to 25°C. After 7 days, each cotyledon was rated for disease severity on a 0–5 scale where 1 is <10% leaf area affected and 5 is >75% leaf area affected.

A similar experiment was conducted incorporating a wider range of isolates and cultivars. Two watermelon cultivars (Minilee, Candy Red), 2 rockmelon cultivars (Early Dawn, Hyline) and 1 honeydew (Green Flesh) were pregerminated and transplanted to seedling flats. Each flat contained 4 plants of each variety and was inoculated by 1 of the following isolates: 4412, 4425, 4397 (all ex. North Queensland); 4391, 4884, 4885, and 4886 (all ex. South Queensland). There were 4 replications. The inoculation and incubation procedures were similar to the previous experiment.

Watermelon (cv. Crimson Sweet) and rockmelon (cv. Planters Jumbo) seeds were germinated in vermiculite, then seedlings transplanted 1 per 12.5-cm-diameter pot and raised in a glasshouse until the first true leaf was about 2 cm diameter. A 5 pin inoculator injured the cotyledons and true leaf, then leaves were spray- inoculated to wetness with either sterile water, or a suspension (1×10^8 cfu/mL) of isolate 4391; 4412; 4397 (ex. watermelon, Bowen) or 4425 (ex. rockmelon, Bowen). Plants were kept at high humidity for 30 h and rated for disease severity (0–5) 8 days after inoculation. Each treatment had 5 replicate plants.

Pathogenicity to prickly paddy melon (Cucumis myriocarpus). *Cucumis myriocarpus* is a weed host of *A. avenae* subsp. *citrulli* in South Queensland (unpublished data). Seed was collected from field plants, germinated and plants grown individually in 12.5-cm-diameter pots until they were in early flower production. Two plants were inoculated with either sterile water or suspensions of isolates 4391, 4885, 4887 (ex. South Queensland); 4397; 4999 or 5017 (ex. North Queensland). Inoculated plants were incubated at high humidity for 48 h then observed for symptom development over 4 weeks.

Results

Pathogen isolation and identification

A slow-growing, white, non-fluorescent, Gram-negative bacterium was consistently associated with the disease in seedlings of watermelon, rockmelon and honeydew as well as leaves and fruit from field crops in the Ayr–Bowen district. It was also present in leaves and fruit of field-grown watermelons, as well as leaves of the weeds prickly paddy melon *(C. myriocarpus*) and pie melon (*Citrullus lanatus)* in South Queensland. All isolates were confirmed as *A. avenae* subsp. *citrulli* by Biolog with a similarity of 0.74–0.98 (Table 1).

Seed-borne transmission

Seed collected from the naturally infected rockmelon and honeydew fruit in May 1998 gave seedling emergence values of 94 and 69% respectively. Of the emerged seedlings, 91 and 33% showed blotch symptoms within 11 days of sowing. The presence of *A. avenae* subsp. *citrulli* was confirmed in samples of affected seedlings.

Strain differentiation

Biolog reactions. Although there was some variability in the coloration of particular microplate wells, 2 carbon sources consistently separated isolates from North Queensland and South Queensland (Table 1). All 16 isolates tested from North Queensland did not cause colour change in well G-3 (L-leucine) within 24 h, while all 6 isolates from South Queensland caused the expected strong coloration indicating high ability to utilise L-leucine. Conversely, South Queensland isolates appeared unable to utilise 2-amino ethanol, while North Queensland isolates caused a strong reaction in the H-7 GN MicroPlate well.

Pathogenicity to rockmelon and watermelon. Control plants showed no symptoms and these results were omitted from the statistical analysis (i.e. ANOVA, Genstat 5). The results show that both isolates caused equally severe symptoms on the 2 watermelon cultivars but only isolate 4412 caused damage to the 2 rockmelon cultivars (Fig. 2). With this isolate, cv. Hyline was significantly ($P<0.05$) more susceptible than the 3 other cultivars.

Inoculation of the 3 melon types with the 3 North Queensland isolates caused symptoms on rockmelon and honeydew seedlings at least as severe as those on the 2 watermelon cultivars (Table 2). With the 4 isolates from South Queensland, disease severity on the watermelon cultivars and the honeydew cultivar was significantly ($P<0.05$) higher than on the 2 rockmelon cultivars. For isolates 4391 and 4884, the disease severity on cv. Green Flesh was not significantly

Table 1. Ability of 22 isolates of *A. avenae* subsp. *citrulli* (A.a.c.) to utilise L-leucine and 2-amino ethanol as determined by their reactions in Biolog GN MicroPlates after 24 h incubation

Isolate and host	Ability to utilise 2 carbon sources		Similarity to A.a.c.
	L-leucine	2-amino ethanol	
Isolates from South Queensland			
4391 ex. watermelon	+	—	0.81
4884 ex. *Cucumis myriocarpus*	+	—	0.98
4885 ex. *Citrullus lanatus*	+	—	0.77
4886 ex. watermelon	+	—	0.86
4887 ex. *Cucumis myriocarpus*	+	—	0.92
4888 ex. *Citrullus lanatus*	+	—	0.83
Isolates from North Queensland			
4397 ex. rockmelon	—	+	0.74
4412 ex. watermelon	—	+	0.81
4425 ex. watermelon	—	+	0.81
4495 ex. rockmelon	—	+	0.77
4936 ex. rockmelon	—	+	0.78
4995 ex. rockmelon	—	+	0.77
5014 ex. rockmelon	—	+	0.87
5015 ex. rockmelon	—	+	0.75
5016 ex. rockmelon	—	+	0.81
5017 ex. rockmelon	—	+	0.78
5019-1 ex. honeydew melon	—	+	0.83
5019-2 ex. honeydew melon	—	+	0.77
5037 ex. honeydew melon	—	+	0.68
5039 ex. honeydew melon	—	+	0.77
5046 ex. rockmelon	—	+	0.87
5084 ex. rockmelon	—	+	0.75

different from that on both watermelon cultivars. For isolates 4885 and 4886, it was significantly less than on cv. Minilee but not different from cv. Candy Red.

Isolate 4391 from South Queensland caused significantly (P<0.05) more damage to watermelon cv. Crimson Sweet than isolates 4412 or 4425 but was non-pathogenic to rockmelon cv. Planters Jumbo. Isolates 4397, 4412 and 4425 caused severe symptoms on the rockmelon plants (Table 3).

Pathogenicity to prickly paddy melon (Cucumis myriocarpus). Although all isolates were pathogenic to *C. myriocarpus*, isolates 4397, 4999 and 5017 caused infections only on young leaves. Isolates 4391, 4885 and 4887 caused severe symptoms of water soaking and, eventually, leaf spotting on both young and old leaves (Fig. 3). After 4 weeks in a glasshouse, disease symptoms caused by the first group of isolates were barely discernible while plants inoculated with the second group showed severe leaf spotting.

Discussion

The identification of *A. avenae* subsp. *citrulli* as a damaging pathogen of rockmelon and honeydew, shows it must now be considered as a serious threat to all melon crops rather than just watermelons. The severity of symptoms on rockmelon and honeydew fruit in North Queensland fields exceeds that previously described.

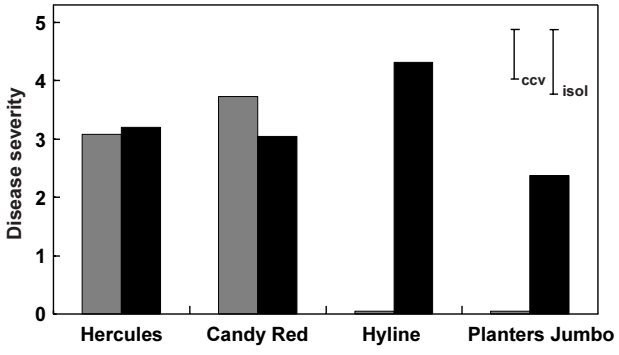

Figure 2. Disease severity in watermelon (cvv. Hercules and Candy Red) and rockmelon (cvv. Hyline and Planters Jumbo) seedlings inoculated with 2 isolates of *A. avenae* subsp. *citrulli*. Isolate 4391 is from South Queensland (shaded bars) and 4412 is from North Queensland (solid bars). Vertical bars represent l.s.d. (P = 0.05) between cultivars (cvv) and between isolates (isol).

Table 2. Severity (0–5 scale) of blotch symptoms on watermelon, rockmelon and honeydew seedlings following inoculation with three isolates of *A. avenae* subsp. *citrulli* from North Queensland and four isolates from South Queensland

The l.s.d. values allow comparison between cultivars for each isolate

Cultivar	North Queensland isolates			South Queensland isolates			
	4412	4425	4397	4391	4884	4885	4886
			Watermelon				
Minilee	3.75	3.92	4.05	4.92	4.80	4.95	4.87
Candy Red	2.85	3.50	3.50	4.70	4.37	4.70	4.15
			Rockmelon				
Early Dawn	3.77	3.72	4.05	2.92	2.25	2.55	2.15
Hyline	4.57	4.32	4.75	2.20	1.72	1.52	1.55
			Honeydew melon				
Green Flesh	4.37	4.75	4.72	4.27	3.70	3.67	3.65
l.s.d. (P = 0.05)	0.77	0.70	n.s.	0.94	1.20	1.10	0.58

The change has been brought about by the development of new strains with increased virulence to honeydew and rockmelon. These strains were initially found in nurseries, suggesting seed-borne introduction, and we have shown that, as for watermelon (Frankle *et al.* 1993), the disease is readily seed-borne in naturally infected rockmelon and honeydew fruit. Whether the bacteria are carried within the seed coat, as well as externally, was not determined but propagules remained viable during 3 months storage of the seed at room temperature. The reason outbreaks have been observed only in North Queensland could be due to the particular conditions in North Queensland nurseries which are, perhaps, more favourable for disease spread. Seedlings are grown on raised benches but, unlike most other nurseries, there are no overhead shelters. This allows long periods of leaf wetness to occur. In addition, irrigation is by overhead boom which favours splash dispersal of inoculum.

Table 3. Disease severity (0–5 scale) caused by four isolates of *A. avenae* subsp. *citrulli* to watermelon cv. Crimson Sweet and rockmelon cv. Planters Jumbo seedlings following wound inoculation

Isolate and host	Crimson Sweet	Planters Jumbo
	Isolate from South Queensland	
4391 ex. watermelon	3.5	0.5
	Isolates from North Queensland	
4397 ex. rockmelon	2.1	3.5
4412 ex. rockmelon	1.3	3.3
4425 ex. watermelon	1.4	3.4
Water control	0	0
l.s.d. (P = 0.05)	1.5	0.6

Wen *et al.* (1997) showed isolates collected from North Queensland nurseries could be distinguished from South Queensland watermelon isolates on the basis of genomic DNA fingerprinting analysis. Both groups of isolates could also be distinguished from 5 isolates of US origin. These genetic differences between the 2 groups of Queensland isolates can be characterised by differences in ability to use the carbon sources L-leucine and 2-amino ethanol, as well as differences in pathogenesis to rockmelon, watermelon and the weed species *C. myriocarpus*. The low pathogenicity of watermelon isolates such as 4391 to rockmelon in our tests may explain why *A. avenae* subsp. *citrulli* has previously been considered a threat only to watermelons.

Isolates collected from North Queensland nurseries and fields over a 2-year period, had similar pathogenic characteristics, indicating that strains involved in the 1998 field epidemic are similar to those collected from nurseries as early as January 1996. It is clear that weather conditions that allow bacterial colonisation of leaves, and later, flowers and fruit, are a requirement for field expression of this disease. During 1996 and 1997, infected seedlings from North Queensland nurseries failed to initiate field epidemics due to dry field conditions, but in 1998 wet conditions favoured infection, with substantial losses. Hopkins (1993) has made similar observations in watermelon crops. It is interesting to note that the first recorded field infection of honeydew fruit also occurred in 1996 in an isolated field in Texas

Figure 3. Representative plants of *Cucumis myriocarpus* showing greater susceptibility to the South Queensland isolate 4391 (right) than the North Queensland isolate 5017 (left).

(Isakeit *et al*. 1997). Although tests confirmed the pathogenicity of isolates to both honeydew and watermelon, information about the similarity of these isolates to others from watermelon was not presented. The described fruit symptoms were, however, less severe than those observed in North Queensland fields.

During 1996–98 we have detected no blotch in watermelon seedlings or crops which could be directly attributed to seed-borne contamination with a watermelon strain of *A. avenae* subsp. *citrulli.* This is probably due to the introduction of grow-out tests by seed companies to detect contaminated seed lots. In 3 Bowen nurseries, blotch has occurred on cotyledons of rockmelon and honeydew seedlings since January 1996 and has occasionally spread to watermelon seedlings (isolates 4412, 4425). Only the rockmelon strain has been isolated from nurseries. The apparent success of grow-out tests as a method for limiting the occurrence of the disease in watermelons suggests that consideration should now be given to its introduction for screening rockmelon and honeydew seed lots.

Acknowledgments

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