VG97031
Management of bacterial fruit blotch of watermelon

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Queensland Department of Primary Industries

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VG97031

This report is published by the Horticultural Research and Development Corporation to pass on information concerning horticultural research and development undertaken for the vegetable industry.

The research contained in this report was funded by the Horticultural Research and Development Corporation with the financial assistance of the Queensland Fruit and Vegetable Growers.

Cover price: $22.00 (GST Inclusive)
HRDC ISBN 0 7341 0155 4

Published and distributed by:
Horticultural Research & Development Corporation
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Sydney NSW 2000

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HRDC Project No.: VG 97031

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Purpose of Project: Bacterial fruit blotch (*Acidovorax avenae* subsp. *citrulli*) has been a relatively minor disease of watermelons in Queensland with outbreaks occurring every few years. In 1996, severe outbreaks occurred in watermelon crops and we identified *A. avenae* subsp. *citrulli* as the cause of a seedling disease of melons in three Bowen nurseries. In 1997 (and subsequently) bacterial fruit blotch was a widespread and damaging field disease of watermelons, rockmelons and honeydew melons. The purpose of this project was to identify the factors involved in these outbreaks and find control strategies. Of particular interest was the extension of the host range from watermelon to include rockmelon and honeydew and the apparent seed-borne introduction of the disease.

Funding Sources: The Melon Sub-Committee of the Queensland Fruit and Vegetable Growers provided the funding for this project. Matching funds were contributed by the Horticultural Research and Development Corporation. The Queensland Department of Primary Industries provided staff and research facilities.

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INDUSTRY SUMMARY

Bacterial blotch of watermelon (*Acidovorax avenae* subsp. *citrulli*) has been a relatively minor disease in Queensland since the first recording in 1968. In 1996 we identified it in seedlings of watermelon, rockmelon and honeydew in nurseries at Bowen. With wet field conditions in 1997 and subsequently, bacterial blotch caused widespread losses in these crops. Our studies (1997-2000) were aimed at control of the disease. The major findings were:-

- There are at least two strains of the bacterium. We recognised a previously unreported strain which attacked rockmelon and honeydew as well as the traditional host, watermelon.

- The disease is seedborne. Seed collected from diseased fruit of rockmelon and honeydew produced high percentages of infected seedlings, even after six months storage. A large number of outbreaks occurred in commercial nurseries suggesting seed borne transmission. In most cases, nursery outbreaks originated in rockmelon seedlings. (Watermelon seed is screened for disease –rockmelon is not).

- Contaminated seed can be cleaned. Clean seed is essential for nursery plantings. A hot water treatment of 55°C for 20 minutes almost completely eradicated the bacterium. Seed should be sown within 48h. This treatment lowered the germination rate in low vigour seed lots but not in seeds of normal vigour.

- Field carryover is probable on weeds and volunteer seedlings. The weeds, prickly paddy melon and pie melon, are susceptible to blotch. We also found infected volunteer watermelon seedlings occurring naturally in fields. Studies showed buried crop trash was unlikely to be a source of contamination for later crops.

- Prevention is better than cure. Copper fungicides provide adequate control in low disease situations but are inadequate when extended wet weather prevails. Even low levels of foliar disease may lead to extensive fruit loss. An experimental fungicide (Bion) gave promising results for disease control in watermelon.

Further reading:

O'Brien, R.G. and Martin. H.L. (1999). Bacterial blotch of melons caused by strains of *Acidovorax avenae* sub.sp. *citrulli*. *Australian Journal of Experimental Agriculture* **39**: 479-85.

O'Brien, R.G. (1998). Bacterial fruit blotch of watermelon. QDPI Agdex 264/633.

TECHNICAL SUMMARY

In the last decade, bacterial fruit blotch caused by *Acidovorax avenae* subsp. *citrulli* has devastated watermelon crops in the USA. In Queensland, severe field outbreaks in melons occurred in 1996 in south Queensland and 1997 in north Queensland.

Identification of *Acidovorax avenae* subsp. *citrulli* as the cause of a bacterial disease in seedlings, indicated the field disease may be originating as a seedborne disease. In most cases, affected seedlings were rockmelon or honeydew rather than the usual host, watermelon. Comparison of isolates collected from melons in north Queensland with those from watermelons in south Queensland showed they could be differentiated into two types: a 'watermelon strain' with poor pathogenicity to rockmelon predominated in south Queensland. while a 'rockmelon strain' pathogenic to both watermelon and rockmelon predominated in north Queensland. The watermelon strain was much more pathogenic to the weed host, prickly paddy melon (*Cucumis myriocarpus*). The two strains could be differentiated by genomic DNA fingerprinting analysis as well as differential reactions in Biolog microplates.

Seed collected from field-infected fruit of rockmelon and honeydew produced infected seedlings after six months storage, confirming the seedborne nature of the disease. Hot water treatment (55°C for 20minutes) was more successful than chlorine dip in eradicating internal contamination. There was some loss of germination if treated seed was not sown within 48 h.

Although weeds such as prickly paddy melon (*Cucumis myriocarpus)* and pie melon (*Citrullus lanatus*) as well as volunteer melon seedlings were found carrying the disease, buried trash quickly lost its infective ability. Infected samples of leaves, rind and externally contaminated seeds buried in soil lost infectivity after two weeks.

Sprays of copper hydroxide, copper oxychloride and cupric ammonium carbonate significantly reduced the severity of the leaf spot phase of the disease but, under severe conditions, did not reduce the fruit blotch phase of the disease. Addition of ferric chloride failed to improve the performance of copper compounds. In glasshouse experiments, the systemic resistance promoter, Bion (0.05 – 0.1 g/L), significantly reduced disease severity in watermelon from 4.56 (untreated) to 0.44 on a 0-6 scale of disease severity. Bion was phytotoxic to honeydew and rockmelon.

In 1999, *Acidovorax avenae* subsp. *citrulli* caused a severe foliar disease in a cucumber crop suggesting a further extension of its host range. Further studies, using molecular techniques, should be undertaken to identify whether new strains are threatening a wider range of cucurbit crops.

1. GENERAL INTRODUCTION

Causal organism and symptoms. A photograph of bacterial fruit blotch on watermelon was published in the DPI's "Handbook of Plant Diseases" in 1978. The photograph was, however, taken in 1967 which verifies the first known Australian outbreak of the disease (at Bowen) occurred at the same time as the first published account of the disease by Crall and Schenck (1969) in Florida. The use of the descriptive name "bacterial fruit blotch" was also first carried in the DPI publication. The disease has therefore been known to occur in both Australia and the USA for over 30 years.

The bacterium causing fruit blotch is currently known as *Acidovorax avenae* subsp. *citrulli*. It is a gram negative non-fluorescent bacterium which produces small white circular colonies on Kings B medium. Prior to 1992 the fruit blotch disease was attributed to *Pseudomonas pseudoalcaligines* subsp. *citrulli*, a bacterium which Schaad *et al.* (1978) showed responsible for seedling lesions and fruit spots on watermelon. Willems *et al.* (1992) showed there were differences in fatty acid profiles between *P. pseudoalcaligines* and the organism causing fruit blotch symptoms in watermelon crops and renamed it *Acidovorax avenae* subsp. *citrulli*.

The bacterium can be seedborne and symptoms develop on cotyledons as they expand, about 7-10 days after sowing. In the humid environment of a seedling production area, the first symptom is watersoaking on the undersurface of cotyledons (Fig 1). Cucurbit cotyledons often have natural watersoaking at high humidity, but this will disappear once atmospheric relative humidity falls below 95%. Watersoaking caused by bacterial infection does not disappear. Diseased areas gradually dry out and turn brown over a period of days, often maintaining a water soaked margin. With continued high humidity, the infection will travel down the cotyledon to the hypocotyl to cause seedling collapse. We have noticed that the infected areas on watermelon cotyledons are generally darker than on rockmelon cotyledons (Fig.4).

In the field, foliar symptoms are not conspicuous. Leaf infection is seen as small brown, angular lesions which often extend along veins. Under most conditions these will be barely noticeable and will not give warning of damaging fruit losses which can occur later. If there are extended wet periods, leaf symptoms may become noticeable due to the high infection level. Watersoaking and a thick white bacterial exudate on infected areas are often seen. With returning dry conditions, leaf lesions become papery and torn. The bacterial exudate dries to a flaky encrustation. Heavily infected leaves appear tattered (Fig. 4). Infection may sometimes extend from the leaf to the petiole and stem. These infections occur as thin brown strands of infected tissue (Fig.2).

The most important phase of the disease is fruit infection. "Bacterial fruit blotch" is an accurate descriptive term for the occurrence on watermelon. On young fruit, the whole surface area may appear watersoaked. Such fruit usually drop off the vine. Infection of older watermelon fruit is seen as small watersoaked areas which enlarge rapidly under humid conditions to cover large areas of the fruit surface (Fig.3). The watersoaked blotch generally has an irregular shape and irregular margin. Internally, a reddish lesion occurs in the rind. This is of various shapes and extent but is typically in the outer rind beneath the skin. Cavities often form in the affected area . As fruit matures, lesions often become flecked with red brown areas and skin cracking may occur (Fig. 4). Until this stage, fruit are sound and firm. Once cracks allow entrance of secondary soft rot bacteria, the contents of the melon,

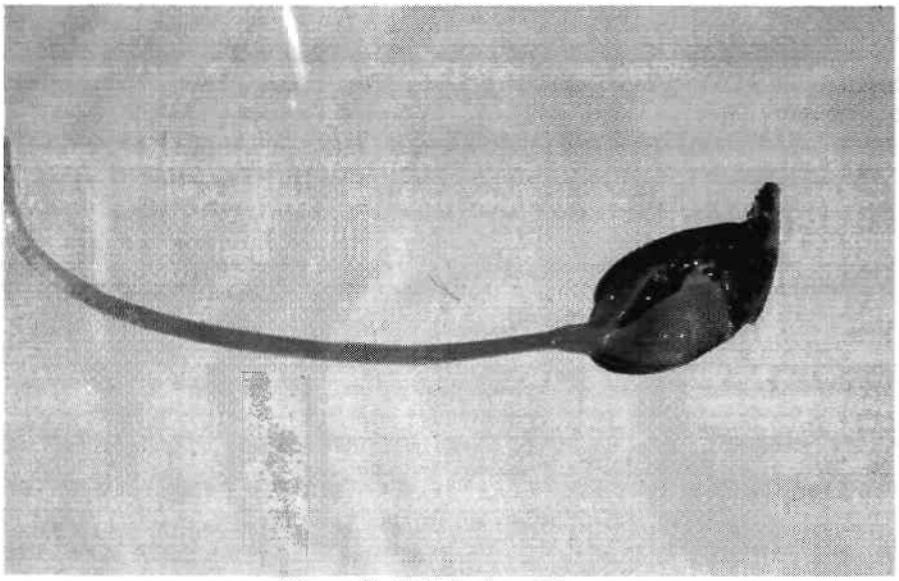


Figure 1. Infected seedling

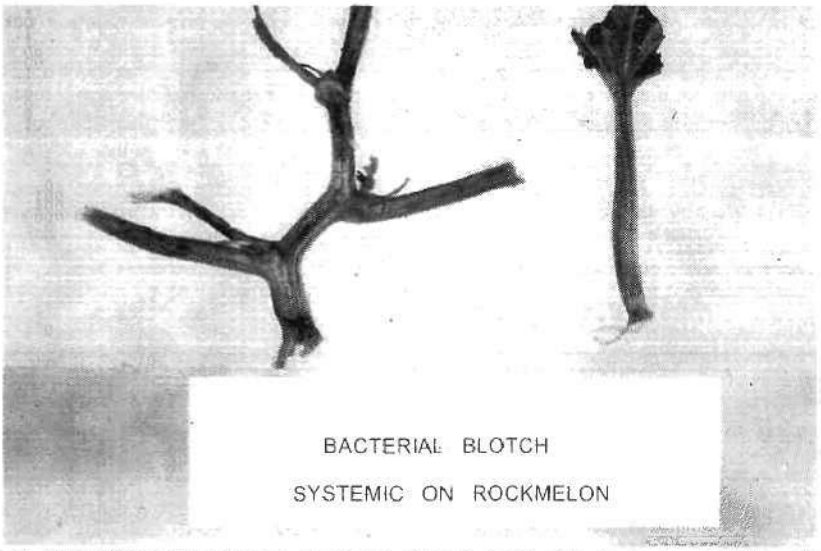


Figure 2. Infection of petioles and stems of rockmelon by *A. avenae* subsp. *citrulli*

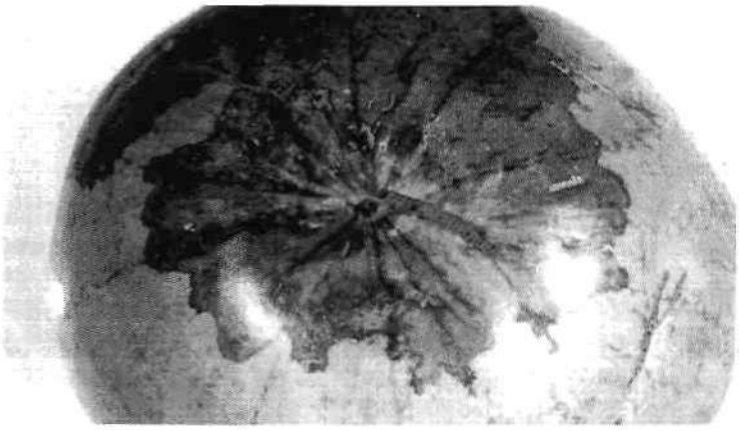
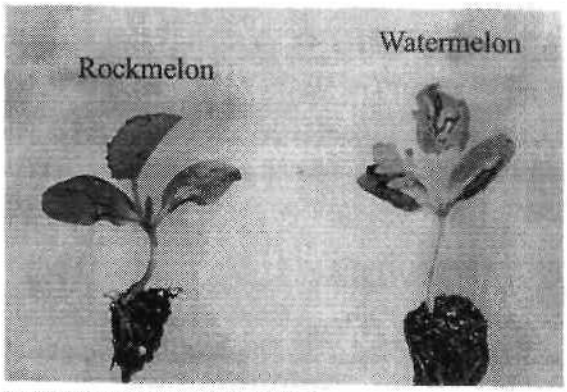
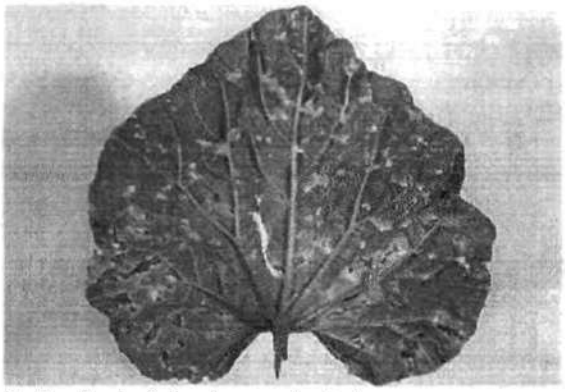


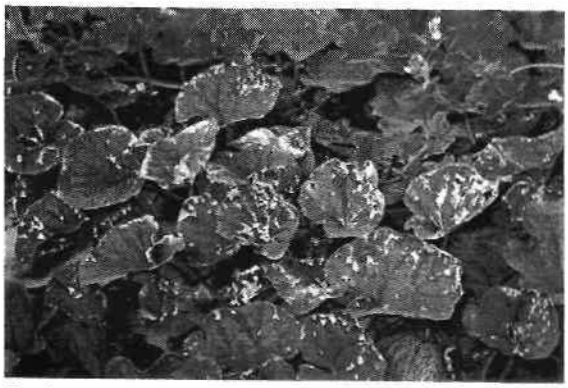
Figure 3. Watersoaking symptoms on watermelon fruit



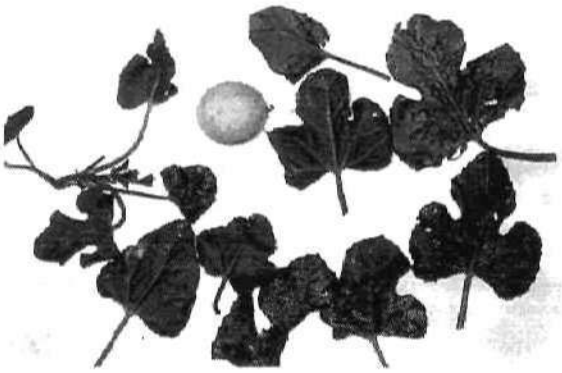
Spots are darker on watermelon



Rockmelon leaf showing angular lesions



Rockmelon - old infections

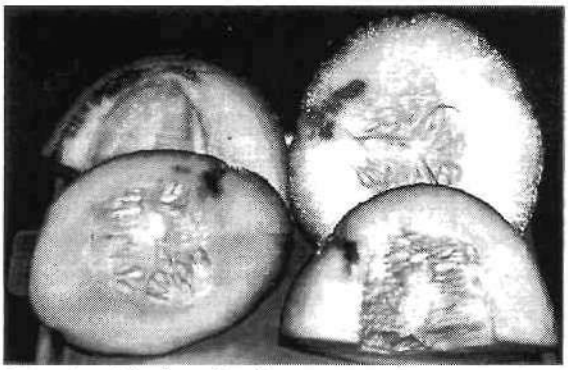


Prickly paddy melon - a susceptible weed

Blotch on young rockmelon fruit

Watermelon fruit with mature infection

Small lesions on mature rockmelon



Internal cavities in rockmelon

Figure 4. Symptoms of bacterial blotch on melons

breakdown and turn watery. Gas generated may cause a proportion of melons to rupture, with a loud hiss of liquid and gas escaping. These are known as 'exploding melons'.

Symptoms in rockmelon and honeydew fruit are different from those in watermelon due to differences in skin structure and, perhaps, susceptibility. In rockmelon, young fruit develop large watersoaked areas on the smooth green skin but once netting occurs, the lesion is much smaller. The infection site remains as a smooth island in the normal netted skin (Fig.4). It may be slightly depressed. In some fruit the surface blemish is small and difficult to detect when harvesting or grading. Internally, a firm red-brown infection in the rind may be quite extensive. Cavities often develop (Fig.4). Once again, breakdown does not occur until soft rot bacteria enter through skin cracks. Without careful scrutiny of harvested melons, apparently sound fruit will decay during marketing.

The smooth green flesh of honeydew fruit shows infection as localised lesions, somewhat similar to those of anthracnose (*Colletotrichum* sp.). Lesions are initiated as small watersoaked spots which develop into depressed, circular or elongated lesions, generally 1-2 cm diameter (Fig 5). The rockmelon strain may cause less extensive surface lesions than the watermelon strain but lesions extend deeper into the fruit to form cavities (Fig. 6).

Disease cycle. Studies by Frankle *et al.* (1993) showed that bacteria enter young watermelon fruit through stomata. As fruit mature, a wax layer is deposited on the skin which plugs stomata, making it more difficult for bacteria to gain entrance. Although no studies have been carried out with rockmelon and honeydew, it is suspected that young fruit of these melons would also be more susceptible to infection than older fruit. Once infection has occurred, internal development of the infection brings bacteria into close contact with developing seed.

Seed transmission of the disease was recognised in the field studies carried out by Goth and Webb (1975) and confirmed by Sowell and Schaad (1979). Contamination, either internal or external, can cause the seedling phase of the disease. Both Sowell and Schaad and Rane and Latin (1992) demonstrated a reduction, but not eradication, by surface sterilising seed coats with chlorine. The latter authors showed bacteria were present internally in embryo tissue.

A high proportion of seed from naturally infected watermelons will initiate infected seedlings. Rane and Latin (1990) found a transmission level of about 40%. In humid glasshouse conditions where seedlings are crowded together and irrigated by overhead sprays, spread of the disease is rapid. If seedlings are irrigated from below, disease spread is much slower, being dependent on plant to plant contact rather than water splash transmission of the bacteria (Hopkins, 1994).

In the field, transmission also depends on conditions of high humidity and rain splash dispersal. Foliar symptoms are usually not devastating to growth but the disease will spread rapidly if rainfall and temperature are favourable. Hopkins (1993) found one infected seedling enabled transmission to 40 out of 50 neighbouring plants. Fruit infection occurs as described previously and the disease cycle is completed.

Disease carryover from season to season on plant debris is unlikely as Rane and Latin (1990) could only recover the causal organism from buried trash for one week. Field carryover via infected seed from discarded fruit is, however, a distinct possibility. Cucurbit weed hosts may also perpetuate the disease and Isakeit *et al.* (1998) reported naturally occurring fruit

Figure 5. Honeydew with surface lesions caused by the watermelon strain of *A. avenae* subsp. *citrulli*

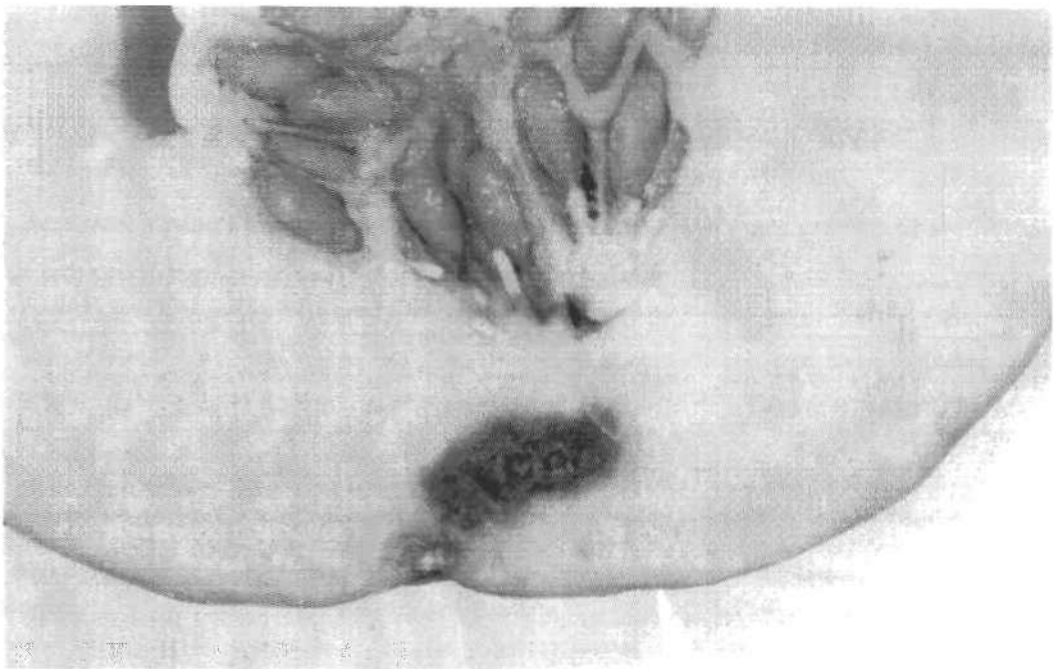


Figure 6. Honeydew showing internal cavities caused by the rockmelon strain of *A. avenae* subsp. *citrulli*

infections of a citron melon (*Citrullus lanatus* var. *citroides*) in Texas. This weed is common in melon areas of south Texas and is probably similar to pie melon (*Citrullus lanatus*) which grows extensively in the Chinchilla district.

Other hosts. At the commencement of this project, fruit blotch was recognised to be a field disease of watermelon only. Symptoms on rockmelon fruit were described as water soaked pits on the fruit surface (Latin 1996) while on honeydew fruit, lesions were reported as circular, 3-10 mm diameter and not extending into the flesh (Isakeit *et al.* 1997). A report by Assouline *et al.* (1997) described the quarantine interception of *Acidovorax avenae* subsp. *citrulli* on tomato and eggplant seeds as well as on watermelon seeds. Leaf spots developed on seedlings raised from the seeds. In 1998 (Langston *et al.* 1999), *Acidovorax avenae* subsp. *citrulli* was identified from a fruit rot affecting pumpkins in Georgia, USA. The organism was pathogenic to watermelon in pathogenicity tests.

Control. Since seed transmission is the most important mechanism in the perpetuation of the disease, screening to detect the organism in seed lots has become an established practice. Seed companies in the USA commenced using "grow out" tests on watermelon seed lots in 1995. In these tests, a sample of 10 000 – 45 000 seeds is germinated under conditions favourable for disease development (RH >55%; temp. 25-32°C). Any infected seedlings are recognised and contaminated seed lots are then withdrawn from sale.

Treatment of seed to eradicate infection has also been attempted. Hopkins *et al.* (1996) found that natural fermentation of seed for 24 h and seed soak in hydrochloric acid were very effective in reducing both internal and external contamination. Natural fermentation has the drawback that it would have to be performed at the time of seed extraction and could not be implemented by a seedling producer. Rane and Latin (1992) did not have the same success with hydrochloric acid. Hot water treatments have been used with success to control other bacterial seedborne diseases, e.g. black rot of crucifers and Wall (1989) found a seed treatment of 50°C for 30 minutes greatly reduced carryover in watermelon seeds.

The ability of the disease to spread quickly through crowded seedling production areas led to the recommendation by seed companies (Jackson pers. comm.) that direct sowing in the field would avoid this situation and isolate infected seedlings for the first few weeks of growth.

As with other bacterial diseases, copper compounds are currently the only option for chemical control in the field. Since most infection of melon fruit occurs during the early stages of development, Frankle *et al.* (1993) have advocated careful spray application at this time. The efficacy of copper sprays can be inadequate during weather conditions favourable for bacterial disease development. The addition of ferric chloride improved the bactericidal ability of copper sprays against *Xanthomonas campestris* pv. *juglandis* in walnut (Lee *et al.* 1993) and could be beneficial for blotch control also.

Field hygiene measures are also important. Rane and Latin (1990) showed a poor ability of the bacterium to survive burial, indicating that ploughing in crop trash is a sound practice. The survival of the bacterium via volunteer melon and weed cucurbit hosts also demands attention.

History of the disease. Sporadic outbreaks of the disease occurred in watermelon crops in Queensland and the USA in the period 1967-1987. This sporadic nature indicates outbreaks were probably initiated through batches of contaminated seed rather than field carryover.

The current fruit blotch epidemic commenced in the Mariana Islands in 1987 (Wall and Santos 1988). By 1989 epidemics had occurred in watermelon crops in Florida, South Carolina and Indiana (Latin and Hopkins 1995). The disease occurred in Oaklahoma in 1991 (Jacobs *et al.* 1992) and Texas in 1993 (Black *et al.* 1994) and was also confirmed in Delaware, Arkansas, Georgia and North Carolina. Rane and Latin (1992) confirmed there was seed contamination in a commercial seed batch of watermelon cv. Prince Charles.

In Australia, fruit blotch occurred in cv. Baby Lee at Bowen in June 1995 and in nurseries at Bowen in both rockmelon and watermelon cultivars through 1996. Field outbreaks in both watermelon and rockmelon crops occurred at Bowen and Ayr and in watermelon at Chinchilla in 1996. The response of growers in the USA was to initiate lawsuits against seed companies which resulted in suspension of sales or sale only on the basis of indemnity from prosecution. Some Australian distributors followed suit.

Aims of project. While the severe epidemics in the USA were thought due to contaminated watermelon seed, early outbreaks in Queensland seemed to be from both watermelon and rockmelon seed. The main objectives were to:-

- determine whether there are strains of *Acidovorax avenae* subsp. *citrulli* which have adapted to rockmelon or other hosts
- establish methods for the production of clean seedlings
- improve disease control in the field
- determine whether the disease can carryover on trash or cucurbit weeds

2. HOST RANGE OF *ACIDOVORAX AVENAE* SUBSP. *CITRULLI* AND DIFFERENTIATION OF STRAINS

INTRODUCTION. At the commencement of this project we were observing extensive infection in rockmelon and honeydew seedlings — something which had not been reported elsewhere. This indicated the possibility of a new strain, or new strains, with wider host ranges than previously observed. In this section we report some of the experiments we conducted to determine whether there is more than one strain of *Acidovorax avenae* subsp. *citrulli* and whether other cucurbits are at risk.

2.1 Strain Identification

MATERIALS AND METHODS.

Pathogen isolation and identification

Bacterial isolations were made from seedling leaves, leaves and fruit from field grown plants and from bacterial lesions on weed hosts in the Chinchilla district. 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 ultra-violet light. Single colonies with these characteristics were selected and identified using the Biolog system (Biolog, Hayward CA).

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, we noticed consistent differences 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 isolates 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 approx. $3x10^8$cfu/mL were prepared and dispensed to GN MicroPlates. After incubation for 24 h at 30^0C, 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 $1x10^8$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 was deliberately wounded and we did not try to cause the inoculum to infiltrate the leaves. Glasshouse temperatures ranged from 14-25^{0}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 pre-germinated 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 ($1x10^8$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. There were 5 replicate plants per treatment.

*Pathogenicity to prickly paddy melon (*Cucumis myriocarpus). *Cucumis myriocarpus* is a weed host of *A. avenae* subsp. *citrulli* in south Queensland (Fig.2). 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.

PCR diagnostic test.

A specific PCR-based diagnostic test for *A. avenae* was developed by A. Wen (University of Queensland) and used to compare isolates 4391 and 4419 (ex watermelon, Chinchilla); 4397, 4412, 4422, 4425, 4431 (ex watermelon and rockmelon, Bowen); American isolates ATCC 29625, ICMP 6521, ICMP 6522 (ex watermelon); American isolates ICMP 7713, ICMP 7714 (ex *Cucumis* melon). The protocol for the test was:

Culture isolates and Biolog GN identification\
↓
DNA extraction
↓
rep PCR

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).

Strain differentiation

Biolog reactions. Although there was some variability in the coloration of particular microplate wells, there were 2 carbon sources which 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/watermelon. There were no symptoms on control plants and these results were omitted from the ANOVA using Genstat 5. The results (Fig. 7) show that both isolates caused equally severe symptoms on the 2 watermelon cultivars but only isolate 4412 caused damage to the 2 rockmelon cultivars. 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 higher ($P<0.05$) than on the 2 rockmelon cultivars. For isolates 4391 and 4884, the disease severity on cv. Green Flesh was not significantly 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 s. Qld. 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).

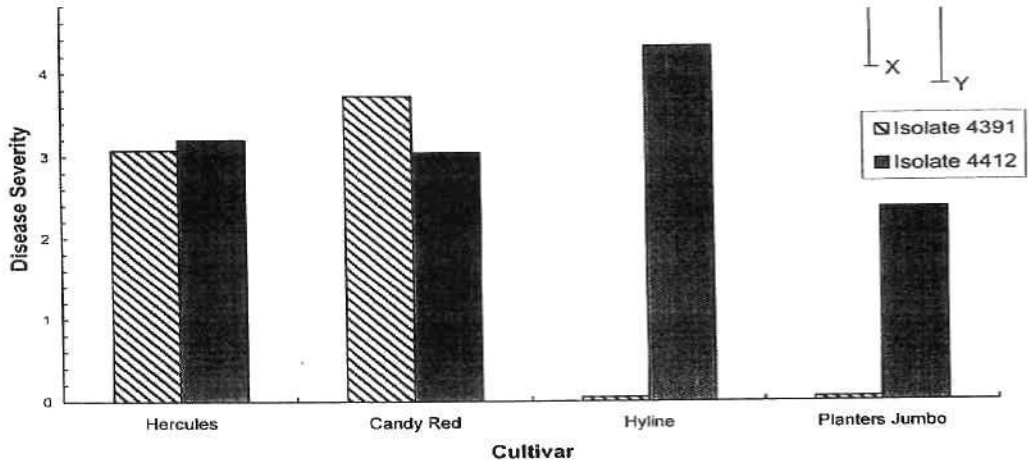


Figure 7. Disease severity in watermelon (Hercules, Candy Red) and rockmelon (Hyline, Planters Jumbo) seedlings inoculated with two isolates of *A. avenae* subsp. *citrulli*. Isolate 4391 is from south Queensland and 4412 is from north Queensland. Vertical bars represent l.s.d. ($P = 0.05$) between cultivars (X) and between isolates (Y)

Figure 8. Representative plants of *Cucumis myriocarpus* showing greater susceptibility to the watermelon strain (isolate 4391) on the right than the rockmelon strain (isolate 5017) on the left

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

Isolate, host*, district	Ability to utilise 2 carbon sources		Similarity to *A.a.c.*
	L-leucine	2-amino ethanol	
4391 ex. Wm. s.Qld.	+	-	0.81
4884 ex. C.m. s.Qld.	+	-	0.98
4885 ex.C.l. s.Qld.	+	-	0.77
4886 ex. Wm. s.Qld.	+	-	0.86
4887 ex. C.m. s.Qld.	+	-	0.92
4888 ex. C.l. s.Qld.	+	-	0.83
4397 ex. Rm. n.Qld.	-	+	0.74
4412 ex. Wm. n.Qld.	-	+	0.81
4425 ex. Wm. n.Qld.	-	+	0.81
4495 ex. Rm. n.Qld.	-	+	0.77
4936 ex. Rm. n.Qld.	-	+	0.78
4995 ex. Rm. n.Qld.	-	+	0.77
5014 ex. Rm. n.Qld.	-	+	0.87
5015 ex. Rm. n.Qld.	-	+	0.75
5016 ex. Rm. n.Qld.	-	+	0.81
5017 ex. Rm. n.Qld.	-	+	0.78
5019-1 ex. Hd. n.Qld.	-	+	0.83
5019-2 ex. Hd. n.Qld.	-	+	0.77
5037 ex. Hd. n.Qld.	-	+	0.68
5039 ex. Hd. n.Qld.	-	+	0.77
5046 ex. Rm. n.Qld.	-	+	0.87
5084 ex. Rm. n.Qld.	-	+	0.75

*Wm.,Watermelon; Rm., Rockmelon; Hd., Honeydew; C.m., *Cucumis myriocarpus;* C.l., *Citrullus lanatus;* n.Qld., north Queensland; s. Qld., south Queensland.

Table 2. Severity of blotch symptoms on watermelon, rockmelon and honeydew seedlings following inoculation with three isolates of *A. avenae* subsp. *citrulli* from north Queensland and four from south Queensland

*Melon cultivar	Disease severity (0-5)						
	n. Qld isolates			s. Qld isolates			
	4412	4425	4397	4391	4884	4885	4886
Wm. Minilee	3.75	3.92	4.05	4.92	4.80	4.95	4.87
Wm. Candy Red	2.85	3.50	3.50	4.70	4.37	4.70	4.15
Rm. Early Dawn	3.77	3.72	4.05	2.92	2.25	2.55	2.15
Rm. Hyline	4.57	4.32	4.75	2.20	1.72	1.52	1.55
Hd. 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	NS	0.94	1.20	1.10	0.58

*Wm., Watermelon; Rm., Rockmelon; Hd., Honeydew

The lsd values allow comparison between cultivars for each isolate.

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

Isolate	Disease severity (0-5)	
	Crimson Sweet	Planters Jumbo
4391 ex. watermelon (s. Qld)	3.5	0.5
4397 ex. rockmelon (n. Qld)	2.1	3.5
4412 ex. rockmelon (n. Qld)	1.3	3.3
4425 ex. watermelon (n. Qld)	1.4	3.4
Water control	0	0
l.s.d. ($P = 0.05$)	1.5	0.6

Figure 9. Bacterial blotch affecting field-grown cv. Jarrahdale pumpkin (top) and cv. Jetset cucumber at Gumlu March 1999

Pathogenicity to prickly paddy melon (C. 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.8). 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.

PCR diagnostic test.

Genomic DNA fingerprinting analysis showed that the Australian isolates had a very similar but distinct fingerprint to American isolates of *A. avenae* subsp. *citrulli*. Two *A. avenae* subsp. *citrulli* isolates from watermelon in Chinchilla (isolates 4391 and 4419) were different from *A. avenae* subsp. *citrulli* isolates from Bowen (4397, 4412, 4422, 4425, 4431). These results indicate there are at least three genetically different strains of *A. avenae* subsp. *citrulli*.

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.

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 at night. In addition, irrigation is by overhead boom which favours splash dispersal of inoculum.

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 American 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 explains 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, is 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 wet conditions in 1998 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 (Isakeit *et al.* 1997). Although tests confirmed the pathogenicity of isolates to both honeydew and watermelon, similarity of these isolates to other isolates from watermelon was not presented. The described fruit symptoms were, however, less severe than those observed in north Queensland fields. In subsequent studies using PCR technology, (Walcott *et al.* 1999) has described two groups of USA isolates: A (watermelon) and B (cantaloupe).

During 1996-99 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 urgent consideration should be given to its introduction for screening rockmelon and honeydew seed lots.

2.2 Host Range

INTRODUCTION. It has been generally recognised (Latin 1996) that *Acidovorax avenae* subsp. *citrulli* can infect other cucurbit species besides melons, although disease symptoms are mild.

At the commencement of the project, we discovered strains with high virulence to rockmelons and honeydew, which, conceivably, could have a wider host range than the typical watermelon strains. As the project progressed we found *A. avenae* subsp. *citrulli* as the cause of a field disease in cucumbers and pumpkins at Gumlu (Fig.9). Overseas workers (Langston *et al.* 1999) reported an outbreak of bacterial blotch (*A. avenae* subsp. *citrulli*) in pumpkin cvs. Lumina and Prize Winner. These natural field infections indicate the pathogen can become severe in hosts not usually considered susceptible. The reason may be new pathogenic strains, a particularly susceptible cultivar or very favourable environmental conditions.

In our host range studies we screened several cucurbit types as well as vegetable species, e.g. beans, tomatoes, capsicum which are commonly grown in melon production areas. Both wounding and non-wounding inoculation methods were used.

MATERIALS AND METHODS. Isolates of *A. avenae* subsp. *citrulli* were routinely cultured on Kings B medium. Inoculum was produced from 24-48 h cultures and was standardised to approximately 1 x 10^8 cfu/mL. The wetting agent Tween 80 was added at the rate of 1 drop per 200 mL.

Plants were raised in seedling flats and transplanted to larger pots as required. Tests were conducted in a cooled glasshouse with intermittent misting available to maintain high humidity.

Experiment 1. Fifteen different cucurbit cultivars were spray inoculated with four different isolates of *A. avenae* subsp. *citrulli*. After one week, disease severity on the two cotyledons and first true leaf of each plant was rated on a 0-5 scale of severity where; 0, no

disease; 1, small spot <3 mm diameter; 2, >1 small spots; 3, 1 large spot >3 mm diameter ± smaller spots; 4, >1 large spot but <50% leaf area affected; 5, spots covering >50% leaf area.

Experiment 2. Twenty-two cucurbit and other cultivars were inoculated with a watermelon strain (4391) and a rockmelon strain (4412) using two different wounding techniques. The young plants (cotyledons + one true leaf) were (a) dusted lightly with carborundum powder then wiped with a cotton bud dipped in inoculum, or (b) two droplets of inoculum were placed on each cotyledon, then a sterile needle passed through the inoculum and into the cotyledon.

There were four replicates of each treatment. After seven days, each cotyledon was rated on a 0-5 scale of severity where; 0, no progressive lesion; 1, inoculation point with a narow zone of progression <10% leaf area; 2, some watersoaking and progression >10 <25% leaf area; 3, >25 <50% leaf area; 4, >50 <75% leaf area; 5, >75% leaf area affected.

Experiment 3. Thirty-one cucurbit cultivars were inoculated by leaf infiltration with four isolates of *A. avenae* subsp. *citrulli* — 4391 watermelon strain; 4425 rockmelon strain; A244 rockmelon strain ex cucumber crop at Ayr; A370, rockmelon strain. Each isolate was infiltrated to give watersoaking at four sites on each cultivar. After 14 days, the inoculation site was rated as:-

0, no movement from inoculation site; well defined margin between infiltrated area and healthy tissue; inoculated area dry and papery

1, slight movement from inoculation site; well defined margin with dark colour suggesting some bacterial activity

3, progressive lesion, greasy appearance or water soaking around margin

A mean figure for each isolate/cultivar combination was deduced.

Experiment 4. All previous experiments used small seedlings. In this test we examined the effect of plant age on susceptibility. Seed of watermelon, rockmelon, honeydew, pumpkin and cucumber varieties (see Table 4) were sown so that plants were aged 2, 3, 4, 5 and 6 weeks at time of inoculation. This glasshouse trial was laid out in three separate blocks of 125 plants (5 cultivars x 5 plant ages x 5 replications). Each block was spray inoculated with a different isolate of *A. avenae* subsp. *citrulli* (4391, 4425 and A244). Eight days after inoculation, disease severity was assessed on each leaf on a 0-5 rating scale where

0 – no apparent infection
1 – light infection — few small spots
2 – light infection — few small elongated spots
3 – moderate infection — >10 <25% leaf area affected
4 – severe infection — >25 <50% leaf area affected
5 – very severe infection — 50% leaf area affected

Mean severity figures per plant and treatment were calculated.

RESULTS

Experiment 1. The disease severity figures in Table 4 show that overall, watermelon was the most susceptible species followed by rockmelon. Squash, pumpkin, zucchini and cucumber developed some spots but symptoms were non-progressive. Two exceptions are high pathogenicity to cv. Jarrahdale by isolate 4391 and high pathogenicity to cv. Green Bush Hybrid by isolate 4397.

Table 4. Susceptibility of 15 cucurbit varieties to four isolates of *Acidovorax avenae* subsp. *citrulli*

Cucurbit variety	Disease severity 0-5*				
	4391	4412-1	4397	4425	Mean
1. Cucumber cv. Crystal Salad	0	1.0	1.3	0.3	0.65
2. Cucumber cv. Crystal Apple	0.7	1.3	0	0	0.50
3. Cucumber cv. Long Green	0	0.3	0.3	0	0.15
4. Pumpkin cv. Qld Blue	0	1.0	0	0	0.25
5. Pumpkin cv. Jarrahdale	3.3	1.0	0.3	1.0	1.40
6. Pumpkin cv. Early White Bush	0	0	1.0	0.3	0.32
7. Squash cv. Green Bush Hybrid	0.7	0.3	3.7	1.3	1.50
8. Squash cv. Early White Bush	0	1.3	0.7	1.0	0.75
9. Rockmelon cv. Planters Jumbo	0	3.0	3.3	3.3	2.40
10. Rockmelon cv. Planters Jumbo	0	2.0	1.0	3.7	1.67
11. Watermelon cv. Crimson Sweet	5.0	0.3	3.0	2.3	2.65
12. Watermelon cv. Candy Red	3.0	2.0	2.7	2.3	2.5
13. Watermelon cv. Warpaint	5.0	3.3	3.0	3.0	3.57
14. Watermelon cv. Continental Sweet	5.0	3.3	3.3	3.3	3.72
15. Zucchini cv. Blackjack	1.0	2	0	0	0.75

*Mean figures for ratings on two cotyledons and the first leaf
0 = no symptoms
1 = small spot <3 mm diameter
2 = >1 small spot
3 = 1 large spot > 3 mm ± smaller spots
4 = >1 large spot < 50% leaf area affected
5 = spots covering >50% leaf area

Experiment 2. Due to the separation of plants inoculated with different isolates, the experiment was analysed as two different sets of data, i.e. plants inoculated with isolate 4391 and those inoculated with 4412. In both cases, inoculation with carborundum/cotton bud gave more severe symptoms than the leaf prick method (Table 6). Several cultivars did not show significant symptom development with either isolate. These included bean, zucchini, pumpkin, capsicum, squash and cucumber cv. Crystal Salad. Other cultivars showed significant symptom development with both isolates. These include tomato cv. Floradade, watermelon cvs. Hercules, Candy Red, Minilee and Warpaint; rockmelon cultivars Early Dawn, Laguna and Planters Jumbo; and honeydew cv. Green Flesh. The rockmelon cv. Hiline was very susceptible to isolate 4412 but not 4391. Cucumber cvs. Jade and Fancy Pak and eggplant cv. Black Night were affected by 4391 but not 4412 (Table 5).

Table 5. Susceptibility of 22 cucurbit, tomato, bean and egg fruit cultivars to two isolates of *Acidovorax avenae* sub.sp. *citrulli*

Plant	Cultivar	Disease Severity (0-5)	
		4391	4412
Bean	Labrador	0	0.00
Zucchini	Black Jack	0	0.06
Squash	Green Buttons	0	0.06
Capsicum	Wonder Belle	0.06	0.12
Zucchini	Regal Black	0.12	0.37
Cucumber	Crystal Salad	0.12	0.37
Pumpkin	Jarradale	0.31	0.31
Pumpkin	Qld Blue	0.31	0.44
Rockmelon	Hiline	0.50	3.75
Pumpkin	Butternut	0.50	0.50
Egg Fruit	Black Night	0.69	0.00
Rockmelon	Early Dawn	0.87	2.12
Tomato	Floradade	1.44	1.37
Cucumber	Jade	1.62	0.56
Cucumber	Fancy Pack	1.69	0.31
Rockmelon	Planters Jumbo	1.81	3.94
Watermelon	Warpaint	3.00	1.81
Watermelon	Hercules	3.06	1.87
Honeydew	Green Flesh	3.06	3.75
Watermelon	Candy Red	3.50	1.87
Watermelon	Minilee	3.50	2.12
Rockmelon	Laguna	3.69	4.75
	LSD (*P = 0.05*)	0.59	0.72

Table 6. Mean differences in disease severity of bacterial blotch induced by two different inoculation techniques

Isolate	Mean Disease Severity 0-5		LSD
	Cotton Bud	Leaf Prick	*P = 0.05*
4391	1.494	1.222	0.177
4412	1.517	1.233	0.213

Experiment 3. The mean disease ratings (Table 7) show the melon varieties were more susceptible than other cucurbit types.

Cucumber: Isolate A244 was generally more virulent on this host than other isolates.
Zucchini: All cultivars resistant.
Squash: Early White Bush showed low susceptibility but, in general, squash cultivars were resistant.
Pumpkin: The three important commercial cultivars were resistant, cv. Baby Bear was slightly susceptible.
Watermelon: All cultivars susceptible to all isolates.

Honeydew: All cultivars susceptible to all isolates.
Rockmelon: Isolate 4391 was non-pathogenic. All cultivars were susceptible to the other three isolates.

Table 7. Susceptibility of cucurbits to four isolates of *Acidovorax avenae* subsp. *citrulli* (Mean of 5 reps)

Cultivar		Disease severity 0-2				
		4391	4425	A244	A370	Total
CUCUMBER	Jade	0	1	0.5	1	2.5
	Fancipak	0.8	0	0.8	0	1.6
	Crystal Salad	0	0.8	1.0	0	1.8
	Redlands Long White	0	0	2.0	1	3.0
ZUCCHINI	Pot Black	0	0	0	0	0
	Regal Black	0	0	0	0	0
	Black Jack	0	0	0	0	0
SQUASH	Green Buttons	0	0	0.8	0	0.8
	Table Gem	0	0.2	0	0	0.2
	Table Queen	0	0	0	0	0
	Early White Bush	1	0.5	0.2	0	1.7
	Pacifica	0	0.5	0	0	0.5
PUMPKIN	Butternut Large	0	0.5	0	0.2	0.7
	Queensland Blue	0	0	0	0	0
	Jarrahdale (W.A.)	0	1	0	1	2.0
	Baby Bear	0.8	0	2	1	3.8
WATERMELON	Candy Red	2	1	2	2	7.0
	Warpaint	2	1	1.5	2	6.5
	Pharoah	2	2	2	2	8.0
	Red Tiger	2	2	2	2	8.0
HONEYDEW	Green Flesh	1.2	2	2	2	7.2
	Dewette	2	2	2	2	8.0
	Field Collection	2	2	2	2	8.0
ROCKMELON	Hales Best	0	2	2	0.5	4,5
	Laguna	1	2	2	2	7.0
	Planters Jumbo	0	2	2	2	6.0
	Hammersley	0	2	2	2	6.0

The major difference between isolates is still based on pathogenicity to rockmelon. There is some evidence that isolates such as A244 are slightly more virulent to cucumbers and also some cultivars e.g. Baby Bear and Early White Bush may be more susceptible than other cultivars in their group.

Experiment 4. The disease severity figures are based on the mean severity per leaf. Plants at different ages had produced different numbers of leaves at the time of rating. These are shown in Table 8. A summary of the disease severity ratings is shown in Table 9. Some of the main points are:-

- Isolate 4391 was of low virulence to rockmelon but was the most pathogenic of the three isolates to pumpkin

- All isolates were of low virulence to cucumber
- With few exceptions, the difference in disease severity between plants of different ages was not large. There was a trend towards cotyledons being more severely affected than true leaves. This is most noticeable in watermelon with isolates A244 and 4425, and in pumpkin.

Table 8. Mean number of leaves produced per plant at time of disease assessment

Plant Variety	Number of leaves produced on plants of different ages				
	2 wks	3 wks	4 wks	5 wks	6 wks
Watermelon cv. Candy Red	3	4	6	8	13
Pumpkin cv. Qld Blue	4	5	6	8	9
Cucumber cv. Crystal Salad	4	6	9	11	12
Rockmelon cv. Planters Jumbo	3	5	6	8	15
Honeydew cv. Green Flesh	3	5	6	10	13

Table 9. The severity of blotch symptoms on plants of different ages after inoculation with three different isolates of *A. avenae* subsp. *citrulli*

		2 wks	3 wks	4 wks	5 wks	6 wks	Av.
WATERMELON							
Candy Red	4391	2.6	1.28	2.86	2.2	2.4	2.27
	A244	3.5	0.76	1.2	1.24	0.88	1.52
	4425	4.1	0.88	2.24	1.67	1.7	2.12
PUMPKIN							
Qld Blue	4391	2.6	1.32	0.8	1.24	1.4	1.47
	A244	1.0	0.64	0.54	0.08	0.12	0.48
	4425	2.6	0.8	0.54	0.2	0	0.83
CUCUMBER							
Crystal Salad	4391	0	0.24	0.7	0.19	0.86	0.4
	A244	0.52	0.7	0.88	0.42	0.34	0.57
	4425	0.16	0.2	0.78	0.26	0.82	0.44
ROCKMELON							
Planters Jumbo	4391	0.2	0.2	0.16	0.54	0.2	0.26
	A244	3.3	2.86	3.08	3.48	2.42	3.03
	4425	2.7	2.0	3.72	3.06	2.62	2.82
HONEYDEW							
Green Flesh	4391	2.1	1.9	3.5	2.78	1.08	2.27
	A244	2.2	1.84	3.4	2.96	3.08	2.70
	4425	0.4	1.68	2.58	1.5	1.18	1.47

DISCUSSION.

The host range studies confirmed our previous claim that there are two pathogenic strains of *A. avenae* subsp. *citrulli* based on virulence to rockmelon. Although in some tests it may have appeared that an isolate was pathogenic to a non-melon cultivar (e.g. 4391/Jarrahdale, Expt 1), it was not confirmed in other tests, e.g. 4391/Jarrahdale, Expt 2. Isolate A244 , collected from a diseased cucumber crop in Gumlu, were capable of infecting

the test cucumber varieties. In experiment 3, isolate A244 was generally more virulent on cucumber than other isolates but this was not the case in experiment 4. In summary, cucurbits, apart from the melon types, were poor hosts for the isolates tested, except under exceptionally favourable circumstances. There are no references in the literature which detail the results of inoculation tests similar to the ones we conducted. In most cases it is stated that melons are the only significant host. In cases where new hosts have been claimed, e.g. pumpkin (Langston *et al.* 1999), honeydew (Isakeit *et al.* 1997), citron melon (Isakeit *et al.* 1998), the only pathogenicity tests reported have been to confirm pathogenicity to watermelon. Langston *et al.* (1999) used PCR amplification to compare the organism from pumpkin with a standard watermelon isolate and found the two were identical. This suggests the outbreak in pumpkin in the USA may have been similar to the outbreak in cucumbers and pumpkin in Ayr where extremely favourable weather conditions caused predisposition to disease. Some cultivars of particular cucurbit types may also be more susceptible than others. This was reported for watermelon by Goth and Webb (1981) who tested 38 cultivars which ranged from completely susceptible to immune. It is conceivable that the blotch outbreaks in cucumbers at Ayr and pumpkin in Georgia were due to the varieties being more susceptible than others.

Species apart from cucurbits are also reluctant hosts. In one test we included tomato, eggplant, capsicum and bean. Tomato and eggplant developed small lesions on cotyledons. Assouline *et al.* (1997) also reported tomato and eggplant as hosts and claimed *A. avenae* subsp. *citrulli* was carried on the seeds. From the relatively low disease severity which occurred in our tests (Expt 2), it is unlikely tomato and eggplant would be subject to field disease problems with known strains of the pathogen.

3. PATHOGEN SURVIVAL

INTRODUCTION: The sporadic history of the disease in Queensland from 1967-1996, suggests *A. avenae* subsp. *citrulli* has difficulty surviving between successive crops. DPI records show we identified the disease in 1967, 1975 and 1986 before the current problems began in 1995. If *A. avenae* subsp. *citrulli* could survive on weed hosts, trash, or machinery etc., a more constant presence would be expected. The disease was similarly sporadic in the USA until 1989 and Latin and Hopkins (1995) also postulated the cause as seed borne introduction.

Sowell and Schaad (1979) found that 110 of the 740 P.I. watermelon accessions in the USA National collection were carrying the bacterium with the seed and produced infected seedlings. Thus, it is plausible that new watermelon varieties derived from P.I.s may also be carrying *A. avenae* subsp. *citrulli* and the periodic release of these could contribute to sporadic outbreaks.

In this section we examined seedborne inoculum, weed hosts and survival in buried plant trash as possible mechanisms for perpetuating disease outbreaks.

MATERIALS AND METHODS:

Seedborne 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 storage periods of 14 and 21 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°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.

Cucurbit weed hosts

A survey (11 November 1997) in the Chinchilla district showed that wild cucurbits and volunteer watermelon seedlings growing on old watermelon land were affected by a tan-brown leaf spot. Bacterial streaming was seen under the microscope and isolations onto Kings Medium B were made from two samples of pie melon (*Citrullus lanatus*), two samples of prickly paddy melon (*Cucumis myriocarpus*) and one sample of volunteer watermelon. Bacterial colonies similar to *A. avenae* subsp. *citrulli* were selected and identified by Biolog GN microplates.

Survival of **A. avenae** *subsp.* **citrulli** *in plant trash*

In this experiment, infected plant remains were buried for 10 weeks. Tests were conducted at 2, 5 and 10 weeks to determine whether bacteria were still viable.

Infected plant material for the test was produced:

Leaves – Rockmelon and honeydew leaves were spray inoculated, incubated, and harvested after seven days. Infected leaves were chopped into 1 cm^2 pieces.

Fruit – Honeydew and rockmelon fruit were surface sterilized, wounded and inoculated by immersing in a bacterial suspension for two minutes then incubating for 24 h. Bacteria were injected into the seed cavity. The rind was cut into thin strips and air dried for seven days. Before burial the strips were cut into approximately 1 cm^3 pieces.

Seeds – Seeds from the above fruit were air dried.

Whole fruit – Two immature honeydew fruit with naturally occurring blotch infection were used in one test (burial for five weeks).

Soil was collected from a melon farm at Chinchilla and determinations made so we could maintain moisture levels in pots at 80% of field capacity. Pots (12.5cm. diam.) were lined with plastic and filled with soil.

Each inoculum type (leaves, skin, seeds) was divided into six portions then placed in six small mesh bags. Bags were buried (3 per pot) and duplicate pots placed in controlled environment cabinets set at 15°C and 25°C. The moisture content of each pot was restored to 80% of field capacity each week.

One of the three samples in each pot was extracted after 2, 5 and 10 weeks and tested for infective ability. The test was designed so that germinating rockmelon seeds pushed their way up through the infected sample. Five 200 mL pots were filled with layers of 30 mL vermiculite, 80 mL UC mix, five seeds of rockmelon cv. Planters Jumbo, 20 mL vermiculite, 1/5 of the chopped infected sample and 20 mL vermiculite. A set of five control pots was also included. Pots were placed in a glasshouse and seedlings raised until cotyledons were fully developed. Counts of infected seedlings were then made.

In addition, at the two weeks sampling, a small portion of each sample was macerated with 2 mL sterile water and streaked over KB agar. Colonies similar to those of *A. avenae* subsp. *citrulli* were purified and identified using Biolog. Some macerate was also filtered to remove large particles then injected into three sites on young rockmelon leaves. Lesions which were in any way similar to those of bacterial blotch were examined and isolations made to confirm presence of *A. avenae* subsp. *citrulli*.

RESULTS:

Seedborne transmission

Seed collected from the naturally infected rockmelon and honeydew fruit in May 1998 gave seedling emergence figures of 94% and 69% respectively after 14 weeks storage. Of the emerged seedlings, 91% and 33% showed blotch symptoms within 11 days of sowing.

After 21 weeks storage, the emergence figures for rockmelon and honeydew were 90% and 74% respectively. The percentage of emerged seedlings which were diseased were 96% and 84% respectively.

Microscopic examination of several water soaked seedling leaves showed the presence of bacteria. Isolations and Biolog tests confirmed the presence of *A. avenae* subsp. *citrulli*.

Cucurbit weed hosts

In the survey, wild cucurbits were common in any area where soil had been recently disturbed. They were found growing in crops as well as along fence lines and old cultivated land. They are summer growing annuals which are frost sensitive.

On old watermelon land, prickly paddy melon was severely affected by bacterial leaf spotting. Pie melon seemed less susceptible. Leaf spotting was not found on vines growing away from old watermelon land.

The results of the Biolog identification showed the causal organism was *A. avenae* subsp. *citrulli* (Table 10).

Table 10. Identification of bacterial pathogen from wild cucurbit hosts

No.	Host	Location	Biolog ID	Similarity
4884	Prickly Paddy Melon	Site 1	*Acidovorax avenae* subsp. *citrulli*	0.977
4887	Prickly Paddy Melon	Site 2	*Acidovorax avenae* subsp. *citrulli*	0.956
4885	Pie Melon	Site 1	*Acidovorax avenae* subsp. *citrulli*	0.985
4888	Pie Melon	Site 2	*Acidovorax avenae* subsp. *citrulli*	0.918
4886	Watermelon	Site 2	*Acidovorax avenae* subsp. *citrulli*	0.977

Survival of Acidovorax avenae *subsp.* citrulli *in plant trash*

Buried plant material consisting of fruit skins and leaves inhibited the germination of test seedlings in the first evaluation at two weeks. In later tests at 5 and 10 weeks, germination was satisfactory. No infection similar to bacterial blotch occurred on test seedlings. (In a preliminary test of the method, fresh leaf material caused infection in 60 percent of test seedlings).

No colonies of *Acidovorax avenae* subsp. *citrulli* were identified on the KB agar plates following macerate streaking.

No leaf spots similar to bacterial blotch resulted from macerate injection of rockmelon leaves.

DISCUSSION: Our studies indicate that it is extremely unlikely that *Acidovorax avenae* subsp. *citrulli* will survive from season to season on buried plant trash. This is in agreement with Rane and Latin (1990) who were able to detect *Acidovorax avenae* subsp. *citrulli* in buried samples for up to one week but not for longer periods.

Field carryover on cucurbit weeds and volunteer melon plants is certainly possible and we found naturally occurring lesions of bacterial blotch on these hosts in the Chinchilla district. The mechanism for carryover is most likely via infected seed because these plants are frost sensitive and do not normally survive the winter in this area. For a weed population to perpetuate itself as a source of inoculum would require: over-wintering via infected seed, germination and infection of seedlings, followed by seasonal conditions suitable for foliar and fruit infection. Thus, it seems probable that during wet seasons this cycle could be maintained, but during dry seasons it could be broken due to poor fruit infection. Although weeds are found extensively in southern districts they may not be able to provide a continuous source of inoculum, hence, there are intervals between outbreaks.

The disease is well adapted to survival in seed of commercial melon types as we reported previously (Section 3). This strong adaptation to seedborne infection indicates that sporadic outbreaks could be due to infected seed lots. It is almost certainly the way the outbreaks of the new "rockmelon strain" of bacterial blotch in north Queensland were initiated. Although we have isolated *Acidovorax avenae* subsp. *citrulli* from melon seedlings in Bowen nurseries and in fields on many occasions since 1996, it has always been the new rockmelon strain. It is probable we have not found the "watermelon strain" due to the introduction of grow-out tests by seed companies to detect contaminated watermelon seed lots. Some unscreened seed lots of rockmelon or honeydew in all probability carried the new strain of the bacterium. We have made these findings clear to seed companies in Australia, the USA and Holland and there is now confirmation from pathologists in the USA that they have also found the rockmelon strain (Walcott *et al.* 1999). The onus is now on seed companies to include these melon types in their testing programs.

4. DISEASE CONTROL

INTRODUCTION: Since *Acidovorax avenae* subsp. *citrulli* is well adapted to seedborne transmission, it is essential that infected seed lots be detected by a grow-out test or molecular-based system. If seed has not been screened in this way, an effective treatment to eradicate infection should be available. Hopkins *et al.* (1996) claimed fermentation of seeds for 24 h-48 h in watermelon juice followed by 1% HCl for five minutes reduced disease incidence but did not eradicate the pathogen. Hot water treatment (50 C for 30 min.) was suggested by Wall (1989).

In the field, control measures are aimed at minimising spread of the disease, particularly before and during fruit set. Unfortunately there are few chemicals effective against bacterial diseases. Copper products such as cupric hydroxide, copper oxychloride and cupric ammonium carbonate are the most widely used. Their efficacy can be low during periods of wet weather but Lee *et al.* (1993) found the addition of Fe enhanced their toxicity to the bacterium *Xanthomonas campestris* pv. *juglandis*. A drawback to the use of copper sprays is that they can slow the development of some crops and there was concern amongst growers that fruit set could be affected. Some new products such as Bion (Novartis Pty Ltd) have been successful against a range of pathogen (including bacteria) by activating the resistance mechanisms of the host.

In this section we evaluated various seed treatments (particularly hot water seed soaks) and screened copper compounds and additives in field trials for efficacy and crop safety.

MATERIALS AND METHODS

Seed treatments to eradicate Acidovorax avenae *subsp.* citrulli

Several watermelons cv. Red Tiger showing signs of blotch infection were collected from the Jandowae district where melons are produced under dryland conditions. These were kept for a week before seed was extracted to allow the disease to progress. Seed was extracted before secondary rots became well established.

The seed from about 10 large melons was recovered on a sieve after washing away pulp. This was dried on blotting paper and stored in bottles until the experiment commenced (2 months). The seeds were then divided into seven lots, each held loosely in a muslin bag. One bag of seed was immersed for 20 minutes in each of the following:-

1. 1% Hydrochloric acid – (10 mL/L of 36.5%HCl)
2. 3000 ppm Sodium hypochlorite (3 mL/L of 100 g/L av. Cl solution)
3. 3000 ppm Calcium hypochlorite (1 g/L $CaOCl_2$)
4. Hot water – 55°C
5. Hot water – 50°C
6. 2% Agrigard (20 mL/L)
7. Sterile water – 23°C

Seed was dried overnight and the first trial was sown the following day (Experiment 1). A second trial (Experiment 2), using the same treated seed was sown two weeks later.

Experiment 1 (7 treatments x 4 reps; RB; plot size, 42 seeds). A "Kwik Pot" seedling tray liner (42 cells per tray) was a plot. The containers were filled with UC mix and one seed sown per cell. Trays were watered, covered with clear plastic lids and randomised on glasshouse benches. Germination was slow due to cold conditions and two reps were placed in CECs (25°C 12/12) after 10 days.

Seedlings were examined two weeks after sowing, and the number of diseased and total seedlings were noted. Four days later a second rating was made in which severity of infection was rated 0-3; 0, nil; 1, trace; 2 moderate (<25% leaf area affected); 3, severe >25% leaf area affected.

Experiment 2 (7 treatments x 8 replications; RB; plot size, 20 seeds). Due to the cool ambient temperature, the second experiment was redesigned to fit inside two growth cabinets. Kwik Pot seedling tray liners (20 cells per tray) were used. A row of five cells with four seeds in each cell was a plot. Seven trays (4 replications) were placed in each cabinet.

Diseased seedlings were removed as soon as they were observed. A final count was made 14 days after sowing. The best technique for observing diseased plants was to remove covers for 1-2 days, replace overnight, remove in the morning and wait two hours for normal water soaking to disappear, then rate those with permanently water-soaked lesions.

Effect of hot water treatment (HWT) on seed germination

In this series of tests, we screened 46 melon lines to determine whether HWT is liable to cause reductions in germination of watermelon, rockmelon or honeydew.

Seed samples were received from the following companies for testing:- Novartis, South Pacific Seeds and LeFroy Valley.

Watermelon: Charleston Grey, Warpaint, Candy Red, Minilee, Hercules, WM584, WM706, WM466, WM778, WM393, WM738.
Rockmelon: Hiline, Early Dawn, Laguna, Planters Jumbo, Hales Best, Eastern Star, Sweet Success, Meteor, Glacier, Oakley, Premier, Colusa, Saratoga, RM663, RM684, RM580, RM701, RM717, RM735, RM759, RM695, RM694, RM710, RM696, RM699, RM697, RM698, RM736, RM738, RM766, RM769, RM776, RM777.
Honeydew melon: Green Flesh, Moonshine.

HWT technique:

Forty-seed lots of each variety were immersed in a waterbath with a constant temperature of 55°C (+/- 0.5°C) for 20, 25 or 30 minutes; in room temperature water (~ 25°C for 30 minutes or left on the bench for 30 minutes. Heat-treated seeds were immediately immersed in room temperature running water for five minutes after HWT.

Seeds were then spread out to dry, and put in a fumehood to promote evaporation overnight and stored for 2 days.

Plantcon clear plastic containers were filled with 100 ml of vermiculite, seed was sown and then covered with another 100 ml of vermiculite and moistened with 65 ml of distilled water.

Containers were placed in a CEC at 25°C 12/12 for germination. Counts of germinated seeds were made after 4, 7 and 11 days.

Chemical control of bacterial blotch in field plots

Experiment 1. A field trial was established at Redlands Research Station to evaluate copper compounds and the additives Bion, Sporekill and ferric chloride for control of bacterial blotch in watermelons. Watermelon seedlings (cv. Red Tiger) were supplied by a commercial nursery. One third of seedlings were inoculated (isolate 4391; spray inoculum 1 x 10^8 cfu/mL; incubated at high humidity for 48 h) in the glasshouse before transplanting. Inoculated seedlings were evenly spaced through the trial area.

Experimental design was 10 treatments x 4 replications in a randomised blocks layout. Seedlings were spaced 1 m apart along the row and rows were 2 m apart. Plot size was 1 row x 9 m. The spray treatments are listed in Table 14. The chemicals were:

Liquicop- cupric ammonium carbonate, 9% Cu
Kocide - 500g / kg Cu, as cupric hydroxide
Copper oxychloride - 500g / kg Cu, as copper oxychloride
Sporekill - quaternary ammonium compound
Bion - 500g / kg Benzo (1, 2, 3) thiadiazole - 7 - carbothionic acid - S - methyl ester
Ferric chloride - Fe Cl_3. $6H_20$

Chemicals were applied at 7-10 day intervals by a gas pressurised 1m wide boom spray. Spray volumes increased with crop growth from 200 L/ha to 1000 L/ha. The concentration of chemicals in the sprays remained constant.

Overhead irrigation was used to assist disease spread.

Plots were rated for disease severity on two occasions: early fruit set and three weeks later. Five runners were selected at random in each plot and eight consecutive leaves on each runner rated for disease severity by the scale:

0 - no disease
1 - isolated small spots < 1% leaf area
2 - spots extending over 1 - < 5% leaf area
3 - spots extending over 5 - < 10% leaf area
4 - spots extending over 10 - < 25% leaf area
5 - spots extending over 25 - < 50% leaf area
6 - spots extending over > 50% leaf area

From these figures, we calculated the % of leaves showing any disease and the mean leaf disease severity (0-100).

Disease ratings (fruit). Melons were assessed for disease at the first harvest (30/12/98) and eight days later. All fruit > 30 cm long were included. A scale of 0-4 was used.

0 - no blotch lesions
1 - small spot to 25 mm diameter
2 - affected area 2.5 - 10 cm

3 - affected area > 10 cm but fruit firm
4 - fruit rotted

From the combined figures of the two ratings, we obtained total fruit number per plot, the % which showed any disease and the mean disease severity per fruit.

Experiment 2. The object of this experiment was to examine the effect of standard and double standard concentrations of copper hydroxide (Kocide) on fruit set in melons. The trial was not inoculated but a natural epidemic of bacterial fruit blotch occurred which allowed further observations on disease control with Kocide.

Seedlings of watermelon (Red Tiger) rockmelon (Eastern Star) and honeydew (Dewcrisp) were obtained from a nursery and transplanted to the experimental site at Redlands Horticultural Research Station.

The experimental design was of randomised blocks with split plots. The 3 main treatments were sprays and the 3 sub treatments were varieties, with 3 replications. Plot size was 1 row x 8 m. Plant spacing was 0.5 m for rockmelon and honeydew and 0.1 m for watermelon.

Plants were grown on white plastic mulch with trickle irrigation. When downy mildew appeared late in the growing season, 2 sprays of Ridomil MZ were applied. Powdery mildew was not a problem.

Treatments

Sub treatments	Main treatments
1. Honeydew cv. Dewcrisp	1. Untreated
2. Watermelon cv. Red Tiger	2. 2 g/L Kocide*
3. Rockmelon cv. Eastern Star	3. 4 g/L Kocide

* Kocide : 500 g / kg Cu as cupric hydroxide

Sprays were applied by a gas powered 1 m wide boom spray with 4 Hardi No. 12 nozzles. Pressure was 6 bar. Spray volumes increased with crop growth from 200 L / ha to 1000 L / ha. This was achieved by varying the nozzle number and speed of the boom.

Data collection. A rating for foliar phytotoxicity was made when plants were in full flower. Whole plots were given a single rating 0-5.

0 - no visible phytotoxicity
1 - no severe phytotoxicity on any leaf. Light symptoms on < 1 leaf per metre of row
2 - as above > 1 leaf per metre
3 - 1-3 leaves per plot with > 25% < 50% leaf area damaged
4 - > 3 leaves per plot with > 25% < 50% or < 3 leaves with phytotoxicity > 50% leaf area
5 - > 3 leaves per plot with > 50% damage

This scale was designed specifically for the low level of damage apparent in this trial.

It was considered a count of total fruit produced would indicate whether copper sprays were affecting fruit set. There was no visible evidence of blossoms being affected.

Two ratings were made of bacterial blotch severity on leaves (6 and 9 weeks). A 0-6 scale was used to rate individual leaves.

0 - no spots
1 - spot < 1% leaf area affected
2 - 1-5% leaf area affected
3 - 5-10% leaf area affected
4 - 10-25% leaf area affected
5 - 25-50% leaf area affected
6 - > 50% leaf area affected

In each plot, 5 runners were selected at random and 8 consecutive leaves rated on each. The first leaf rated was the youngest fully expanded leaf on the vine.

Fruit were harvested as they matured. A disease severity rating (0-4) was given to each fruit

0 - no blotch lesions
1 - small spot to 25 mm diameter
2 - spot 25-100 mm diameter
3 - affected area large (> 100 mm) but fruit still firm
4 - fruit rotted

The effect of Bion on susceptibility of watermelon and honeydew to bacterial blotch

Plants of honeydew cv. Green Flesh and watermelon cv. Crimson Sweet were grown singly in 15 cm diameter pots until six weeks old. They were staked and side runners trimmed.

A single spray of Bion at rates of 0.01, 0.025, 0.05 and 0.1 g/L was applied on 23 September 1999. There were three replicate plants in each treatment. On 4 October, plants were spray inoculated with isolate 4425 (3×10^8 cfu/mL). Tween 80 was added to the inoculum at the rate of one drop/200 mL. Plants were maintained at high humidity for 48 h.

A rating of disease severity was made on 15 October 1999 by selecting the three most affected leaves and rating them on a 0-6 scale of severity.

0 - no disease
1 - < 1% leaf area affected
2 - 1 - < 5% leaf area affected
3 - 5 - < 10% leaf area affected
4 - 10 - < 25% leaf area affected
5 - 25 - < 50% leaf area affected
6 - > 50% leaf area affected

Bacterial blotch is not an aggressive foliar pathogen and a rating of 2 and above would cause concern in a field crop since severe fruit damage could occur.

RESULTS:

Seed treatments to eradicate *A. avenae* subsp. *citrulli*.

Experiment 1. Agrigard reduced seedling emergence and vigour but also reduced the incidence of blotch. Calcium hypochlorite was moderately effective in reducing blotch but hot water (55°C) gave best emergence and disease control. (Table 11)

Table 11. The effect of seven seed treatments on % diseased seedlings, mean disease severity and % germination. Seed sown 48 hours after treatment.

Treatment	% Infected	Mean Disease Severity 0-3	% Germination
Hydrochloric acid	74	2.4	81
Sodium hypochlorite	69	2.3	76
Calcium hypochlorite	53	2.0	69
Hot water - 55°C	30	1.2	86
Hot water - 50°C	75	2.5	82
Agrigard	55	1.7	26
Sterile water	74	2.2	64
LSD (P=0.05)	16.1	0.57	8.3

Experiment 2.

Table 12. The effect of seven seed treatments on % of diseased seedlings and % germination. Seed sown 14 days after treatment.

Treatment	% Infected	% Germination
Hydrochloric acid	28	64
Sodium hypochlorite	39	67
Calcium hypochlorite	60	67
Hot water - 55 C	11	74
Hot water - 50 C	10	74
Agrigard	18	34
Sterile water	36	53
LSD (P=0.05)	18.1	19.6

Germination % was reduced overall (Table 12) compared to the first experiment. The hot water treatments gave highest emergence while Agrigard reduced emergence. These 3 treatments also gave best disease control.

Effect of hot water treatment on seed germination

For most seed lots, HWT did not affect germination (Table 13). Some rockmelon lines did, however, show reductions of between 10-30%. These were: Eastern Star, Sweet Success, RM696, RM736 and RM717. For RM696 (30% reduction), the overall vigour and germination of the seed was lower than for other lines.

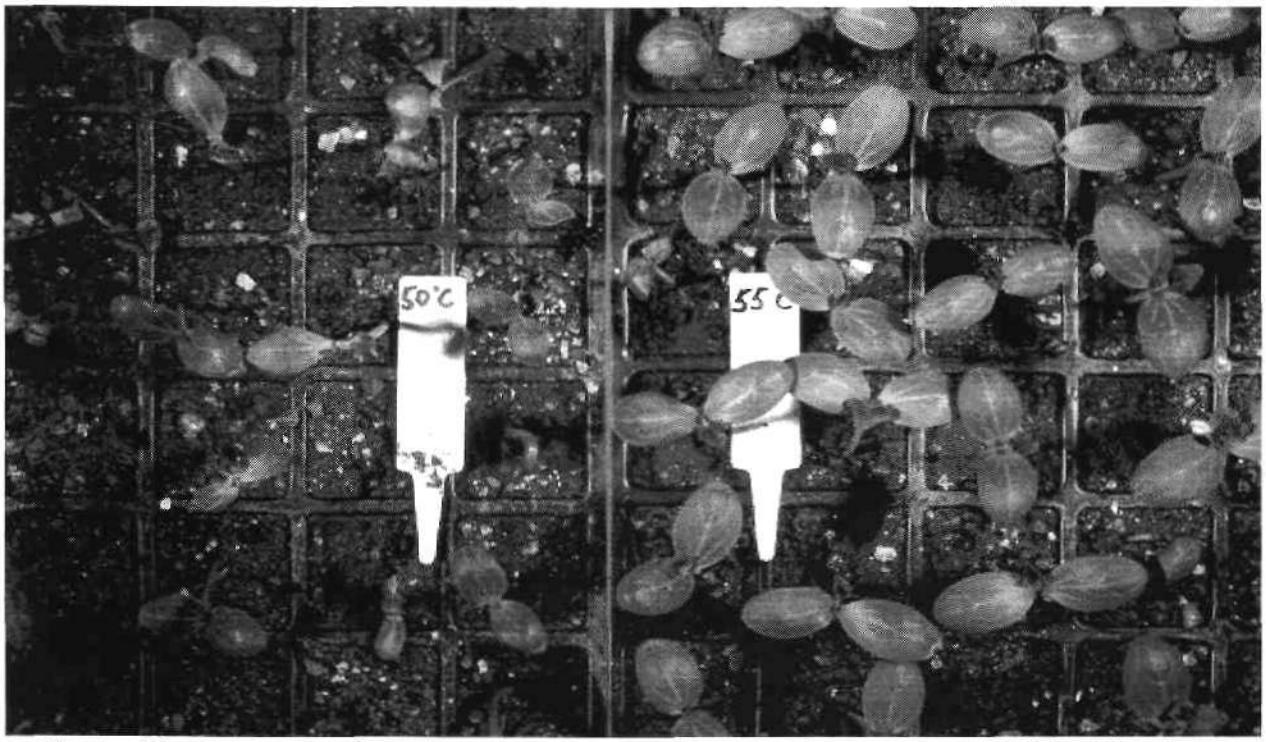


Figure 10. Treating infected watermelon seed by a hot water dip at 55°C for 20 min (right) is more effective than treating at 50°C (left)

Table 13. The effect of 3 hot water seed treatments on the germination of 46 varieties of melons. Figures are means of 11 watermelon cultivars, 33 rockmelon cultivars and 2 honeydew cultivars.

	% Germination			
Untreated	**Watermelon**	**Rockmelon**	**Honeydew**	**Mean of 46**
Water soak 25°C - 30 min	91.2	95.5	88.7	94.5
HWT 55°C - 20 min	95.0	92.0	98.7	95.2
HWT 55°C - 25 min	93.7	90.5	95.0	93.0
HWT 55°C - 30 min	93.7	91.7	97.5	92.5

Chemical control of bacterial blotch in field plots

Experiment 1. Weather conditions were showery and very conducive to development of blotch. The inoculated plants developed extensive foliar disease in the first few weeks which then spread to nearby plants.

The copper sprays significantly reduced disease severity on leaves. (Table 14) There was no significant benefit in adding Sporekill, $FeCl_3$ or Bion to the standard chemicals. There was a general trend for the addition of Sporekill and $FeCl_3$ to reduce the efficacy of the standard and for Bion to improve it. These trends were non-significant.

The disease development in fruit was high (Table 15). The range of % infected fruit was 65-79% with no significant differences between treatments. Similarly, mean fruit disease severity varied from 1.5 - 2.25 with no significant differences between treatments.

Table 14 . The effect of chemical sprays on the control of bacterial blotch on watermelon leaves as indicated by the % of leaves with lesions and the mean severity on each leaf. Two ratings were made during the early fruit development stage.

Treatment	% Infected Leaves		Disease Severity 0-100	
	24/11/98	11/12/98	24/11/98	11/12/98
1. Liquicop, 5 mL/L	24.4 abc	35.6 abc	5.6 ab	7.6 abc
2. Liquicop, 5 mL/L + Sporekill, 1 mL/L	32.5 bc	41.2 bc	7.6 ab	9.2 bc
3. Kocide, 2 g/L	19.4 ab	36.2 abc	4.5 ab	8.2 abc
4. Kocide, 2 g/L + $FeCl_3.6H_20$, 0.25 g/L	35.0 cd	41.2 bc	8.2 ab	8.7 abc
5. Copper oxychloride, 4 g/L	17.5 a	25.0 a	3.4 a	4.9 a
6. Copper oxychloride, 4 g/L + $FeCl_3.6H_20$, 0.25 g/L	25.6 abc	26.9 ab	5.0 ab	5.9 abc
7. Bion (CGA245704), 0.05 g/L	46.9 de	65.0 d	15.2 c	15.7e
8. Bion, 0.05 g/L + Kocide, 2 g/L weekly application	14.4 a	25.0a	3.2 a	5.2 ab
9. Bion, 0.05 g/L + Kocide, 2 g/L fortnightly application	35.0 cd	43.1 c	9.8 bc	9.7 cd
10. Control	64.4 e	59.4 d	21.6 d	13.6 de
LSD ($P = 0.05$)	14.0	16.0	6.3	4.0

Table 15. The effect of various spray treatments on fruit number and the mean disease severity (0-4) of fruit blotch on fruit

Treatment	Mean number of fruit/plot	Diseased fruit (%)	Mean fruit disease severity (0-4)
1. Liquicop (cupric ammonium carbonate), 5 mL/L	22.7	69.7	1.72
2. Liquicop, 5 mL/L + Sporekill, 1 mL/L	17.5	64.7	1.47
3. Kocide (copper hydroxide), 2 g/L	22.7	68.5	1.80
4. Kocide 2 g/L + $FeCl_3.6H_20$, 0.25 g/L	20.2	67.5	1.80
5. Copper oxychloride, 4 g/L	23.2	73.2	1.87
6. Copper oxychloride, 4 g/L + $FeCl_3.6H_20$, 0.25 g/L	22.5	66.5	1.47
7. Bion (CGA245704), 0.05 g/L	22.2	73.7	2.10
8. Bion, 0.05 g/L + Kocide, 2 g/L weekly application	18.0	78.7	1.82
9. Bion, 0.05 g/L + Kocide, 2 g/L fortnightly application	21.0	77.2	1.92
10. Control	18.7	78.7	2.25
LSD ($P = 0.05$)	N.S.	N.S.	N.S.

Experiment 2. Plants established well and grew rapidly. Weather was frequently showery.

There was a very low level of leaf damage due to copper (Table 16). The ratings indicate that 1-2 leaves per metre were slightly affected by spray.

Rockmelon was not affected by bacterial blotch while honeydew leaves were more susceptible than those of watermelon (Tables 17and 18). Both spray concentrations (2 & 4 g/L) reduced the % infected leaves as well as the mean leaf area destroyed. There was no significant difference between the two treatments. The rate of increase of disease between the two ratings was lower in plots sprayed with 4 g/L.

Watermelon plots produced significantly fewer fruit than rockmelon or honeydew but fruit production was independent of spray treatment (Table 19).

Table 16. Influence of copper (Kocide) sprays and melon type on phytotoxicity symptoms

Kocide treatment	Phytotoxicity (0-6)	Melon Type	Phytotoxicity (0-6)
0	0.44 a*	Honeydew	0.67 a
2 g/L	1.11 b	Watermelon	0.89 a
4 g/L	1.56 b	Rockmelon	1.56 a
LSD (*P = 0.05*)	0.61		1.50

*Means in the same column with the same subscript are not significantly different at P = 0.05.

Table 17. The influence of Kocide and melon variety on % leaves infected with bacterial blotch at 6 and 9 weeks after planting

Kocide treatment (g/L)	% diseased leaves		Melon type	% diseased leaves	
	6 wk	9 wk		6 wk	9 wk
0 g/L	53.67 b	77.50 b	Honeydew	51.1	61.56 c
2 g/L	28.00 a	44.33 a	Watermelon	19.8	37.89 b
4 g/L	24.67 a	27.33 a	Rockmelon**	0	0 a
LSD (*P = 0.05*)	25.42	24.05	LSD (*P = 0.05*)	NS	17.91

*Means in the same column with the same subscript are not significantly different at P = 0.05

Table 18. The effect of copper (Kocide) sprays and melon type on mean severity of bacterial blotch on leaves

Ratings made 6 and 9 weeks after planting

Kocide treatment (g/L)	% leaf area destroyed		Melon type	% leaf area destroyed	
	6 wk	9 wk		6 wk	9 wk
0 g/L	17.55 b	24.85 b	Honeydew	14.4 a	18.49 b
2 g/L	6.38 a	11.30 a	Watermelon	5.0 a	9.08 a
4 g/L	5.45 a	5.20 a	Rockmelon**	0	0
LSD (*P = 0.05*)	9.54	9.75	LSD (*P = 0.05*)	28.72	3.99

*Means in the same column with the same subscript are not significantly different at P = 0.05
**Rockmelon was excluded from the analysis since its rating was 0

Table 19. The influence of Kocide sprays and melon type on the number of fruit produced per plot and mean disease severity on each fruit

Kocide treatment	No. fruit per plot	Mean disease severity	Melon type	No. fruit per plot	Mean disease severity (0-4)
0	52.2 a	1.54 b	Honeydew	67.44 b	1.66 b
2 g/L	54.1 a	0.61 a	Watermelon	27.78 a	0.41 a
4 g/L	61.2 a	0.46 a	Rockmelon	72.33 b	0.53 a
LSD (*P = 0.06*)	9.52	0.29	LSD (*P = 0.05*)	10.69	0.69

*Numbers in the same column with the same subscript are not significantly different at P = 0.05

The effect of Bion on the susceptibility of watermelon and honeydew to bacterial blotch

The results in Table 20 show that honeydew was adversely affected by the two higher rates of Bion. Plants were low in vigour, leaves turned yellow and, in some cases, became necrotic. Watermelon was more tolerant and no phytotoxic symptoms were seen.

The effect of Bion on disease control in honeydew was masked by the phytotoxicity symptoms. In watermelon, control plants showed high levels of disease which were progressively reduced by increasing rates of Bion up to 0.05 g/L.

Table 20. Severity of foliar symptoms of *Acidovorax avenae* sub.sp. *citrulli* on watermelon and honeydew treated with Bion at rates from 0-0.1 g/L

Treatment	Disease Severity (0-6*)
Watermelon – Bion 0.1 g/L	0.44 a
Watermelon – Bion 0.05 g/L	0.44 a
Watermelon – Bion 0.025 g/L	1.22 a
Watermelon – Bion 0.01 g/L	2.44 b
Watermelon – Control	4.56 c
LSD *(P = 0.05)*	0.96
Honeydew – Bion 0.1 g/L	— (Phytotoxic)
Honeydew – Bion 0.05 g/L	0.33 (Phytotoxic)
Honeydew – Bion 0.025 g/L	1.11
Honeydew – Bion 0.01 g/L	1.44
Honeydew – Control	1.44

*0 = no disease symptoms, 6 = >50% leaf area diseased

DISCUSSION. A large proportion of the commercial melon crop is produced from transplanted seedlings. In the crowded conditions of nurseries, bacterial blotch can spread rapidly. The availability of clean seed for nurseries is the most important step in control of field losses due to fruit blotch. In 1994, a committee of experts in the USA chaired by Dr R E Stall, recommended that seed companies test their watermelon seed lots by way of "grow out" tests. The minimum sample size was to be 10,000 seeds which would detect an infestation level of 0.1% at the 95% confidence level. While the implementation of this recommendation reduced the risk in watermelons, other melon types were not screened since they were believed to be not at risk.

Outbreaks in rockmelons and honeydew seedlings in Bowen nurseries showed this was not so and as an interim measure to provide a method for nurseries to disinfest seed, we undertook the seed treatment tests described in this section. Hot water treatment (55°C for 20 minutes) gave best disease control and also best emergence. The treatment did not completely eradicate the disease but indicted that with further refinements, e.g. longer time, the treatment may be more effective. Increasing the immersion time to 25 or 30 minutes did not increase seed damage. Other possible treatments such as hydrochloric acid and chlorine were ineffective and highlighted the fact that much of the inoculum is carried internally. The quaternary ammonium compound Agrigard was successful in reducing the % infection but it also reduced germination. It is probable that its strong detergent action allowed it to penetrate the seed causing damage to both the embryo and the bacterium. The control treatment (soaking in sterile water) had quite low germination rates in both treatments which must be due to higher pre-emergence activity by the bacterium. The two plantings of treated seed showed that in most treatments, germination rates declined appreciably during the two weeks after seed was treated. Thus, seed treated by HWT, should be sown as soon as possible after treatment and certainly within 48 h.

In the study on the effect of HWT on germination of 46 seed lines, we used 48 h as the standard interval between treatment and sowing. Five of the 46 lines showed appreciable (>10%) reduction in germination by the 55°C/30min treatment. Vigorous seed lines were

unaffected. A small scale trial should therefore be undertaken before committing large quantities of expensive seed to hot water treatment.

Copper sprays are recommended for field control in the USA (Latin and Rane 1991) and in our field trials we tested the three types of available copper compounds as well as some additivies.

The early establishment of the disease through the trial area and conducive weather conditions resulted in a severe test of treatments. The two main findings from the experiment were that copper sprays will reduce foliar disease severity but the level of foliar disease has to be very low before fruit losses can be avoided (Tables 14 and 15). None of the spray additives significantly altered the level of disease control on foliage. Bion, however, appeared to have more promise giving a non-significant improvement rather than the non-significant reduction with Sporekill and $FeCl_3$.

It is clear that economic control of fruit blotch requires practically complete control of the foliar phase of the disease. Current chemicals are not capable of this high standard of control under heavy disease pressure.

Latin and Rane (1991) also warned that treating watermelon crops with copper on a regular basis could result in significant yield reductions. In our second field trial we intended to judge the effect of copper hydroxide on fruit set at normal and above recommended rates in the absence of blotch. However, natural infection occurred with inoculum moving from the watermelon spray trial only 5 m away. The isolate used to inoculate the watermelon trial was 4391, a watermelon strain, hence, rockmelon plots were unaffected. The honeydew cultivar Dewcrisp was very susceptible to both the foliar and fruit phases of the disease. On fruit, circular depressed lesions developed from small water soaked spots. Extensive blotching (as on watermelon) did not occur. This may be due to the isolate being a watermelon strain rather than the rockmelon strain which is even more aggressive on this host.

In this experiment, copper sprays were effective in limiting the spread of disease in foliage. The higher rate (4 g/L) of Kocide gave consistently lower disease severity ratings than the standard (2 g/L) rate but differences were not significant ($P = 0.05$). Sprayed plots also showed reduced disease severity on fruit (Table 19).

Phytotoxicity was very mild with only a few leaves in the whole trial area affected. There were no significant differences in the number of harvested fruit per plot (Table 19) but the trend was for sprayed plots to have more fruit. From this we concluded that copper sprays are safe to use on melon crops.

The use of copper sprays in the field may not guarantee an adequate level of disease suppression. There is a need for improved chemical control of bacterial blotch. Bion, which systemically activates natural resistance mechanisms in plants showed some promise in the field trial which was supported in the glasshouse test. This product needs further evaluation because both the beneficial and phytocidal effects depend greatly on the interaction between plant and chemical. Evidence from the glasshouse test shows that honeydew cv. Greenflesh is sensitive to damage from Bion while there was a very satisfactory disease reduction induced in watermelon cv. Crimson Sweet. Further studies may require the assessment of individual cultivars for their interaction with this promising new product.

5. GENERAL DISCUSSION

The early identification of a rockmelon strain of *Acidovorax avenae* subsp. *citrulli* in this project was important because it alerted seed companies to the vulnerability of rockmelon and honeydew to bacterial blotch. We made direct contact with a wide range of colleagues in seed companies and universities providing them with details of our research and isolates of *Acidovorax avenae* subsp. *citrulli* so they could conduct their own tests. An outbreak of bacterial blotch in rockmelon seedlings and field crops was recently observed in Georgia, USA (Walcott *et al.* 2000). These workers now agree (Walcott *et al.* 1999) that *Acidovorax avenae* subsp. *citrulli* is composed of two major strains, one from watermelon and the other from other hosts including rockmelon. Although further studies need to be done to determine how Australian isolates of *Acidovorax avenae* subsp. *citrulli* compare genetically with those from overseas outbreaks, we are hopeful that, with the confirmation of two strains in the USA, seed companies will introduce detection tests in all melon seed lots.

As an interim measure, hot water treatment of seed will reduce seed contamination but may also reduce the germination rate in poor quality or low vigour seed. Treated seed should be sown as soon as possible.

Although our observations indicated that seed-borne inoculum was the most common way the disease carries over, survival on cucurbit weed hosts (*C. lanatus* and *C. myriocarpus*) is also possible. These species are widespread in the Chinchilla district and are susceptible to the watermelon strain. They may not be so important in the life cycle of the rockmelon strain since we found *C. myriocarpus* was much less susceptible to this new strain. Survival on plant trash is unlikely, provided it is ploughed in after the final harvest.

The field trials and on-farm observations showed that relatively minor foliar infestations of blotch may lead to severe fruit losses. Copper sprays, although useful, may not give the degree of control necessary to prevent these losses in wet conditions. New products such as Bion require further development. Prevention, through the use of clean seed and seedlings, trash burial and wild host destruction, is far preferable to chemical field control.

Melon types (watermelon, rockmelon, honeydew) appear to be the preferred host of *Acidovorax avenae* subsp. *citrulli*. Occasional outbreaks in pumpkin or cucumber (Martin and O'Brien 1999 ; Langston *et al.*1999) may be due to particularly favourable environmental conditions. Our studies did not indicate a new pathological strain. Further comparisons between isolates using molecular techniques may detect differences in isolates, which could then be compared in pathological tests.

6. TECHNOLOGY TRANSFER

During the project we kept various sections of industry informed of our activities. Regular contact with seedling producers was maintained via our disease diagnosis service. Growers were advised of how to identify and control the disease by a colour brochure, "Bacterial fruit blotch of melon". A meeting with seed company representatives in 1998 advised them of the seed-borne nature of the disease and urged them to advise parent companies of the new threat to rockmelons. In addition we made direct contact with key people in the parent companies.

Direct contact was maintained with the following overseas workers:-

Dr German Hoyos, Novartis Seeds, USA (email: german.hoyos@seeds.Novartis.com)
Dr Jeffrey B Jones, University of Florida, USA (jbjones@nersp.nerdc.ufl.edu)
Dr Pieter Vandenberg, Seminis Vegetable Seeds, USA
Dr Mike Meadows, Novartis Seeds, USA (email: mike.meadows@seeds.Novartis.com)
Dr Robert de Vogel, Nunhems Zaden BV., The Netherlands (email: R.deVogel@nunhems.com)
Dr Chet Kurowski, Harris Moran Seeds, USA (email: ckurowski@hmsc.com)

Publications arising from this project

Technical

Martin, H.L., O'Brien, R.G., and Abbott, D.V. (1999). First report of *Acidovorax avenae* subsp. *citrulli* as a pathogen of cucumber. *Plant Disease* **83**; *965*

O'Brien, R.G., and Martin, H.L. (1999) Bacterial blotch of melons caused by strains of *Acidovorax avenae* subsp. *citrulli*. *Australian Journal of Experimental Agriculture* **39:** 479-85.

Conference papers

Horlock, C.M., and O'Brien, R.G. (1999). Hot water treatment reduces seed-borne bacterial blotch in melons. APPS Conference, Canberra.

Martin, H.L., and O'Brien, R.G. (1999). Bacterial fruit blotch (*Acidovorax avenae* subsp. *citrulli*): a new pathogen of cucumber. APPS Conference, Canberra.

Wen, A., Hayward, A.C., Chakraborty, S., Sly, L.I., Fegan, M., and O'Brien, R.G. (1997). Rapid identification and detection of *Acidovorax avenae* in melon. APPS Conference, Perth.

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Extension

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O'Brien, Rob, and Wen, Aimin (1997) Bacterial fruit blotch of watermelon. *Australian Melon Runner* **4**: 15.

7. RECOMMENDATIONS

- Seed companies continue to be encouraged to introduce tests to detect *Acidovorax avenae* subsp. *citrulli* in seed lots of all melon crops.

- In the absence of quality assurance from seed companies, nurseries treat seed with hot water (55°C for 25 m). Small scale test for germination reduction to be carried out prior to large scale treatment.

- Field control to be based on preventive measures (clean seedlings, trash destruction, elimination of volunteer seedlings and cucurbit weeds). Spraying with copper compounds is recommended but may not adequately control field outbreaks during wet conditions.

- Further development of Bion for blotch control is recommended.

- Detailed studies of genetic differences between isolates of *Acidovorax avenae* subsp. *citrulli* (both local and overseas) should be initiated to detect isolates with possibly wider host ranges.

8. ACKNOWLEDGEMENTS

We appreciate the support given by the melon industry of Queensland and, in particular, the growers of the Chinchilla district and the seedling producers of both north and south Queensland. We are also indebted to seed companies for donations of seed and their willingness to try to understand the nature of the disease. Throughout the project we collaborated with overseas blotch workers to our mutual benefit.

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