VG108
Queensland fresh market tomato breeding project

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Queensland Department of Primary Industries

Know-how for Horticulture™

VG108

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

The research contained in this report was funded by the Horticultural Research and Development Corporation with the financial support of the Bowen District Growers Association, Bundaberg & District Fruit & Vegetable Growers and Qld Fruit & Vegetable Growers.

Cover Price $20.00

HRDC ISBN 1 86423 509 8

Published and Distributed by:

Horticultural Research and Development Corporation
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7 Merriwa Street
Gordon NSW 2072

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QUEENSLAND FRESH MARKET

TOMATO BREEDING

VG108

HORTICULTURAL RESEARCH & DEVELOPMENT CORPORATION

The Research Arm of the Australian Horticultural Industries

DPI QUEENSLAND DEPARTMENT OF PRIMARY INDUSTRIES

Queensland Fruit & Vegetable Growers

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ACKNOWLEDGMENTS

The project was jointly funded by Queensland Fruit and Vegetable Growers and the Horticultural Research and Development Corporation.

Outcomes of Queensland Fresh Market Tomato Breeding

1. Three inbred lines with tolerance to bacterial wilt were developed at Bundaberg Research Station. It is expected that they will be released to seed companies for F_1 hybrid production.

2. Two F_1 hybrids with resistance to Fusarium wilt races 1, 2 and 3 were developed at Bowen Research Station. Subject to testing in 1995, they will be made available to industry through DPI's release procedure.

3. A bioassay for resistance to Potato Tuber Moth, *Phthorimaea operculella*, was developed. The technique demonstrated resistance in four accessions of *Lycopersicon hirsutum*.

4. A rapid and reliable seedling test for resistance to bacterial wilt was developed following a series of experiments investigating the effect of temperature, plant age, inoculum density and genotype on symptom expression.

5. Breeding lines with markedly improved soluble solids contents, flavour and post-harvest shelf-life were developed by backcrossing.

6. Four breeding lines and two F_1 hybrids with tolerance to bacterial canker were developed at Redlands and Applethorpe Research Station.

Breeding for Resistance to Potato Tuber Moth

I.R. Kay

Introduction

The potato moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is an important pest of tomatoes in the major tomato growing districts of Queensland. The larvae mine in the leaves and in the fruit, and it is the fruit damage that results in large economic losses. Losses of over 60% of fruit have been common despite the frequent application of insecticides, and Kay (1993) showed in field trials that insecticides did not control the pest.

Host plant resistance is one component of integrated pest management systems. Resistance to lepidopteran insects has been reported in tomatoes (see Stoner (1992) for a bibliography), while Juvik *et al.* (1982) reported that two *Lycopersicon hirsutum* accessions were resistant, and a *Solanum pennellii* accession was partially resistant, to *P. operculella.* Accordingly we decided to test several *L. hirsutum* accessions for their resistance to *P. operculella*, with a view to their possible use as parents in a breeding program to develop *P. operculella* resistant tomato cultivars.

Methods

(i) Field Trial

Four *L. hirsutum* accessions (LA 1777, LA 407, LA 1265, LA 1252) and a commercial *L. esculentum* cultivar (Floradade), which is susceptible to *P. operculella* , were grown in two concurrent trials at Bundaberg Research Station in 1992. Each trial consisted of the five lines planted in a 5 by 4 replicated block design with plots of 1 row by 8m. Seedlings were planted into the field in mid October and the leaf assessments were done in early-mid December. Both trials were treated 3 times a fortnight with fungicides and occasionally with dicofol. One trial, designated as the "sprayed" trial, was treated 3 times a fortnight with alternating applications of sulprofos and methamidophos, while the second trial, designated as "unsprayed", was not treated with insecticides.

In each trial a single trap baited with *P. operculella* pheromone was used to monitor *P. operculella* population levels. The traps were cleared three times a week, the moths counted, and the catches recorded as weekly catches.

Resistance was assessed by comparing the number of larval mines in leaves of the different plant lines. Ten leaves per plot were collected at random from the lower parts of the plants (from ground level to 0.25m) and the number of larval mines counted. The numbers of mines per plot were bulked, and these data, following ln (x + 1) transformation, were subjected to analysis of variance . Leaves from the middle of the plants (0.4-0.6m above the ground) were sampled similarly but the data were not analysed as they were very variable.

Bioassays were attempted using leaves from the unsprayed trial. Leaves were cut and returned to the laboratory where their cut ends were wrapped in moist cotton wool before they were placed in plastic containers. Five neonate *P. operculella* larvae (<24h old) from a laboratory colony were placed on leaflets of each leaf, and the containers were placed in a temperature cabinet at 25°C with a 12L : 12D photoperiod. There were four replicates. It was intended to count the number of mines after a period of time (7d) and to weigh the larvae after about 10 days.

(ii) Bioassay - variable temperature

Several larval bioassays were carried out on whole plants in a laboratory with little temperature control as constant temperature facilities were not available. The methods were similar in each case. Plants of the four *L. hirsutum* lines and Floradade were grown in pots in a greenhouse until they were large enough to be used in the bioassay. Five neonate *P. operculella* larvae from a laboratory colony were placed on the upper surface of leaves of each plant. In the first bioassay five plants of each line were used (i.e. 25 larvae were used), and after 14 days larvae that could be found were collected and weighed. In the second bioassay three plants of each line were used (i.e. 15 larvae) and the larvae were weighed after 10 days. In both cases daily maximum and minimum temperatures in the laboratory were recorded.

(iii) Bioassay - feeding duration

As constant temperature facilities had become available a trial was carried out to determine the length of time that larvae should feed to maximise their weight but minimise losses of larvae through pupation. i.e. we wanted large larvae but wanted to recover as many as possible and not lose them through pupation. Five neonate larvae were placed onto the leaves of each of 20 potted Floradade plants which were held in a constant temperature room at 24 ± 0.25°C and 12L : 12D photoperiod. Five plants were removed after each time period and the larvae on them were recovered, counted and weighed. The time periods (i.e. feeding durations) were 8, 10, 12 and 14 days.

(iv) Bioassay - constant temperature

Five neonate *P. operculella* larvae were placed on leaves of potted plants of the four *L. hirsutum* lines and Floradade. There were six replicates of each line i.e. six plants of each line. The plants were held in the constant temperature room at 24 ± 0.25°C and 12L : 12D. Larvae were recovered, counted and weighed after 12 days. Analyses of variance were done on number of surviving larvae and on mean larval weight.

Results

(i) Field trial

The weekly moth counts from the two pheromone traps are shown in Figure 1. The catches show that the *P. operculella* moth population was high in both trials.

Table 1 shows the number of mines in 10 leaves per plot from the lower and middle sections of the plants. There were significantly ($p<0.05$) more mines in lower Floradade leaves than in lower leaves of any of the *L. hirsutum* accessions in both trials. In the sprayed trial there were significantly ($p<0.05$) fewer mines in lower leaves of LA 1777 than in leaves of the other *L. hirsutum* accessions but there were no differences ($p>0.05$) between these accessions in the unsprayed trial. The number of mines data for middle leaves were not analysed because of their extreme variability but the means, which are shown in Table 1, indicate that Floradade had more mines than the *L. hirsutum* accessions.

The bioassays using cut leaves were unsuccessful. Leaves of the four *L. hirsutum* lines turned brown and started to decompose after only a few days. By day seven they were badly decomposed and larvae could not be found. Floradade leaves were in fair to good condition after seven days and mines and larvae were found.

(ii) Bioassay - variable temperature

Daily temperatures for the 14 day duration bioassay ranged from 22 - 25°C to 24.5 - 27°C. Few larvae were recovered to be weighed in this bioassay (Table 2), even from the commercial cultivar Floradade, although large larval mines were present in the Floradade leaves. Clearly many larvae had grown to full development and had left the mines to pupate, a conclusion supported by the presence of pupae in some pots. The weights of the recovered larvae are not recorded here as obviously they were from slowly developing larvae and so were biased against plants on which larvae developed rapidly and well.

The mean number of surviving larvae and the mean larval weight in the 10 day duration bioassay are given in Table 2. There were no significant differences ($p>0.05$) between varieties in the number of surviving larvae, but larvae from Floradade and LA 407 plants were significantly ($p<0.05$) heavier than larvae from plants of the other varieties. Temperatures during this trial ranged from 21 - 25°C to 21.5 - 25.5°C.

(iii) Bioassay - feeding duration

The mean number of larvae recovered and the mean weight of those larvae for each time period are shown in Table 3. Larval recovery was high (more than 4 out of 5) up to and including day 12, but on day 14 very few larvae were recovered. Obviously most larvae pupated between day 12 and day 14. Mean larval weight also was high at day 12.

(iv) Bioassay - constant temperature

Significantly ($p<0.05$) more larvae were recovered from Floradade than from the *L. hirsutum* lines, except for LA 1265. Very few larvae at all were recovered from the accessions LA 1777 and LA 407. Surviving larvae feeding on Floradade were significantly ($p<0.05$) heavier than larvae from

any of the *L. hirsutum* lines, while larvae feeding on LA 407 were significantly ($p<0.05$) smaller than those on the other *L. hirsutum* accessions.

Discussion

The field trials, done at a time of year when *P. operculella* numbers usually are high, were conducted under heavy *P. operculella* pressure as evidenced by the pheromone trap catches (Figure 1). There were many more mines in leaves of Floradade than in leaves of the *L. hirsutum* lines which indicates that Floradade was more severely damaged, and conversely that the *L. hirsutum* lines were less damaged, by *P. operculella*. It was noted that mines in Floradade leaves appeared more extensive, and the larvae in them larger, than those in the *L. hirsutum* lines, but no objective measurements of these were made. These results indicate the *L. hirsutum* lines are promising as sources of resistance against *P. operculella*.

There are two possible reasons for the higher level of damage in Floradade than in the *L. hirsutum* lines in these trials. The first is that *P. operculella* moths preferred the Floradade plants and preferentially selected them to oviposit on. Juvik *et al.* (1982) reported ovipositional preferences in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) for a range of cultivated and wild *Lycopersicon* lines, but there are no reports of ovipositional preferences in *P. operculella* for different tomato lines. The second possibility is that there was no ovipositional preference but that many larvae failed to mine into leaves of the *L. hirsutum* or died before making an observable mine i.e. there was an antibiosis effect. The observation that mines and larvae in *L. hirsutum* lines were smaller than in Floradade tends to support this. Of course, aspects of both non-preference and antibiosis may be involved.

The bioassay technique, during the experiments reported here, evolved to one that is resonably straight-forward yet reflects the natural feeding behaviour of *P. operculella*. Juvik *et al.* (1982) placed neonate *P. operculella* larvae on excised leaflets, supplied fresh leaflets every 2-3 days and recorded percent survival at 3 days and 14 days. However in our trials the *L. hirsutum* leaflets deteriorated markedly within a few days and comparisons of larval survival on *L. hirsutum* lines and Floradade would have been confounded by the deterioration factor. *P. operculella* larvae usually

remain in a single leaflet and mine it extensively, rather than moving from one leaflet to another as they would be required to do using the Juvik *et al.* (1982) technique, and so their bioassay method requires abnormal behaviour by the larvae.

The 10 day variable temperature bioassay demonstrated that *P. operculella* larval growth rate as measured by weight was slower on most of the *L. hirsutum* lines compared to Floradade. However the larvae on Floradade were still quite small at 10 days, and larger larvae would make comparisons simpler.

The acquisition of a constant temperature room was a major advance. Temperature is the most important factor affecting the developmental rate of insects, so using a constant temperature eliminates a major variable and allows comparison of bioassays run at different times.

The feeding duration trial at 24°C showed that at 12 days larvae had grown to quite a large size but had not reached pupation, so the final bioassay trial was run for 12 days. In this bioassay larval survival on LA 1777, LA 407 and LA 1252 was poor. Juvik *et al.* (1982) also reported very low survival of larvae at 14 days on LA 1777 (2%) and LA 407 (12%) compared to two *L. esculentum* lines (48% and 65%). Larval weight on the four *L. hirsutum* lines was low, again demonstrating that the *L. hirsutum* lines adversely affect the growth and development of *P. operculella* larvae.

The field trial and the bioassays clearly show that the *L. hirsutum* lines are resistant to *P. operculella* larvae, compared to a commercial *L. esculentum* cultivar.

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Figure 1

Weekly pheromone trap catches of *P. operculella* moths during the field trials.

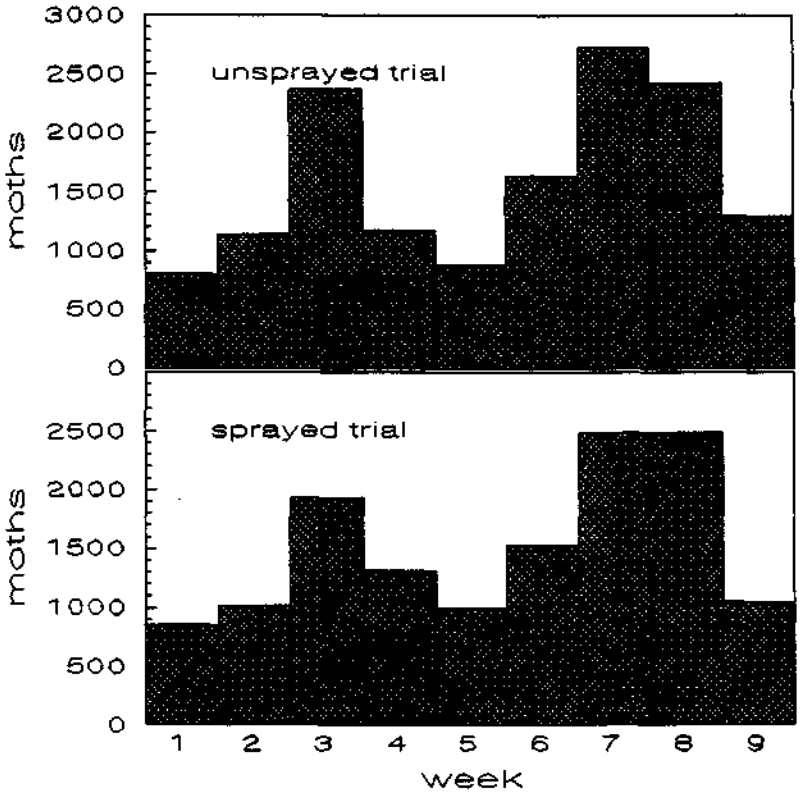

Table 1

Numbers of *P. operculella* larval mines in 10 leaves from the lower and middle sections of tomato plants in the sprayed and unsprayed trials. Values are back-transformed means following ln (x + 1) transformations before analysis.

Tomato line	Number of mines			
	Sprayed trial		Unsprayed trial	
	Lower	Middle	Lower	Middle
LA 1777	2.13 a *	0.50	34.8 a	4.9
LA 407	24.8 b	0.86	19.9 a	0.57
LA 1265	38.6 b	7.3	35.8 a	1.21
LA 1252	21.6 b	2.3	33.1 a	2.3
Floradade	96.4 c	15.3	86.6 b	33.2

* Numbers followed by the same letter are not significantly different (p = 0.05).

Table 2

Mean number of *P. operculella* larvae recovered and mean weight of larvae in the 14 day and 10 day variable temperature bioassays. Values in the 10 day trial are back-transformed means following transformations before analysis.

Tomato line	14 day trial	10 day trial	
	No. larvae recovered	No. larvae recovered *	Larval weight (mg) **
LA 1777	0.2	2.6 a[+]	1.5 b
LA 407	1.8	3.7 a	3.7 a
LA 1265	2.4	3.4 a	1.7 b
LA 1252	2.0	2.2 a	1.5 b
Floradade	1.6	3.4 a	4.7 a

* square root (x + 0.5) transformation; ** ln(x + 1) transformation; + numbers followed by the same letter are not significantly different (p = 0.05).

Table 3

Mean number of *P. operculella* larvae recovered and mean weight of larvae after larvae had fed in Floradade leaves for varying durations.

	Feeding duration (days)			
	8	10	12	14
No. larvae recovered	4.6	4.2	4.2	1
Larval weight (mg)	3.3	7.7	14.5	11.2

Table 4

Number of *P. operculella* larvae recovered and weight of larvae in the 12 day bioassay. Values are back-transformed means following transformations before analysis.

Tomato line	No. larvae recovered *	Larval weight (mg) **
LA 1777	0.3 a[+]	3.3 b
LA 407	0.4 a	0.8 a
LA 1265	2.3 bc	3.8 b
LA 1252	1.1 ab	3.4 b
Floradade	3.4 c	11.6 c

* square root (x +0.5) transformation; ** ln(x + 1) transformation; + numbers followed by the same letter are not significantly different (p = 0.05).

Breeding for Resistance to Bacterial Wilt

J. A. Barnes and M. M. Kelly

Summary

A breeding program was undertaken at Bundaberg Research Station to develop fresh market tomato cultivars with resistance to *Pseudomonas solanacearum* E F Smith biovar 3, the causal organism of bacterial wilt. Bacterial wilt causes substantial losses in susceptible tomato cultivars throughout Queensland during the warmer months of the year. The initial cross was Rodade (resistant female parent) and FDA3 (susceptible male parent). Selections through eight generations have resulted in three inbred lines, BRS 101, 102 and 103, with high tolerance to bacterial wilt, being recommended for commercial release. The lines will be offered to private seed companies on a royalty basis for F1 hybrid production. It is expected that this will result in a number of tomato cultivars with high tolerance to bacterial wilt being made available to industry.

Introduction

Bacterial wilt caused by *Pseudomonas solanacearum* E F Smith biovar 3, causes substantial losses in susceptible tomato (*Lycopersicon esculentum* Mill.) cultivars throughout Queensland during the warmer months of the year. Redlander (Herrington *et al.* 1988), Redlands Summertaste (Herrington and Saranah 1985) and Scorpio (Peterson *et al.* 1983) were released from an earlier Queensland Department of Primary Industries bacterial wilt breeding program. However, they have not received wide acceptance by growers, many considering them to be commercially unsuitable (personal communications).

The aim of this project was to produce bacterial wilt resistant tomato lines with firm, good quality fruit of size around 150 grams.

Materials and methods

Screening for parental material

Two replications of 60 tomato cultivars and/or breeding lines from local and overseas sources and reported to have bacterial wilt resistance were transplanted into a severely infected grower's field of area 0.25 ha on October 10, 1988. Eight plant plots were used. Susceptible cultivars which were planted at frequent intervals throughout the field showed that the infection, while severe, was not sufficiently uniform to enable a successful breeding program to be carried out at the site.

Improving level of field infection

The next 12 months were spent increasing the level of infection over the field. A number of techniques were employed including artificially inoculating plants of susceptible cultivars and ploughing them in, and spreading and incorporating infected plants and soil into sections where the disease expression was less severe.

Crossing and selection

The initial cross between Rodade (seed parent) and FDA3 (pollen parent) was made in April 1989. Rodade (Bosch *et al.* 1985) is a fresh market tomato cultivar with good resistance to bacterial wilt. It has firm fruit of excellent quality. However, fruit size of Rodade is not large enough for the Australian market. FDA3 was developed at Bundaberg Research Station and is an inbred line from a cross between Flora-Dade and Florida 1A. Five hundred second generation (F2) plants were transplanted into the infected field in February 1990 at a plant spacing of 500 mm. The crop was interplanted with the susceptible cultivar, Flora-Dade, a practice which was continued for all future generations. Resistant selections were made only if the Flora-Dade plants either side of the selected plant had died. To increase the selection pressure on the F2 population, a bacterial suspension was injected through the drip irrigation system in March 1990. This was done by soaking the cut stems of 120 infected Flora-Dade plants in 30 litres of water for 3 hours and pumping in the resultant bacterial suspension during an irrigation using a water driven pump.

Sixty-two F2 selections were made in May 1990 and the resultant F3 families were transplanted in October 1990 in 5 replications of 8 plant plots. Selections were based not only on resistance to bacterial wilt but also on agronomic characteristics such as fruit size and shape, bush size, leaf cover, etc. Single plant selections were made from 25 F3 families in December. In all further selection work single replications with a plot size of 40-50 plants was used. The field was mapped for bacterial wilt incidence in November 1990 by counting the number of dead Flora-Dade interplants. Selection continued through 1991 and 1992 and in 1993 detailed testing of 21 F8 lines commenced.

Testing of F8 breeding lines

This comprised growing the F8 lines during the autumn-winter (transplanted February) and spring-summer (transplanted September) production season in both the infected grower's field and a clean field at Bundaberg Research Station. In addition, the F8 lines were artificially inoculated and screened for bacterial wilt resistance in a controlled environment cabinet set at 31°C.

Crop management

Crops were grown on raised, fertilised, polyethylene mulch covered beds with a single drip irrigation tube under the polyethylene. Crops were established from container grown transplants. In-row plant spacing was 500 mm with a between row spacing of 1.5 m. Irrigation was determined by tensiometers and fertigation rates for nitrogen and potassium were determined by testing petiole sap with Merckoquant nitrate and potassium strips. Normal management practice for disease and past control was carried out. At the infected site, the raised beds were fumigated with methyl bromide at the rate of 50 g/m^2 during the mulch laying process. At the clean site, a granular formulation of fenamiphos (Nemacur 100 G, 100 g a.i/kg) was incorporated over a 600 mm wide strip at the rate of 13.3 g/m^2 during the bed shaping operation. These treatments were for root-knot nematode control.

Soil temperature

To allow good plant development, soil temperatures were modified by varying the colour of the polyethylene mulch - white for the February planting to reduce soil temperature and black for the September planting to raise soil temperature.

Results

Screening for parental material

Most of the 60 tomato cultivars and/or breeding lines in the initial field screening showed good resistance to bacterial wilt. However, none were commercially suitable. Many had either quite small fruit, poor fruit shape or soft fruit. Rodade was the most impressive of all the lines and was selected as the resistant parent for a breeding program.

Improving level of field infection

The level of natural field infection was high and very uniform by November 1990 with 96% of all Flora-Dade plants dead 5 weeks after transplanting. Further plant deaths occurred after this original count. This high infection level has been maintained in all subsequent crops as evidenced by the almost total loss of the Flora-Dade interplants. Therefore, a high selection pressure to bacterial wilt has been placed on the progeny of the breeding program.

Testing of F8 breeding lines

Infected site. BRS 101, 102 and 103 had significantly ($P<0.05$) greater yields than FDA3 in the autumn-winter season and FDA3 and Flora-Dade in the spring-summer season (Table 1). In the spring-summer season the yield of FDA3 and Flora-Dade was zero as all the plants had died from bacterial wilt. The yields of BRS 101, 102, 103 and Rodade or Redlander in the case of the autumn-winter season were not significantly ($P>0.05$) different from each other.

BRS 101 had the largest fruit of the 3 breeding lines although the only significant ($P<0.05$) difference was between BRS 101 and BRS 103 in the autumn-winter season (Table 1).

Table 1. Performance of BRS 101, 102 and 103 compared to other fresh market tomato cultivars grown on an infected site at Bundaberg during 1993.

Within a column, values followed by the same letter do not differ significantly at $P = 0.05$.

Cultivar	Autumn-winter 1993[A]			Spring-summer 1993[B]		
	Total yield (kg)	kg/plant	Size (g)	Total yield (kg)	kg/plant	Size (g)
BRS 101	55.6[a]	6.95	127[a]	18.2[a]	3.6	134[a]
BRS 102	52.8[a]	6.6	117[ab]	21.3[a]	4.3	123[a]
BRS 103	55.5[a]	6.9	110[bc]	18.5[a]	3.7	122[a]
Flora-	-	-	-	0.0[b]	0	0[c]
Dade	11.4[b]	1.4	113[abc]	0.0[b]	0	0[c]
FDA3	60.5[a]	7.6	73[d]	17.7[a]	3.5	92[b]
Rodade	52.9[a]	6.6	101[c]	-		-
Redlander	11.9		15	5.7		14
l.s.d. (P=0.05)						

[A]Plot size = 4.0 m (8 plants)
[B]Plot size = 2.5 m (5 plants)

Clean site

BRS 102 gave the highest total yield in both seasons and had a significantly ($P<0.05$) greater total yield than all cultivars except BRS 103 and FDA 3 (Table 2). The total yield of

BRS 101 was not significantly ($P>0.05$) different from that of Flora-Dade, the industry standard.

Rodade had a significantly ($P<0.05$) higher percentage marketable yield and percentage first grade fruit than all other cultivars during both seasons (Table 2). In the autumn-winter season, the percentage marketable yield and percentage first grade fruit of BRS 101 was significantly ($P<0.05$) less than that of BRS 102, 103 and Flora-Dade, all of which were similar. In the spring-summer season the percentage first grade fruit of BRS 101 was significantly ($P<0.05$) less than that of BRS 102, 103 and Flora-Dade. BRS 102 had a significantly ($P<0.05$) greater percentage first grade fruit than either BRS 103 or Flora-Dade. The difference between percentage marketable yield and percentage first grade fruit was much greater in the spring-summer season than in the autumn-winter season.

Fruit size as measured by average fruit weight was significantly ($P<0.05$) less for Rodade than for all other cultivars (Table 2). In the autumn-winter season, BRS 101, 102, 103 and Flora-Dade were not significantly ($P>0.05$) different for average fruit weight. In the spring-summer season, the average fruit weight of Flora-Dade was significantly ($P<0.05$) less than that of BRS 101, 102 and 103.

Table 2. **Performance of BRS 101, 102 and 103 compared to other fresh market tomato cultivars grown on a clean site at Bundaberg Research Station during 1993.**

Within a column, values followed by the same letter do not differ significantly at P = 0.05.

Cultivar	Autumn-winter 1993				Spring-summer 1993			
	Total yield (kg)	% marketable yield	% first grade	size (g)	Total yield (kg)	% marketable yield	% first grade	size (g)
BRS 101	63.7^{b}	86.8^{c}	75.3^{e}	148^{b}	44.5^{cde}	82.3^{d}	53.5^{d}	165^{a}
BRS 102	74.8^{a}	94.3^{b}	90.1^{bc}	144^{b}	57.7^{a}	92.5^{b}	76.1^{b}	154^{b}
BRS 103	67.0^{ab}	95.1^{b}	91.1^{b}	142^{b}	51.8^{abc}	87.4^{c}	63.7^{c}	154^{b}
Flora-Dade	66.0^{b}	92.7^{b}	86.6^{c}	148^{b}	46.9^{bcd}	81.4^{d}	63.6^{c}	143^{c}
FDA3	69.1^{ab}	89.8^{c}	80.1^{d}	170^{a}	54.0^{ab}	92.4^{b}	70.7^{b}	163^{a}
Rodade	50.8^{c}	99.5^{a}	99.3^{a}	94^{c}	38.4^{e}	98.8^{a}	94.0^{a}	107^{d}
l.s.d. (P=0.05)	8.0	2.8	4.0	8	8.4	3.8	6.5	9
Plot size = 4.5 m (8 plants)								

Controlled environment screening

BRS 101, 102, 103 and Rodade were not significantly (P>0.05) different from each other for disease rating but all had a significantly (P<0.05) lower disease rating than Flora-Dade (Table 3). The Flora-Dade seedlings were almost dead while there was some wilting in the other 4 cultivars.

Table 3. **Disease rating[A] on day 12 of tomato seedlings artificially inoculated[B] with *Pseudomonas solanacearum* and grown in a controlled environment cabinet at 31°C.**

Within a column, values followed by the same letter do not differ significantly at P = 0.05.

Cultivar	Disease rating
BRS 101	1.20[a]
BRS 102	2.10[a]
BRS 103	1.55[a]
Flora-Dade	3.40[b]
Rodade	1.50[a]
l.s.d. (P=0.05)	1.13

[A]Disease rating: 0 (healthy), 1 (one leaf wilted), 2 (two to three leaves wilted), 3 (all leaves wilted) and 4 (plant dead).
[B]Inoculum concentration =10^5 CFU/ml

Discussion

The controlled environment screening showed that while BRS 101, 102 and 103 were not completely resistant to *Pseudomonas solanacearum*, they did exhibit a high degree of tolerance (Table 3). They showed a similar tolerance level to Rodade. Bosch *et al.* (1985) found that Rodade was between 72% and 100% resistant to bacterial wilt when tested in the field.

Yield per plant on the infected site was considerably less during the spring-summer season than in the autumn-winter season (Table 1). This could be attributed to the higher soil and ambient temperatures during the spring-summer season (data not presented) causing a

breakdown of resistance. Earlier work showed that resistance to bacterial wilt is reduced at high soil temperatures (Mew and Ho 1977; Peterson *et al.* 1983). This temperature effect could also explain the fact that some of the FDA 3 plants (a susceptible cultivar) survived during the autumn-winter season and produced a low yield while during the spring-summer season they all died and produced zero yield (Table 1).

The percentage of first grade fruit for all cultivars during the spring-summer season was less than that during the autumn-summer winter season (Table 2). This was caused by a higher incidence of the fruit defect, catface, during the spring-summer season. We believe that this was as a result of seasonal conditions such as heavy winds during the flowering and fruit set period. BRS 101 is more susceptible to catface than BRS 102 and 103 and this shows up in the lower percentage of first grade for BRS 101 during both seasons (Table 2).

It is expected that BRS 101, 102 and 103 will be offered to seed companies for hybrid seed production.

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HORTICULTURAL RESEARCH AND DEVELOPMENT CORPORATION

FINAL REPORT: QUEENSLAND FRESH MARKET TOMATO BREEDING PROJECT

(3 YEAR PROJECT)

TECHNICAL REPORT: A glasshouse seedling test for bacterial wilt resistance in tomato

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Summary

A survey of isolates of *Pseudomonas solanacearum* from tomato in Queensland using a DNA-based method of differentiating bacterial strains showed they were a fairly homogenous group, all within the RFLP group 10 of biovar 3. A series of experiments was conducted to evaluate the effect temperature, plant age, inoculum concentration and cultivar on the severity of bacterial wilt in tomato seedlings. This research led to the development of a rapid and reliable seedling test for resistance to bacterial wilt. Three advanced breeding lines with resistance to bacterial wilt will be released from the tomato breeding project being conducted at Bundaberg Research Station.

Introduction

Bacterial wilt of tomato *Lycopersicum esculentum* caused by *Pseudomonas solanacearum* E.F. Smith *biovar* III is a most serious disease of tomato in many tropical, subtropical and warm temperate regions of the world. In many areas where the disease is prevalent, losses are so serious that commercial tomato production is impractical. In coastal areas of Queensland, bacterial wilt limits tomato production in the late spring to early autumn period. In Bundaberg serious crop losses have resulted during this period and the number of new disease outbreaks is on the increase.

Chemical and cultural control of this disease has been attempted with little success (Enfinger *et al.* 1979; McCarter *et al.* 1971.) Grafting using resistant rootstocks has proven successful but is time consuming and expensive. Resistant cultivars appear to offer the most effective means of control. Early research showed the Philippine lines VC9-1 and VC44 were highly resistant to local strains of bacterial wilt. In 1975, Peterson and Inch commenced a breeding program to incorporate this resistance and breed a tomato with a high level of wilt resistance and high yielding commercially acceptable characteristics. This program lead to the release of the wilt resistant cultivar Scorpio which was grown extensively in wilt infested soil in South East Queensland. Improved cultivars to follow were the hybrid Redlands Summertaste (Herrington and Saranah 1985) and the fixed line Redlander, derived from Redlands Summertaste. The current breeding program uses the cultivar `Rodade' as the source of resistance. Rodade was bred in South Africa with resistance to South African strains of bacterial wilt (Bosch *et al.* 1985).

Part of the overseas research effort has been devoted to developing screening techniques to assess resistance. At the Asian Vegetable Research and Development Centre (AVRDC), plants were screened for resistance by wounding stems or leaves with a needle or scissors previously dipped in a bacterial wilt suspension. More recent techniques (Hartman 1991) have included the use of a micropipette to infiltrate wounded tomato stems, and soaking tomato seeds in a bacterial cell suspension for 30 minutes. All methods induced wilt, however they were considered too severe to differentiate resistance. Invasion by the bacterial wilt pathogen using these various techniques was not the natural scheme of bacterial wilt invasion.

In 1991, research commenced into the development of a simple, rapid and reliable method of evaluating large numbers of cultivars and breeding lines for resistance to bacterial wilt. As resistance to the disease has been shown to be temperature sensitive and strain-specific (Krausz and Thurston 1975), a survey of *P. solanacearum* isolates from the tomato growing areas of Queensland was conducted. Temperature control was achieved using fibreglass heated propagation trays. The trays were replaced with a controlled environment cabinet in the final year of the project. The effects of temperature, plant age and inoculum concentration on the development of bacterial wilt in tomato seedlings was investigated and used in developing a seedling test to differentiate susceptible and resistant germplasm.

Materials and Methods

Isolation procedure and preparation of inoculum Cultures of *P. solanacearum* were isolated on tetrazolium media (Kelman 1954) from 0.5 cm^2 discoloured stem pieces suspended in 9 ml of sterile water and streaked onto culture plates. The plates were incubated at 30°C for 3 days. Virulent colonies were removed and stored in 4 ml ampoules of sterile water.

Inoculum was prepared from a mixture of 5 different isolates from the Bundaberg area. Each of the isolates was streaked on tetrazolium agar plates and the plate cultures incubated at 30°C for 72 hours. Bacteria were suspended in distilled water and the suspension was standardised photometrically with a spectrophotometer to an absorbance of 1.7 at 540 mm wave length which approximated a colony density of 10^{10} cfu/ml. The suspension was serially diluted to give concentrations of 10^5 and 10^8 cfu/ml. Concentrations were confirmed by replicated colony counts from 0.05 ml subsamples of serial dilutions spread on tetrazolium plates.

Survey of P. solanacearum isolates Bacterial wilt infected plants were obtained from tomato growing areas where bacterial wilt is a problem. Isolations were conducted and the cultures stored. A collection of 15 different isolates was sent to Dr M. Gillings, N.S.W. Department of Agriculture for DNA fingerprinting using restriction fragment polymorphisms (RFLP's) to ascertain the presence of variant isolates.

Optimum inoculum concentration and seedling age The susceptible cultivar Flora-Dade and the partially resistant Rodade were sown in 60 cell polystyrene trays containing a fertilised peat - vermiculite mix at weekly-intervals to obtain seedlings that were 4, 5 and 6-wks-old. Seedlings were raised in a planthouse where temperatures ranged between 16 and 27°C and plants were watered daily by overhead sprinklers. On the day of inoculation, the inoculum concentrations 10^8 and 10^5 cfu/ml were prepared as previously described. Roots of the tomato seedlings were washed to remove adhering soil, pruned to about 8 cm from the stem base and soaked for 1 hour in bacterial inoculum. The seedlings were than planted into a 1800 mm x 900 mm heated fibreglass propagation tray containing fumigated potting mix (equal parts sand and peat plus fertiliser). The propagation tray was set at 30°C and ambient temperatures were about 25°C day and 17°C night in the planthouse. The seedlings were watered twice daily. Treatments were arranged in a randomised complete block design with 4 replicates. Each plot consisted of 1 row of 5 plants. Daily ratings of wilt severity occurred

from the onset of wilt symptoms. A disease rating scale of 0, healthy; 1, 1 leaf wilted; 2, 2 to 3 leaves wilted; 3, all leaves wilted; 4, plant dead; was used.

Soil temperature and cultigen susceptibility Five-week-old seedlings of Flora-Dade, Rodade and F5 generation material (single plant selections of FDA-3 x Rodade) were root dip inoculated with 10^8 cfu/ml and planted into heated propagation trays set at 26°C, 30°C and 34°C. Ambient temperatures ranged between 24°C day and 14°C night. Cultigens were arranged in a randomised complete block design with 4 replications and 5 plants/plot. The disease assessment commenced with the onset of wilt symptoms.

Optimum inoculum concentration and seeding age under controlled environment conditions The earlier experiment investigating inoculum concentration and seedling age was repeated in a controlled environment cabinet with an ambient temperature of 31°C and soil temperature of 30°C. Seedlings were grown in fumigated potting mix in 15 cm diam plastic pots. Treatments were arranged in a randomised complete block design with 4 replicates. Each plot consisted of 5 plants. Relative humidity was maintained at 90%. The methods used were unchanged from the earlier experiment.

Resistance in advanced breeding lines Five-week-old seedlings of Flora-Dade, Rodade and 8 advanced breeding lines (F8 generation material) were evaluated for resistance to bacterial wilt in a controlled environment cabinet with an ambient temperature of 31°C, soil temperature of 30°C and R.H. of 90%. Seedlings were inoculated with a bacterial suspension of 10^5 cfu/ml concentration. Cultigens were arranged in a randomised complete block design with 4 replications and 5 plants/plot.

Results

Survey of P. solanacearum isolates The fifteen isolates from tomato in Queensland were all within the RFLP group 10 to biovar 3 (Table 1.). The only isolate exhibiting any differences was 4981 from the Mareeba area in far north Queensland.

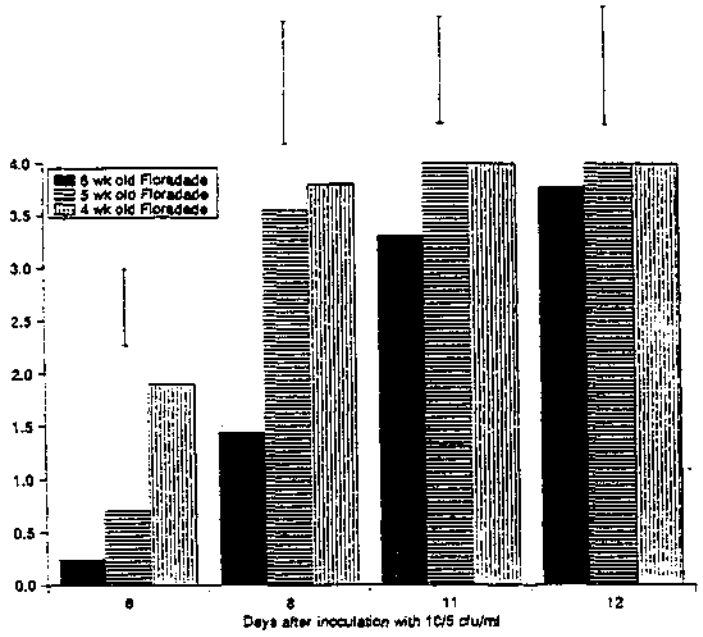
Table 1. Survey of isolates of *P. solanacearum* from tomato growing areas in Queensland

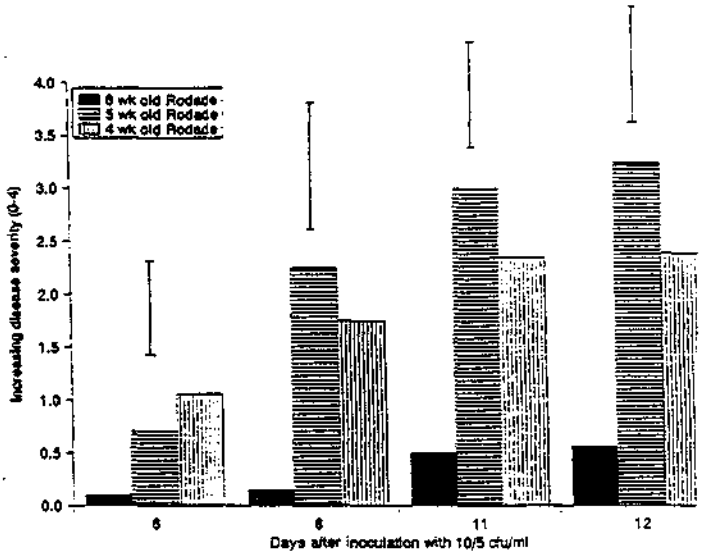
Isolate No.	Locality	Cultivar	Identification	RFLP group
19	Bundaberg	Pirate	L. Vawdrey	10A
20	Bundaberg	Flora-Dade	L. Vawdrey	10A
24	Bundaberg	Pirate	L. Vawdrey	10A
146	Bundaberg	Hawk	L. Vawdrey	10A
322	Bundaberg	Hawk	L. Vawdrey	10A
333	Bundaberg	Flora-Dade	L. Vawdrey	10A
311	Kallangur	Zola	L. Vawdrey	10A
312	Kallangur	Summertaste	L. Vawdrey	10A
314	Stanthorpe	Red Chief	L. Vawdrey	10A
325	Caboolture	C. Supreme	L. Vawdrey	10A
344	Nambour	Hawk	L. Vawdrey	10A
334	Walkamin	?	E. Akiew	10A
4381	Mareeba	?	E. Akiew	10B
5303	Dimbulah	?	E. Akiew	10A
5752	Herberton	?	E. Akiew	10A

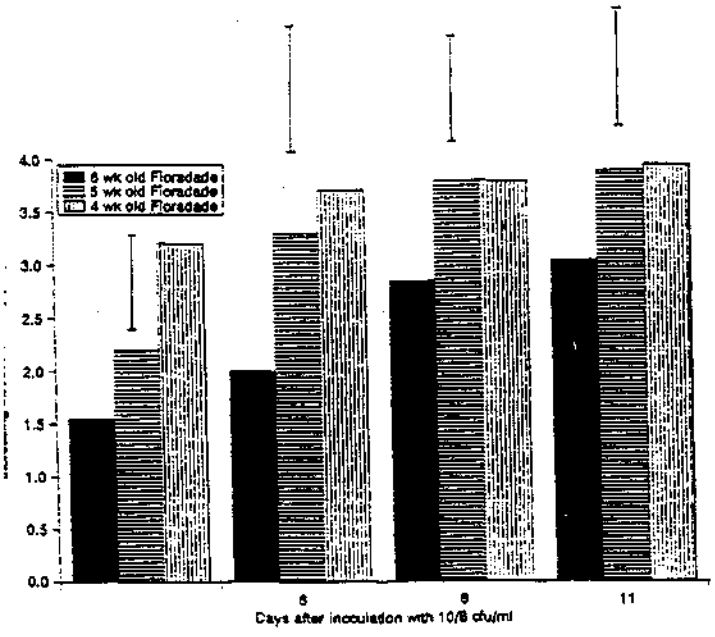
Optimum inoculum concentration and seedling age Disease severity increased with days after inoculation in both Flora-Dade and Rodade (Fig 1.). Disease symptoms appeared earlier where inoculum concentration was high. The first wilt symptoms were noted at day 6. At day 12, there was no significant difference in disease severity ($P<0.05$) between 10^5 cfu/ml and 10^8 cfu/ml in either Flora-Dade or Rodade. However, when Flora-Dade and Rodade were compared at the same inoculum concentration significant differences did occur.

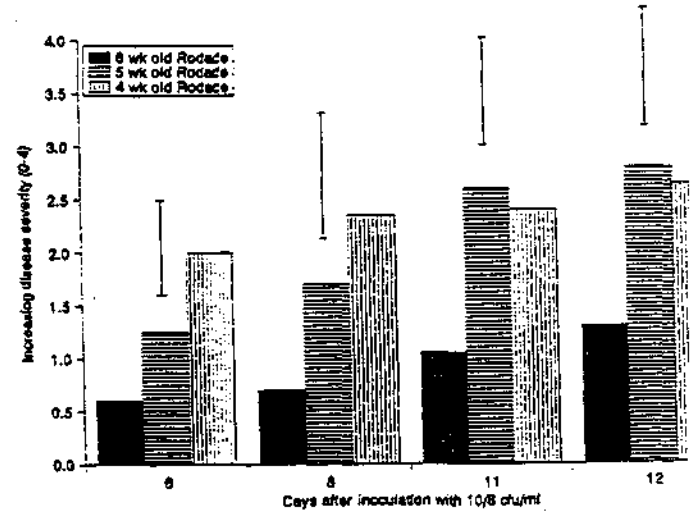
Height of tomato seedlings was influenced by plant age. Older seedlings were taller than younger seedlings. All 4-wk-old Flora-Dade seedlings were dead by day 12 irrespective of the inoculum concentration while some 6-wk-old Flora-Dade plants survived at both inoculum concentrations. Five-wk-old Flora-Dade seedlings inoculated with 10^8 cfu/ml were significantly more wilt affected ($P<0.5$) than 5-wk-old Rodade plants at the same inoculum concentration. Six-wk-old Flora-Dade plants inoculated with 10^5 cfu/ml were more wilt affected than Rodade plants of the same age and the same inoculum concentration. Rodade seedlings of the same age were similarly affected at either concentration.

Figure. 1 Effect of inoculum concentration and plant age on the severity of bacterial wilt in tomato seedlings cv. Flora-Dade and Rodade grown in a heated tray at 30°C in the planthouse. Vertical bars indicate l.s.d. at P<0.05.









Soil temperature and cultigen susceptibility Results of the experiment conducted at 26°C (Table 2) showed Flora-Dade seedlings severely wilt-affected by day 12 and Rodade and the F5 breeding material unaffected. At 30°C, Rodade and the F5 breeding material were less wilt-affected (P<0.05) than Flora-Dade, while at 34°C Rodade and all the F5 material except 47-1-1 were significantly less wilt-affected than Flora-Dade.

Table 2. Severity of bacterial wilt in 10 tomato cultigens inoculated with 10^8 cfu/ml and grown in heated propagation trays at 26, 30 and 34°C.

Tomato Cultigens	Disease Severity[a]		
	26°C	30°C	34°C
Rodade	0	0.85	2.45
Flora-Dade	3.40	3.95	3.70
(F5) 29	0	0	2.15
(F5) 30-1	0	0.15	1.50
(F5) 38-1	0	0.50	1.30
(F5) 38-2	0	0.20	0.95
(F5) 38-3	0	1.9	1.80
(F5) 38-4	0	0.15	1.95
(F5) 45	0	0.20	1.80
(F5) 47-1-1	0	0.80	2.55
l.s.d (P<0.05)	0.11	0.79	1.24
l.s.d. (P<0.01)	0.14	1.06	1.67

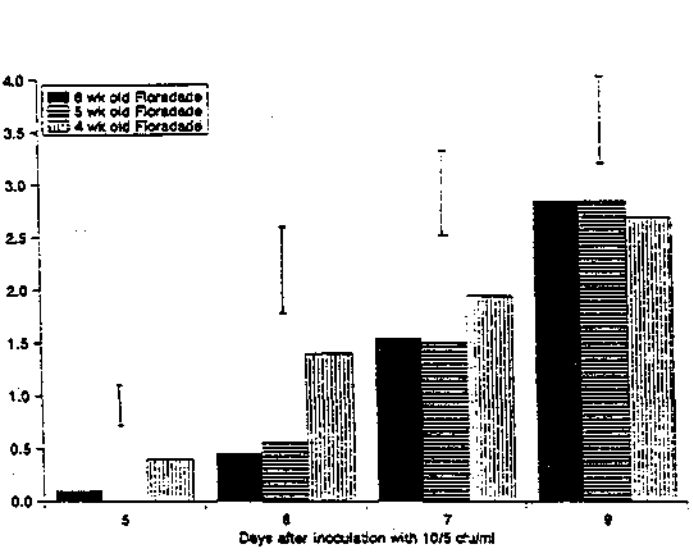
[a]Severity rated on a scale of 0 (no wilt symptoms) to 4 (plant dead)

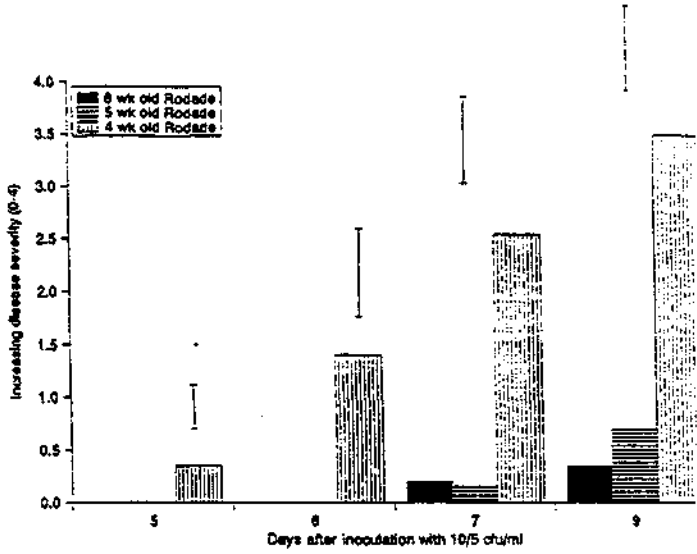
Optimum inoculum concentration and seedling age in a controlled environment Wilt symptoms occurred at day 5 and were most common at the 10^8 cfu/ml concentration (Fig 2.). At day 7, there was a significant difference (P<0.05) between 10^8 cfu/ml and 10^5 cfu/ml concentrations in 4, 5 and 6-wk-old Flora-Dade and Rodade seedlings. At day 9, the significant difference between high and low inoculum concentrations was evident in all except 4-wk-old Rodade seedlings.

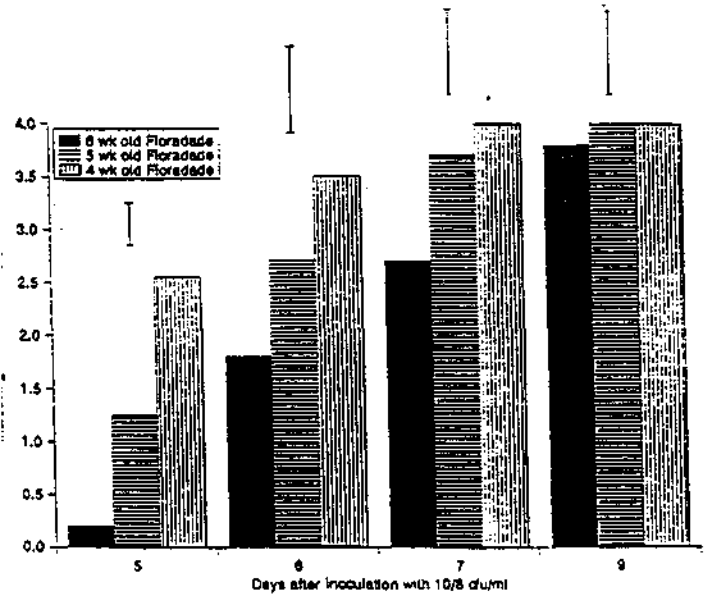
Wilt symptoms appeared on day 5 in 4-wk-old seedlings of both cultivars. At days 7 and 8, 5 and 6-wk-old Rodade seedlings inoculated with 10^5 cfu/ml were significantly less wilt affected (P<0.05) than Flora-Dade plants of similar age.

Resistance in advanced breeding lines Disease severity ratings commenced 6 days after inoculation and increased with time. At day 10 there was no significant difference between cultigens but by day 12, Rodade and 7 of the breeding lines had significantly less wilt (P<0.05) than Flora-Dade (Table 3).

Figure 2. Effect of inoculum concentration and plant age on the severity of bacterial wilt in tomato seedlings cv. Flora-Dade and Rodade grown in a controlled environment cabinet at 31°C. Vertical bars indicate l.s.d. at P<0.05.







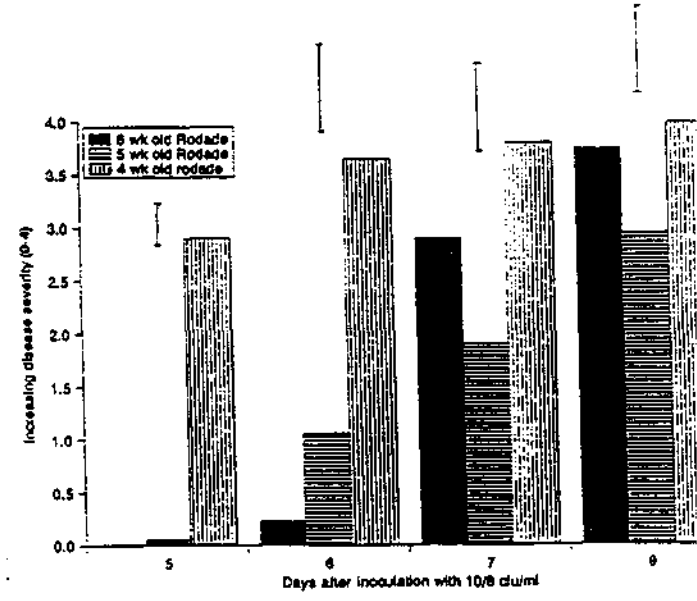


Table 3. Severity of bacterial wilt in 12 tomato cultigens inoculated with 10^5 cfu/ml and grown in a controlled environment cabinet at 31°C.

Tomato Cultigen	Disease Severity[a]	
	Day 10	Day 12
Rodade	1.10	1.50
Flora-Dade	2.7	3.40
26-3-1-1	1.75	2.00
38-2-1-2-1	0.85	1.20
38-2-2-1-1	2.25	3.00
38-4-1-1-1	1.6	2.25
47-1-1-1-1-1	1.55	2.15
47-1-1-3-1-1	2.05	2.10
47-2-1-1-1	2.35	2.80
47-2-2-1-1	2.05	2.40
47-2-3-2-1	1.85	2.05
57-3-1-2-2	1.00	1.55
l.s.d (P<0.05)	n.s.	1.13
l.s.d (P<0.01)	n.s.	1.51

[a]Severity rated on a scale of 0 (no wilt symptoms) to 4 (plant dead).

Discussion

Bacterial wilt is a common disease in the tomato growing areas of Queensland. In the Bundaberg area, long crop rotations with sugar-cane have been used as a means of reducing soil populations of the pathogen. For many small tomato growers, crop rotation with sugar-cane is not a suitable option. The main thrust of our work was to develop a rapid and reliable method of evaluating tomato germplasm for resistance to wilt. This research would culminate in the release of wilt resistant cultivars from the tomato breeding project conducted at Bundaberg Research Station.

The survey of isolates from tomato in Queensland showed they were a fairly homogenous group, all within RFLP group 10 of biovar 3 with no surprise variant isolates. RFLP group 10 is a commonly recovered strain of *P. solanacearum* which is widespread along the east coast of Australia, from Sydney in the south to Darwin in the north (M. Gillings - pers.comm.). Although the survey was from a limited number of samples, it did indicate that variant isolates are uncommon in the tomato growing areas of Queensland. The inclusion of isolate 4981 would be of value in resistance tests although the biological significance of the differences in genomic fingerprints is unknown.

Growing inoculated seedlings in a heated propagation tray allowed the rapid evaluation of a large number of tomato seedlings. In the inoculum concentration and plant age experiment, 6-wk-old plants were considered too large for transplanting and they took too long to develop wilt symptoms. Five-wk-old plants were of suitable size and susceptible and resistant cultivars could be differentiated at a concentration of 10^8 cfu/ml. The effect of soil temperature on cultigen susceptibility was shown in Table 2. The difference in wilt severity between 47-1-1 and the other F5 tomato material at 34°C was significant. Soil temperatures of 30 and 34°C were adopted in the evaluation of tomato germplasm in the heated propagation trays.

Ambient temperature has been shown to have less influence than soil temperatures on the development of bacterial wilt of tomato (Gallegly and Walker 1949). At high soil temperatures wilt development may be more severe if ambient temperatures are also high. The use of a controlled environment cabinet to simulate high soil and ambient temperatures had a profound effect on seedling susceptibility to bacterial wilt. Wilt symptoms developed earlier and 5-wk-old seedlings of resistant and susceptible cultivars could be differentiated at the low inoculum concentration.

To date the progeny of over 100 single plant selections have been screened for resistance to bacterial wilt in either heated propagation trays or the controlled environment cabinet. In a recent experiment F8 breeding lines were inoculated with 10^5 cfu/ml and evaluated in a controlled environment cabinet. Seven of the advanced breeding lines were significantly less wilt-affected than Flora-Dade. Three of these tomato lines will be released as wilt resistant cultivars in the near future..

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Breeding for Resistance to Fusarium Wilt

D.J. McGrath and I.O. Walker

Summary

Inbred lines and F_1 hybrids with resistance to *Fusarium oxysporum f. sp. lycopersici* race 3 (FOL 3) were developed by pedigree selection. The I3 gene from *Lycopersicon pennellii* was transferred to lines with improved horticultural attributes and a separate series of susceptible inbred lines was developed from F_1 hybrids with excellent fruit characteristics. F_1 hybrids were generated from both types of lines and evaluated in a replicated trial at Bowen Horticultural Research Station. Two F_1 hybrids have been chosen for release. They satisfy a market requirement among Fusarium resistant cultivars for medium size fruit with good horticultural and post-harvest characteristics and offer an alternative to the large fruited varieties now available.

Introduction

Genetic resistance to *Fusarium oxysporum f. sp. lycopersici* race 3 (FOL 3) is a requirement for all tomato cultivars grown in the Bowen small crops district. The first resistant cultivar, Tristar, was released in 1988 (McGrath), following the identification and transfer of the I3 gene in *L. pennellii* to *L. esculentum* (McGrath *et al*). Since then, the resistance has been incorporated into a number of F_1 hybrids by seed companies.

These F_1 hybrids are determinate and high yielding, but their fruit is frequently flat, scarred at the blossom end, and over sized. Market reports indicate a preference for fruit of medium to medium/large size rather than the predominantly large fruit produced by current commercial cultivars. There is an industry need for cultivars which produce medium size fruit free from defects and which are well adapted to Queensland environments.

This report describes the development of breeding lines with those requirements and the performance of F_1 hybrids arising from crosses of the best inbred lines.

Materials/Methods

a) Inbred Line Development Nine inbred lines were developed from several sources. FOL 3 resistant lines were obtained by hybridisation of the resistant inbred cultivar Tristar with a commercial F_1 hybrid. The latter was chosen for its deep fruit and freedom from skin cracking and blossom end scar. Progenies of this cross were selfed, subject to pedigree selection and tested for Fusarium resistance in the plant house.

FOL 3 susceptible lines were obtained by selfing and pedigree selection in progenies of another F_1 hybrid with excellent production and fruit characteristics.

A series of partially inbred lines was developed in a similar way from a third F_1 hybrid which showed early and concentrated maturity.

b) Second Cycle Inbred Development Three F_1 hybrids in c) below were inbred and their progenies subject to single plant selection in the field. The disease status of each line was tested in the plant house prior to field evaluation of the next generation. At the close of the project F_3 and F_4 progenies had been obtained through pedigree selection.

c) F_1 Hybrid Development A subset of pairwise crosses among inbred lines obtained in a) was generated for field evaluation. The F_1 hybrids were crosses of susceptible by resistant inbreds, and were designed to provide FOL-resistant genotypes which displayed superior horticultural performance. Single plots of each hybrid were evaluated in the field, after which the best hybrids were retained for replicated field testing in October 1994. Six replications of four F_1 genotypes obtained by crossing 4215, 4611, N91-1-1 and N91-6-2, and three check commercial hybrids were assessed for marketable yield, fruit size and quality and maturity. Size groups corresponding to small (class 1), medium (class 2), medium-large (class 3) and large (class 4) fruit were recognised during grading and data was collected on a size group basis. Marketable yields of 5 plant plots were obtained from 4 harvests at weekly intervals during October 1994.

Results

Inbred Line Development

The designation of inbred and F_4 lines mentioned in a) above is presented in Table 1.

Table 1. Inbred Lines Developed from Several Hybrids

Fusarium Resistance Status	Line	Inbred Generation
Resistant (R)	4215 4611	F_8
Susceptible (S)	N91-1-1 N91-6-2	F_7
S	761-1-1 761-1-2 761-2-1 761-6-1	F_4
R	761-3-1,2,3,4 761-13-1	F_4
Segregating	761-9-1	F_4

Resistant lines 4215 and 4611 were derived from a cross of Tristar by Hybrid 1; susceptible lines N91-1-1 and N91-6-2 were obtained by inbreeding Hybrid 2. The F_4 lines are progenies of Hybrid 3.

The second-cycle F_4 lines referred to in b) above are presented in Table 2.

Table 2. Second-Cycle F_4 and F_3 Lines

Fusarium Resistance Status	Line	Inbred Generation
Susceptible (S)	(N91-1-1 x 4215) -1-1 to 8	F_4
S	(N91-1-1 x 4215) - 4-1 to 5	F_4
Resistant (R)	(N91-6-2 x 4215) -2-1 to 3	F_4
R	(N91-1-1 x 4611) -1 to 3	F_3

The lines indicated in Table 2 represent a range of additional breeding material which will serve as a foundation for regenerating new F_1 hybrids. All selections were chosen for superior fruit quality; priority was given to deep fruit free of blemishes and scars, with smaller stem end scar.

F_1 Hybrid Development

Marketable yield of medium plus medium-large fruit (sizes 2 and 3), large fruit (size 4) and total marketable yield for eight F_1 hybrids is presented in Table 3. This data is also presented for five of the hybrids in Figures 1,2 and 3.

Table 3. Marketable Yield of Size Classes (2 and 3), 4 and Total Marketable Yield For Eight F_1 Hybrids Harvested Four Times

Hybrid/Cultivar	Average Fruit Size (g)	Marketable Yield Classes 2 and 3	Marketable Yield Class 4	Total Marketable Yield Classes 1,2,3 & 4
DPI 1	175	42.3	21.8	67.7
DPI 2	179	46.3	22.8	72.7
DPI 3	165	49.7	14.7	69.1
DPI 4	159	50.9	11.1	68.7
Tornado	201	29.5	37.0	69.6
Eagle	192	29.1	33.5	68.0
Mutiny	192	27.8	24.4	54.8
Delta Lady	202	23.9	30.9	59.1
Lsd (5%)	11	5.7	7.2	8.0

Yield is expressed as Kg per 5 plant plot. DPI 1 is N91-1-1 x 4215; DPI 2 is N91-1-1 x 4611; DPI 3 is N91-6-2 x 4215; DPI 4 is N91-6-2 x 4611

All hybrids produced similar total marketable yields except Mutiny and Delta Lady which were markedly lower. The four DPI hybrids produced much higher yields in the smaller size classes

2 and 3 than the commercial hybrids; however within the DPI hybrid group, numbers 1 and 2, with the common parent N91-1-1, were lower yielding for classes 2 and 3.

The converse was true for the larger size class 4. Although the DPI hybrids were all lower yielding than the commercial hybrids, DPI 1 and 2 yielded significantly more than numbers 3 and 4 in this class.

The average fruit size across four harvests was similar for Tornado, Eagle, Mutiny and Delta Lady (Table 3). DPI 1 and 2 were significantly smaller than the checks and DPI 3 and 4 were significantly smaller again.

Figures 4 and 5 provide estimates of maturity for DPI 1 and 2 respectively, relative to the earliest commercial hybrid, Mutiny. Both DPI 1 and 2 were later maturing than Mutiny.

Discussion

Nearly all commercial F_1 hybrid cultivars resistant to FOL 3 suffer from excessively large fruit size when crops are harvested in late spring and early summer in north Queensland. The four new hybrids developed in this program produced fruit with somewhat smaller average size. DPI 1 and 2 had average fruit size of 170g - 180g which is more acceptable for wholesale markets and retail outlets. Hybrids 3 and 4 had an average size of 160g and were considered unacceptably small. They were rejected on the grounds of size and fruit quality.

Hybrids 1 and 2 produced similar marketable yields to the best commercial hybrids. The marketable yields in different fruit size classes indicated that DPI 1 and 2 yielded relatively more fruit in the medium and medium/large classes and less fruit in the large class than Tornado, Eagle, Mutiny and Delta Lady. Although there was no loss of marketable yield, a greater proportion of that yield was available in the more acceptable size grades.

Several commercial hybrids tend to produce a high proportion of distorted, and scarred fruit. The significantly lower marketable yield of Mutiny and Delta Lady was due to these defects.

The incidence of unmarketable fruit is difficult to predict in cultivars; some cultivars appeared to be more susceptible to adverse climatic conditions because of their broad blossom ends and flatter-shaped fruit. This has been observed with Mutiny and Delta Lady. The four DPI hybrids were selected for deeper fruit shape which is associated with a small blossom end scar. Consequently a much higher proportion of their fruit was marketable, particularly when weather conditions predisposed other cultivars to defects.

The performance of DPI 1 and 2 in formal and informal trials in 1994 indicated that they will be an important addition to the range of FOL 3 resistant cultivars available in Queensland. They produce more fruit in a smaller and more suitable size and the fruit is subject to a lower incidence of defects. In addition the fruit is free from heavily pigmented dark-green shoulders and contains a smaller stem-end scar. Although they were developed principally for use in Fusarium 3 affected soils, these hybrids are likely to be widely acceptable in other environments and cropping systems. Both hybrids will be tested in a wider range of conditions in 1995 and subject to approval, it is proposed that they be formally released in accordance with DPI policy.

Extension

All new F_1 hybrids have been demonstrated to growers at two field days (1993 and 1994) at Bowen Horticultural Research Station. Two producers have also trialed the two hybrids planned for release. Further trials will be conducted across planting dates for the 1995 season in Bowen and Bundaberg.

Commercial Benefits

When F_1 hybrids are released, the release procedure will be in accordance with QDPI policy. The policy provides for royalty payments to the investors in the variety development project, which of course includes HRDC. The details of the policy have been forwarded by Dr R Eisemann and Dr G Behncken. HRDC will be fully informed of developments concerning releases.

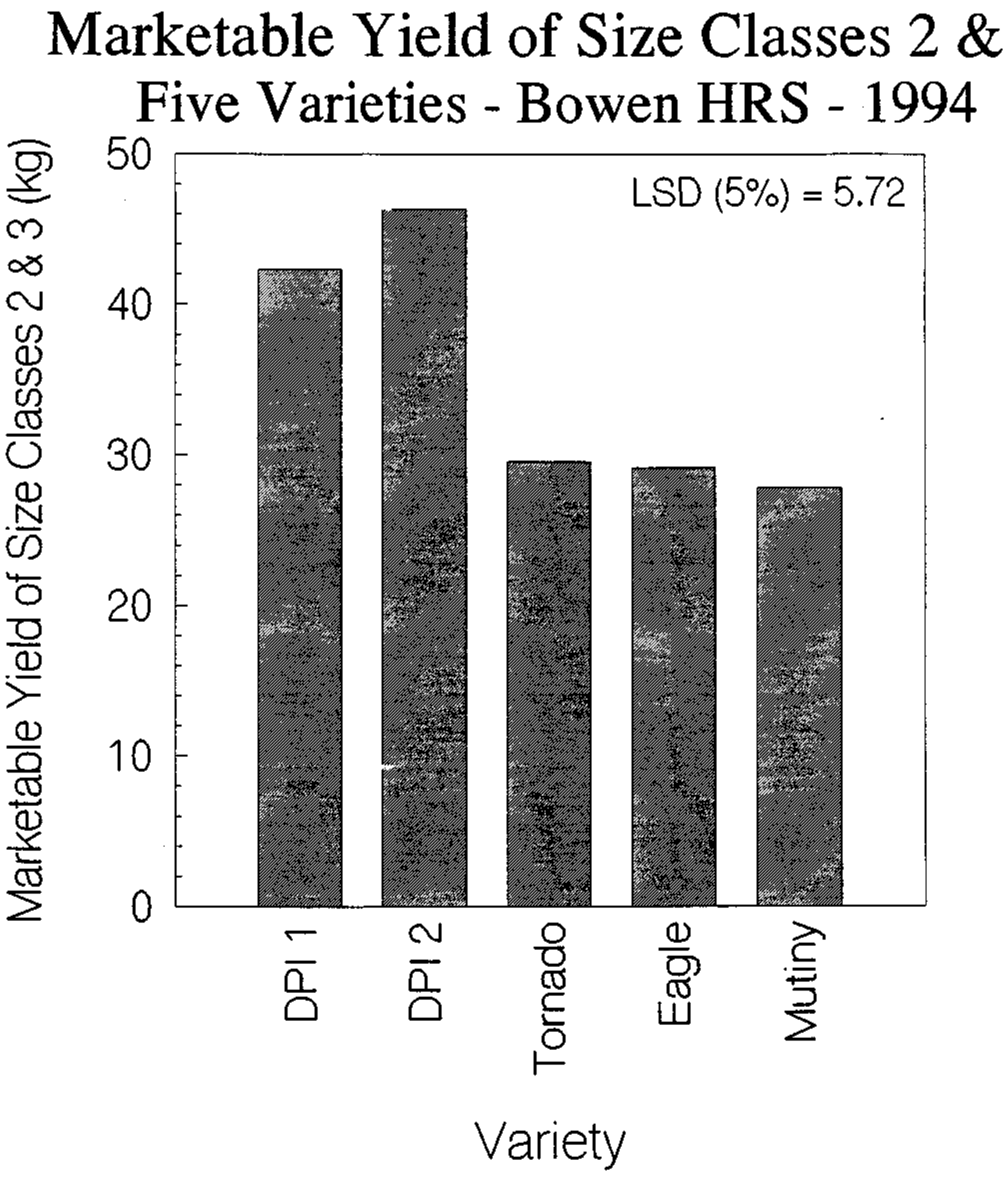

Marketable Yield of Size Classes 2 &
Five Varieties - Bowen HRS - 1994
LSD (5%) = 5.72
Marketable Yield of Size Classes 2 & 3 (kg)
50
40
30
20
10
0
DPI 1
DPI 2
Tornado
Eagle
Mutiny
Variety

Figure 1.

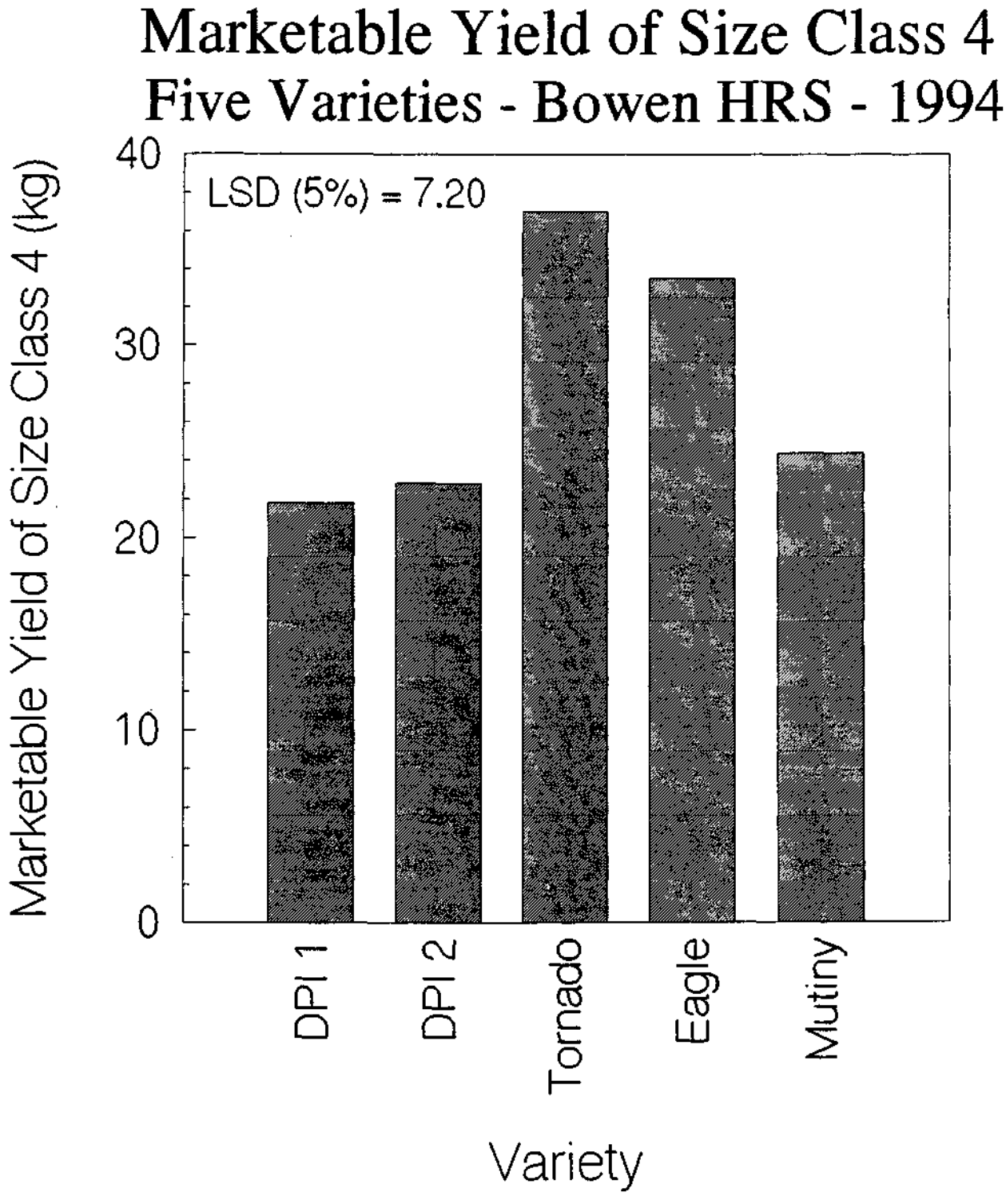

Marketable Yield of Size Class 4
Five Varieties - Bowen HRS - 1994
LSD (5%) = 7.20
Marketable Yield of Size Class 4 (kg)
40
30
20
10
0
DPI 1
DPI 2
Tornado
Eagle
Mutiny
Variety

Figure 2.

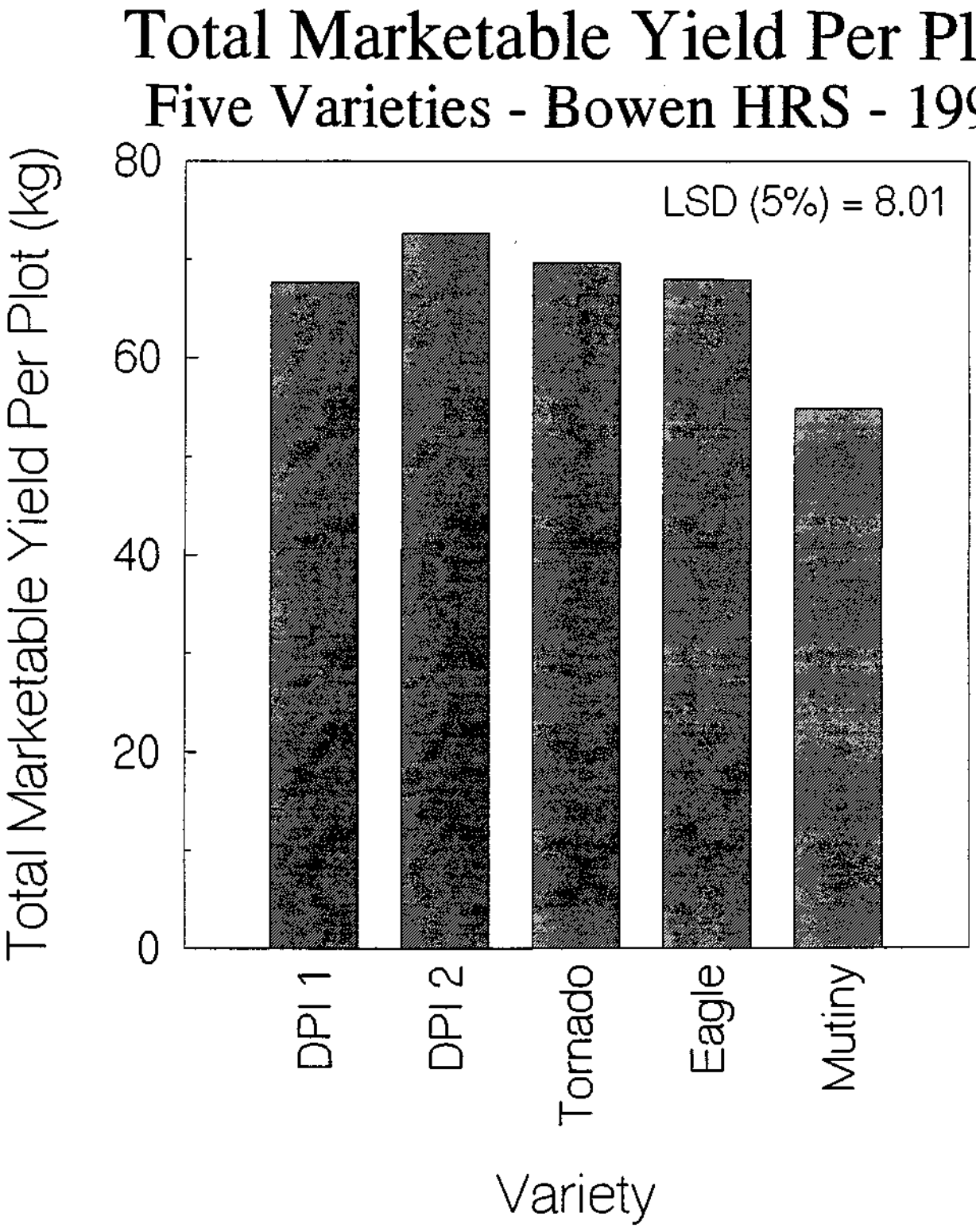

Total Marketable Yield Per Plot
Five Varieties - Bowen HRS - 1994
LSD (5%) = 8.01
Total Marketable Yield Per Plot (kg)
80
60
40
20
0
DPI 1
DPI 2
Tornado
Eagle
Mutiny
Variety

Figure 3.

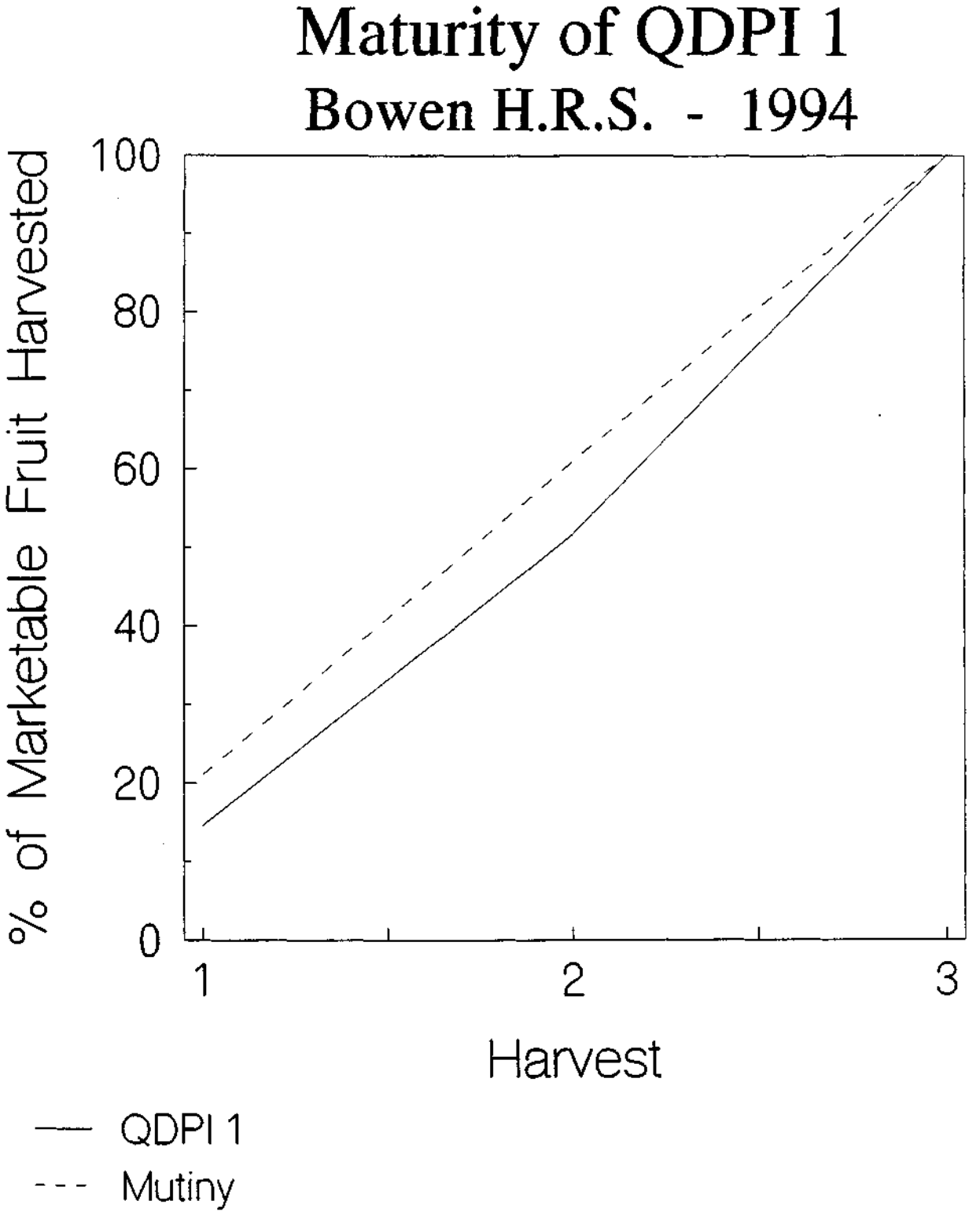

Maturity of QDPI 1
Bowen H.R.S. - 1994
% of Marketable Fruit Harvested
100
80
60
40
20
0
1
2
3
Harvest
QDPI 1
Mutiny

Figure 4.

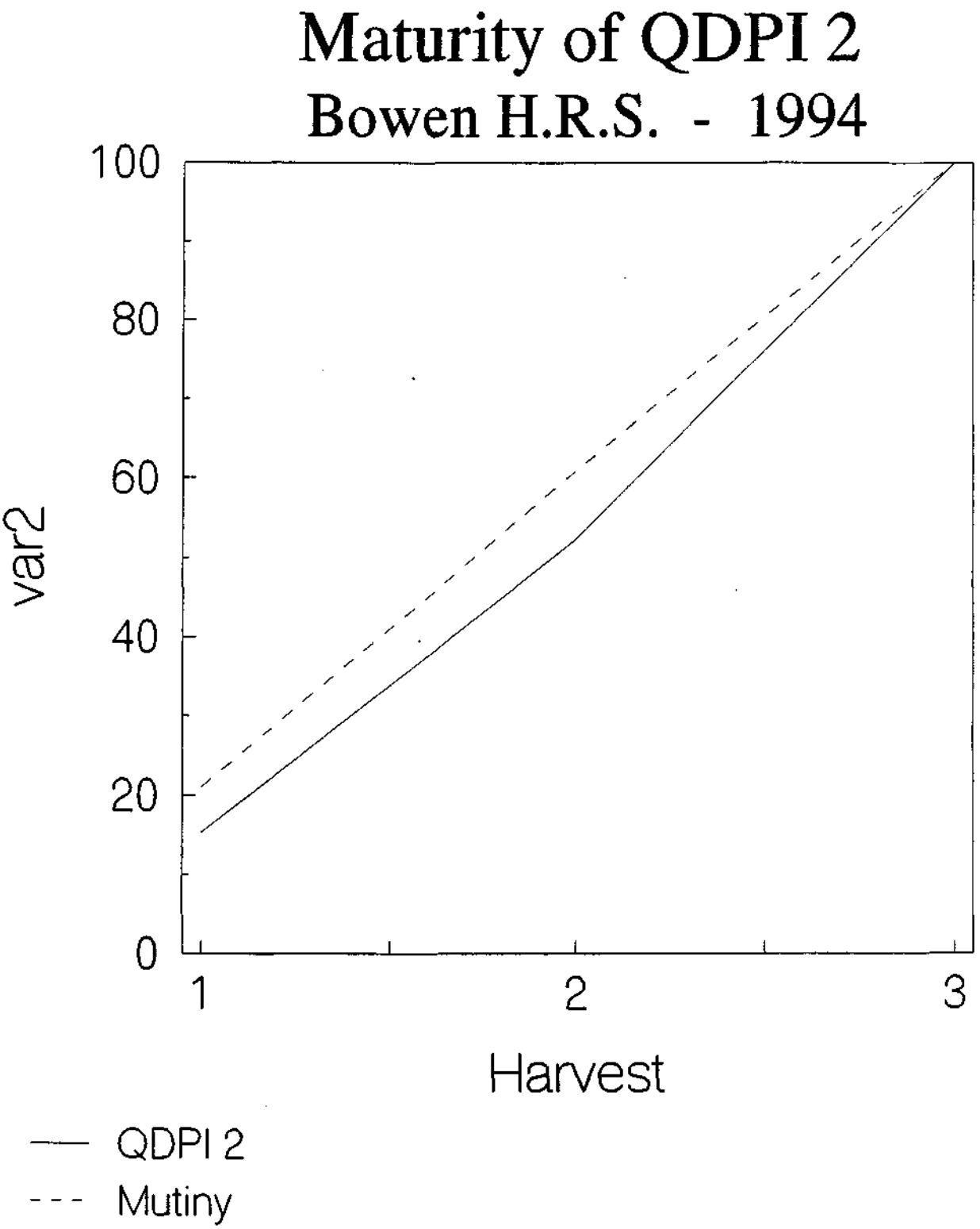
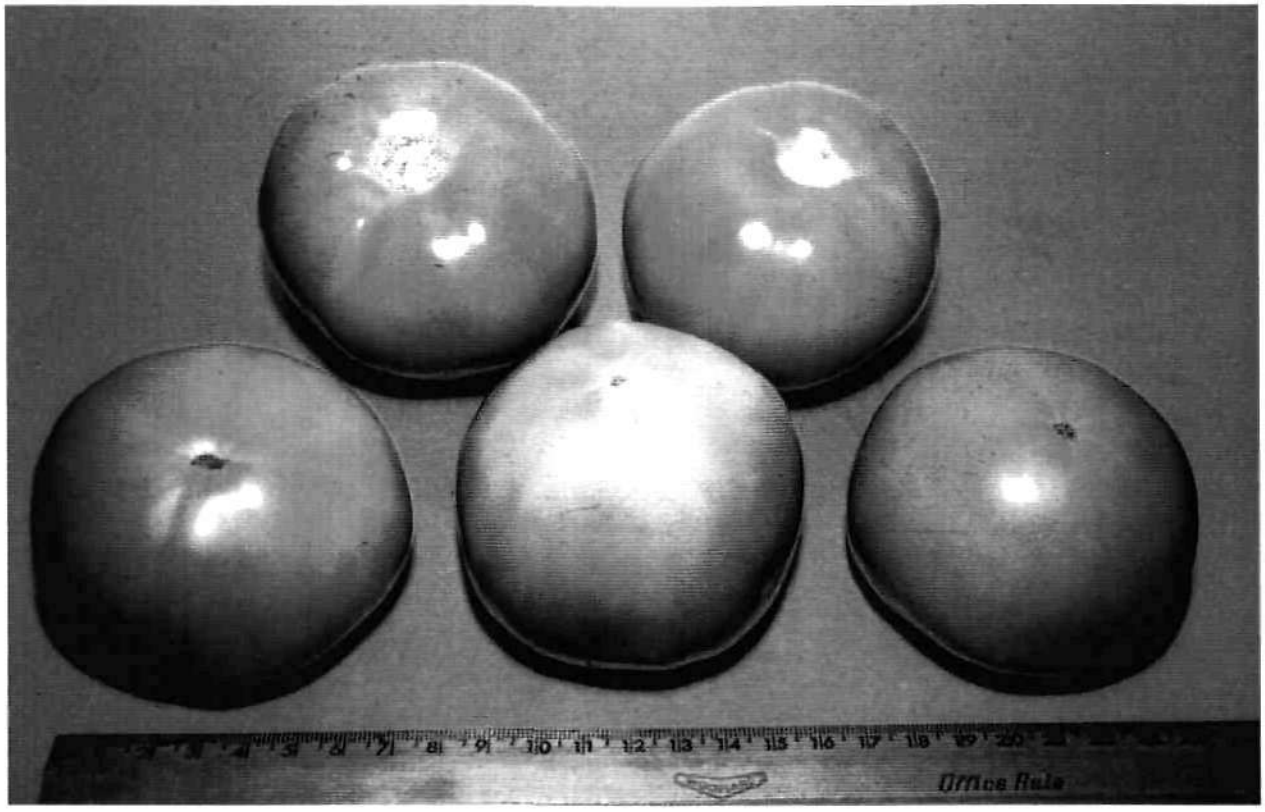

Maturity of QDPI 2
Bowen H.R.S. - 1994
100
80
60
40
20
0
var2
1
2
3
Harvest
QDPI 2
Mutiny

Figure 5.


Office Rule

Breeding For Superior Fruit Quality

D.J. McGrath and I.O. Walker

Summary

Progenies developed by backcrossing and single plant selection expressed high levels of Total Soluble Solids (TSS) compared with check varieties. Selection was practised among F_2 and F_3 Backcross 1 lines to produce genotypes with greater than 6.0 % TSS. By contrast, unimproved varieties expressed TSS between 4.0 and 5.0%. The improved genotypes were segregating for TSS and horticultural characters.

The long shelf-life gene Rin was incorporated into a breeding population. True breeding F_4 lines were selected and backcrossed to adapted lines with disease resistance. Crosses between Rin and high TSS lines will be undertaken when sufficiently advanced lines in both sources are available.

Introduction

Australian fresh market tomato cultivars generally lack the intensity of flavour available in some smaller-fruited European varieties. The problem appears to be a consequence of earlier breeding programs which developed varieties with high levels of multiple disease resistances, fruit firmness and agronomic performance but without satisfactory flavour. It is widely accepted that a greater flavour intensity in Australian cultivars would stimulate demand for tomatoes here and enhance prospects for exports.

The long shelf-life gene Rin has been used extensively in breeding programs overseas. It acts by delaying the degradation of the fruit wall so that it is possible to lengthen the effective shelf-life of fruit. This has been used with great success in the Israeli cultivar Daniella which has displaced many conventional cultivars in Europe.

A strategy of combining Rin and high TSS in a determinate, disease resistant cultivar would address the need for a tomato with excellent flavour able to tolerate shipping to export markets.

The progress in achieving these goals is reported here.

Materials/Methods

A breeding population was established by crossing an adapted genotype with the Japanese cultivar Momotaro. This latter line has a sweet intense tomato flavour, expresses high TSS (>6.0% Brix) but is markedly unadapted to Queensland cropping systems. The F_1 was crossed to an inbred line with excellent horticultural characteristics to produce a large backcross population.

Backcross progenies were field grown at Bowen in 1993 and 1994. Mature fruit (UDA 6) were sampled three times at weekly intervals for Brix determinations. An F_2 population and F_3 lines derived from single F2 parent plants with Brix content greater than 6.0 were grown in 1993 and 1994 respectively, and subject to further cycles of selection.

In 1994, F_3 plants were identified with Brix readings greater than 6.0 and evaluated for flavour by two research staff. Those genotypes with good flavour as reflected in a balance of sweetness and acid on three occasions were selected in the field and struck as cuttings in the plant house. These lines were subsequently crossed to an adapted genotype with determinate growth habit, disease resistance and good fruit firmness. At the close of the project hybrid seed was recovered from these crosses.

An F_1 hybrid heterozygous for Rin was inbred with pedigree selection for the homozygous Rin genotype. Five true breeding F_4 lines were obtained and backcrossed to Fusarium 3 resistant genotypes to generate suitable Rin types adapted to cropping in Bowen. At the close of the project hybrid seed had been recovered from these crosses.

Results

The frequency distributions for Brix content of three backcross 1 F_3 families grown in 1994 is presented in Figures 1, 2 and 3. The families were derived from high Brix F_2 individuals grown in 1993. Each distribution shows a wide range of TSS scores with many segregants expressing Brix levels greater than 6.0. The mean Brix score for the check genotype, 421511, was 4.9.

Each F_3 family contained a few segregants with greater than 7.0% Brix. Line 1-8-2, however, provided the greatest number of high TSS individuals which were selected jointly for sweetness, acid and good internal texture. Table 1 presents the F_3 lines selected, their mean Brix across these samples and fruit colour.

Table 1.

Genotype	Mean % Brix	Colour
1-8-2-4	6.5	Pink
1-8-2-31	6.1	Red
1-8-2-46	7.0	Red
1-8-2-51	6.0	Pink
1-8-5-1	5.7	Red
1-8-17-52	6.1	Red
1-8-17-59	6.1	Red
421511	4.9	Red

Two of the eight selections expressed a pink rather then red skin colour. These were retained for the continued development of pink lines with high TSS. All selections were of indeterminate growth habit.

Three F_4 homozygous Rin individuals were chosen from six F_3 families derived from inbreeding of a commercial Rin hybrid. Each selection was crossed in the F_5 to Fusarium-resistant parents.

Discussion

The results of several years' selection for high TSS in these breeding populations indicate that the inheritance of high TSS is clearly multigenic although the heritability of selection on a single plant basis is high.

By establishing a diverse genetic background for the high TSS breeding population it has been possible to make rapid advances for TSS, fruit size and disease resistance. These attributes have not yet been obtained in a determinate growth habit. At the end of the project further crosses of these indeterminate genotypes to an adapted determinate line were made. The segregation from crosses will provide suitable determinate genotypes with high TSS. It is anticipated that further hybridisation will be required to develop suitable lines.

One feature of this breeding material is variation for fruit colour. Some lines express a pink colour which is inherited from the Momotaro parent. It is inherited simply so that manipulation of the character is straight forward. The association of pink fruit with improved flavour could be a marketing advantage. It is likely that normal red and pink varieties, both with long shelf-life, will be available from this program, so that professional marketing assistance may be necessary to effectively commercialise these products.

Although these breeding lines have been developed with specific disease resistances for Bowen, they will be suitable for cropping elsewhere in Australia and will have wide potential use. It is anticipated that three more years will be required to fully develop F_1 hybrids with long shelf-life, improved flavour, disease resistances and good agronomic performance.

The strategy with the Rin gene is to incorporate it into a separate line of breeding material. By developing a genetic background for Rin which is already known to complement the high solids breeding population it should be possible to produce F_1 hybrids with high levels of heterosis.

Extension and Future Research

The results of this research have been reported formally to Queensland Fruit and Vegetable Growers and informally through growers' inspections of field plots at field days. The development of high solids tomato breeding lines has been widely supported by growers and wholesale market agents.

More formal evaluation of fruit from these lines is planned in 1995/96. It is expected that panel assessments involving the constituents of flavour will be undertaken by the International Food Institute of Queensland in order to characterise the attributes of flavour in high quality lines. This will contribute to decisions concerning the release of hybrids from this program.

HRDC is continuing to fund this project in 1994/95. It may be appropriate for HRDC to be involved with a marketing program as this breeding material is further developed. Unless the consuming public is aware of the specific improvements of this material the advantages of these lines may not be realised.

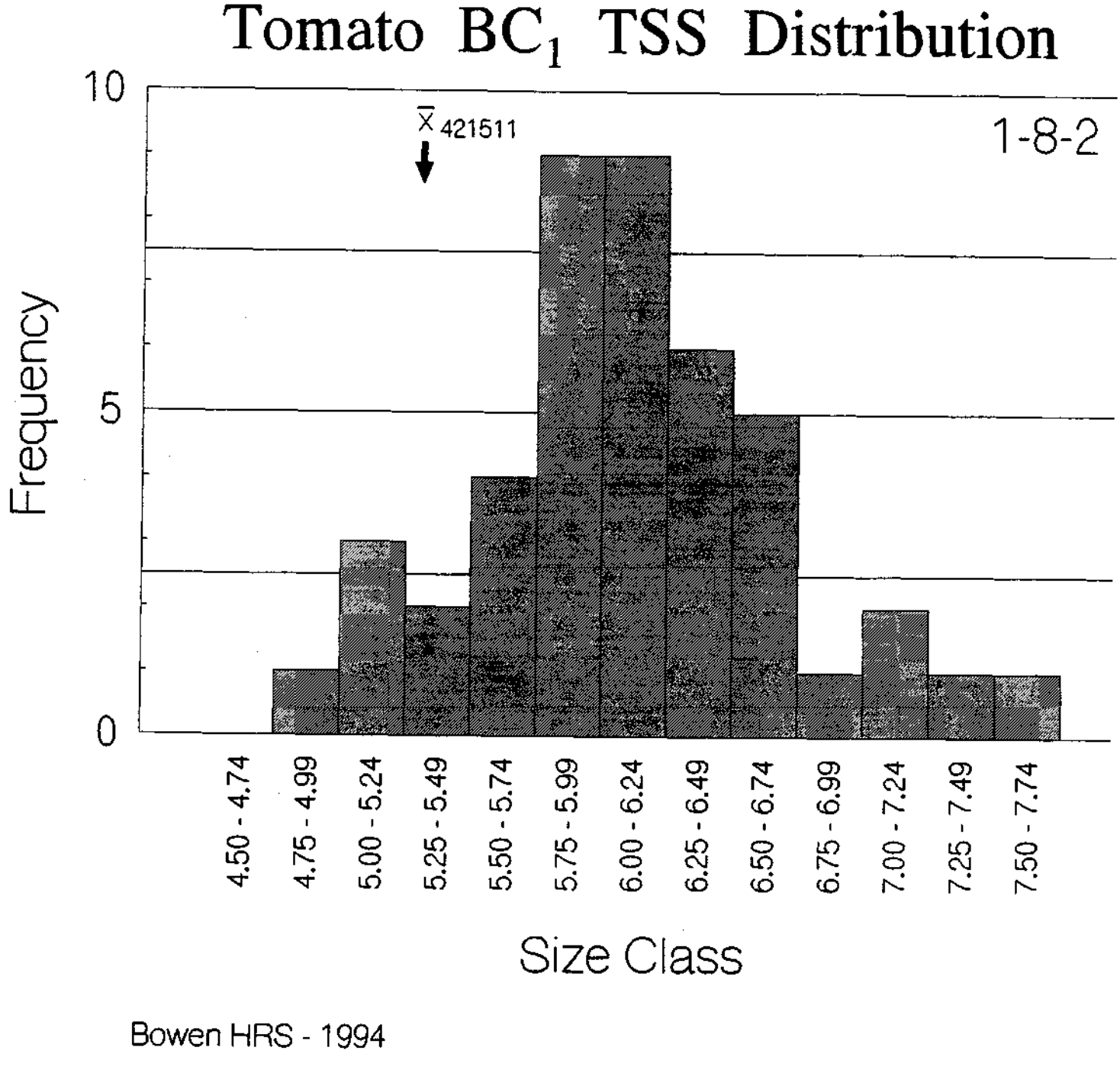

Tomato BC1 TSS Distribution
10
5
0
Frequency
X̄ 421511
1-8-2
4.50 - 4.74
4.75 - 4.99
5.00 - 5.24
5.25 - 5.49
5.50 - 5.74
5.75 - 5.99
6.00 - 6.24
6.25 - 6.49
6.50 - 6.74
6.75 - 6.99
7.00 - 7.24
7.25 - 7.49
7.50 - 7.74
Size Class
Bowen HRS - 1994

Figure 1.

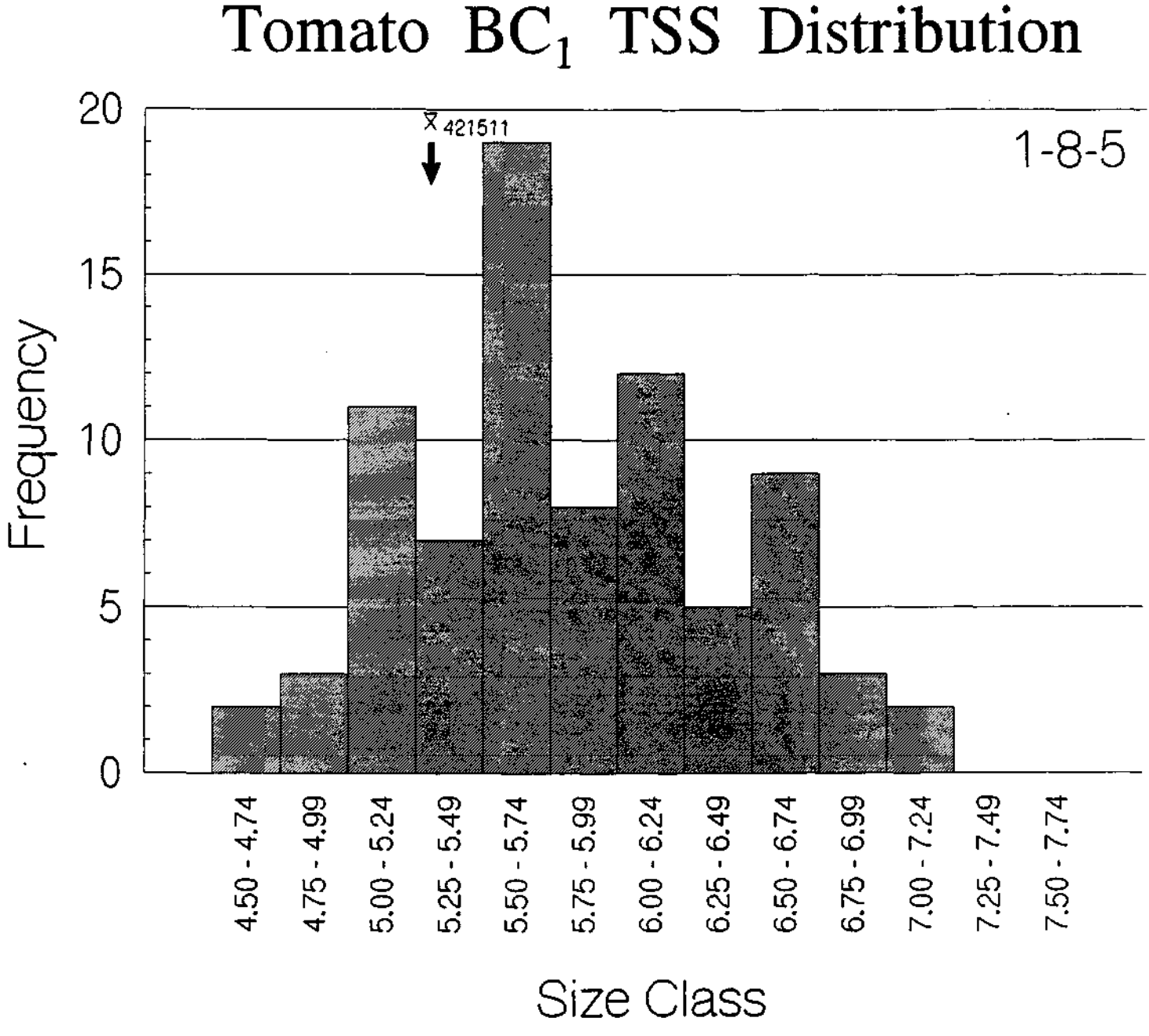

Tomato BC1 TSS Distribution
20
15
10
5
0
Frequency
421511
1-8-5
4.50 - 4.74
4.75 - 4.99
5.00 - 5.24
5.25 - 5.49
5.50 - 5.74
5.75 - 5.99
6.00 - 6.24
6.25 - 6.49
6.50 - 6.74
6.75 - 6.99
7.00 - 7.24
7.25 - 7.49
7.50 - 7.74
Size Class
Bowen HRS - 1994

Figure 2.

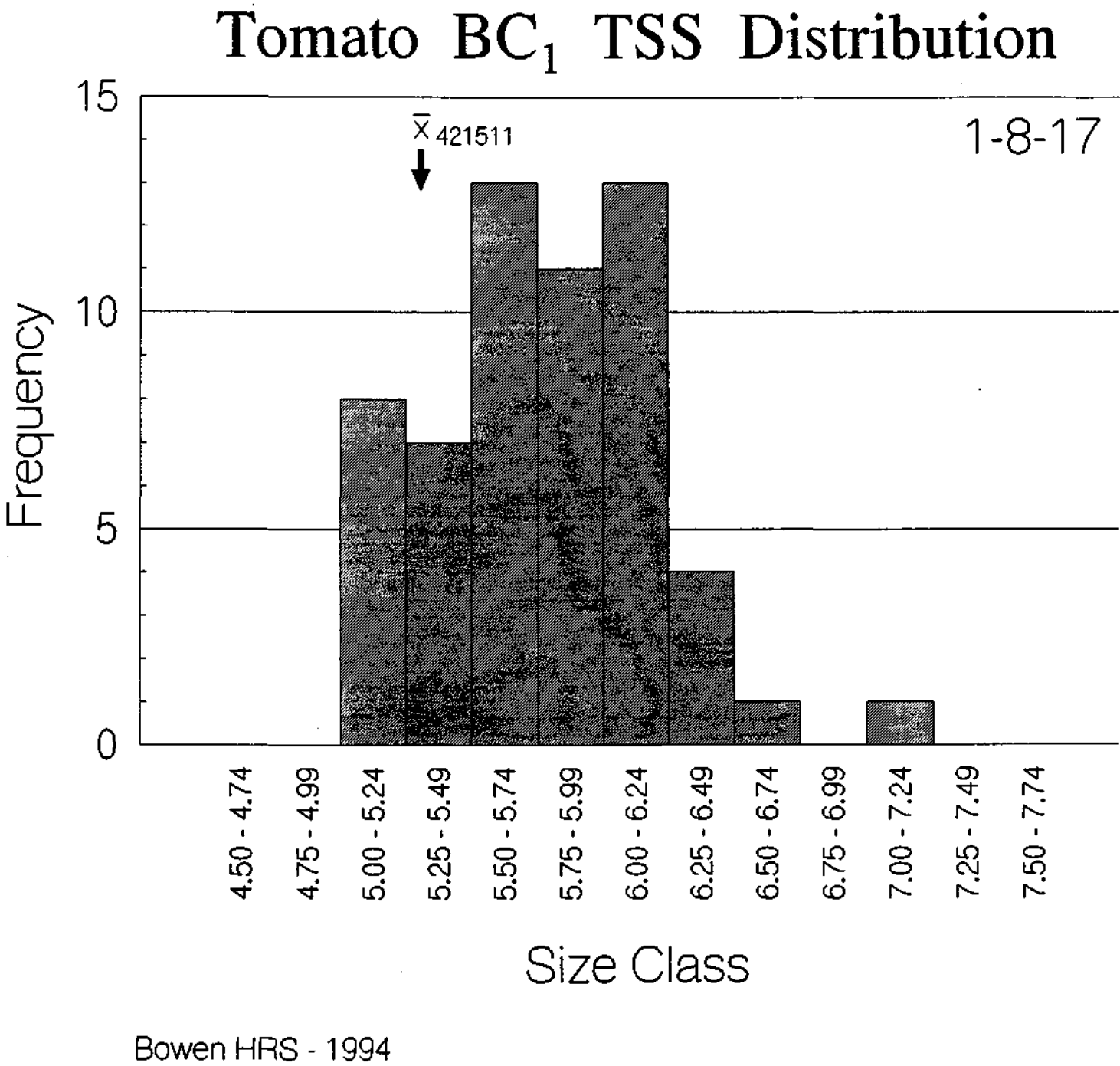

Tomato BC1 TSS Distribution
15
10
5
0
Frequency
X̄ 421511
1-8-17
4.50 - 4.74
4.75 - 4.99
5.00 - 5.24
5.25 - 5.49
5.50 - 5.74
5.75 - 5.99
6.00 - 6.24
6.25 - 6.49
6.50 - 6.74
6.75 - 6.99
7.00 - 7.24
7.25 - 7.49
7.50 - 7.74
Size Class
Bowen HRS - 1994

Figure 3.

Resistance to Bacterial Canker, Bacterial Spot and Verticillium Wilt

Introduction

These diseases are minor causes of losses to the whole industry, but on a local level they are frequently very destructive. Dr A.M. Hibberd was leading this section of the project until 1993 when he was transferred to another project. Progress was therefore more difficult and less advance than anticipated has been made. Nevertheless, a summary of achievements in each area is provided below.

Resistance to Bacterial Canker

Report by J.B. Heaton

Introduction/Materials & Methods

Tomato breeding lines were selected for resistance to bacterial canker caused by the bacterium *Clavibacter michiganense* subsp *michiganense* and for fruit quality in a field trial at Redlands Research Station (RRS) in summer 1993-94.

The twenty lines (designated BC4 F_5 families) grown were inbreds from the fourth backcross which had been designed to combine resistance to disease with good fruit quality. They were derived from plants from the previous generation (BC4 F_4 families) that were selected for resistance to bacterial canker in February 1993 at RRS.

The planting also included six F_1 hybrids that we produced. These hybrids all had as one parent the canker resistant, semi-determinate cultivar Rotam-4 of South African origin. The other parents in the F_1s were three canker resistant inbreds from our own work, and three susceptible inbreds from the work of D McGrath, Bowen Research Station (BRS). Our purpose was to evaluate the F_1s commercial potential.

Six more inbred lines from Bowen were also planted to evaluate fruit quality. Cultivars Floradade (susceptible to canker) and Rotam-4 and H 2990 (both resistant) were included as controls. We grew two replicates of each tomato line and cultivar in our field trial.

The Pathogen

Tomato plants were inoculated in the field late in November 1993 with a mixture of four isolates of the bacterium, Q0978 (from Redland Bay), Q1080 (from Glen Aplin), Q1986 and Q1987 (from Lowood). Pathogenicity of these four isolates were first verified by inoculating seedlings of the susceptible cultivar Buccaneer in a glasshouse at Indooroopilly (courtesy B Stubbings).

The Trial

The tomato plants grew very well. Summer rainfall commenced in January and spread the pathogen. The disease developed rapidly. Plants were rated for severity of bacterial canker on three occasions: 11, 18 and 31 January 1994. We selected plants on 31 January 1994. The ratings were numerical on a scale of 0 to 7 with 0 being no disease symptoms and 7 being plant death (Hibberd *et al* 1992).

Results

1. All ratings of severity of bacterial canker of the tomato breeding lines on 11 and 31 January 1994 are shown in Table 1.

2. Plants of Floradade were severely diseased by the end of January 1994 when they rated 6.1. By comparison, plants of Rotam-4 and H 2990 rated 2.2 and 4.7 respectively. Lower ratings indicated the resistance of these cultivars to bacterial canker.

3. Six of our BC4 F_5 inbred lines (lines 16, 20, 25, 31 and 34) also had effective resistance to bacterial canker. They rated between 3.3 and 4.5 on 31 January 1994.

All tomato lines and varieties had higher canker disease severity ratings on 31 January than on 11 January 1994. Because the disease was most severe on 31 January the susceptible cultivar Floradade, had the greatest increase in canker rating of 3.9 units over that time.

In comparison, our six lines 16, 20, 25, 31 and 34 increased in disease severity ratings by only 2.0 to 3.2 ratings units (Table 1).

The fruit quality of these six BC4 F_5 lines was good, and their quality attributes are briefly listed in Table 2. Seed (now BC4 F_6 generation) was saved.

Two F_1 hybrid lines (line 7 and line 11) also had effective partial resistance to bacterial canker, and rated 4.3 and 4.8, respectively, on 31 January 1994. Their fruit size and quality were very good, and their bacterial canker severity ratings had only slowly increased by 2.9 rating units between 11 and 31 January 1994. The bacterial canker resistance for these hybrids was derived from Rotam-4, their common parent.

Fruit of high quality and size were produced by the six inbred lines from reeding program of D McGrath, Bowen. They were jointless, uniform green, and unaffected by calyx scars, cat face and blossom end rot but all six lines were susceptible to bacterial canker and tomato big bird virus.

Discussion

The only known sources of resistance to tomato bacterial canker are found in the cultivars H 2990 and Rotam-4, which we have used which give a slow rate of canker severity increase, but not immunity (Heaton *et al*, 1991). The slower canker increases, the longer resistant cultivars may produce in comparison to susceptible cultivars.

Worthwhile canker resistance was again confirmed in our FC4 F_5 inbred lines and two F_1 hybrid lines in this trial. These have the potential for sustainable tomato production, and we estimate that two additional harvest are possible after a severe outbreak of bacterial canker occurs. The earlier the outbreak of canker occurs, the more valuable this resistance becomes (Dullahide *et al*, 1983).

Table 1. Ratings of severity of bacterial canker of tomato breeding lines on 11/01/94 and 31/01/94

Tomato lines	**Canker rating ***		**Increase in canker rating * from 11/01/94 to 31/01/94**
	11/01/94	**31/01/94**	
Controls:			
Rotam-4 (resistant)	0.6	2.2	1.6
H 2990 (resistant)	1.2	4.7	3.5
Floradade (susceptible)	2.2	6.1	3.9
BC4-F_5 lines:			
Line 16 BC3-GL45 F_2	1.3	3.3	2.0
Line 20 C661-2 x 52	0.9	3.3	2.4
Line 25 R11-3 x 52	1.6	4.5	2.9
Line 30 C542-1 x 52	1.3	4.5	3.2
Line 31 C542-1 x 52	1.1	4.0	2.9
Line 34 C562-1 x 52	1.5	4.2	2.7
Hybrids (F_1 lines):			
Line 7 (F701 [10-92] x Rotam-4)	1.4	4.3	2.9
Line 11 (Nicks [91-5-1-1] x Rotam-4)	1.5	4.8	2.9

* Rating is on a scale of 0 (no symptoms) to 7 (plant death).

Table 2. Attributes of six F_6 tomato lines with resistance to bacterial canker selected at Redlands Research Station, 31 January 1994.

Line 16	Home garden type, indeterminate (trellis), jointed, small to large fruit. Slight green shoulders, slight grooves, Grosse Lisse 45 flavour, deep red flesh, soft.
Line 20	Home garden type, indeterminate (trellis), jointless, large pointed fruit, soft.
Line 25	Commercial, determinate, jointed, round, red, yellow, firm, large.
Line 30	Commercial, determinate, jointless, flat, red, slight green shoulders, large.
Line 31	Commercial, determinate, jointless, round, red, firm, small.
Line 34	Commercial, determinate, jointless, round, red, firm, large

A Greenhouse Method for Selecting Tomato Seedlings Resistant to Bacterial Canker

Abstract: Hibberd, A.M., Heaton, J.B., Finlay, G.P., and Dullahide, S.R. 1992 A greenhouse method for selecting tomato seedlings resistant to bacterial canker. Plant Dis. 76:1004-1007.

Infiltrations of suspensions of inocula (10^3-10^8 cfu/ml) into leaflets and stems were used to evaluate resistance to *Clavibacter michiganensis* subsp. *michiganensis* in 4- to 5-wk-old tomato seedlings. Disease increased at slower rates in infiltrated tissues of Heinz 2990 than in Floradade and Morden will all concentrations except 10^8 cfu/ml leaflets and 10^3 and 10^4 cfu/ml in stems. Seedlings with low disease severity were selected from a segregating backcross population derived from Heinz 2990 following infiltrations of leaflets and stems with 10^5 and 10^8 cfu/ml, respectively. In field experiments, plants were inoculated by leaf excision with a scalpel dipped in inoculum of 10^8 cfu/ml. Heinz 2990 developed less disease ($P<0.01$) than Floradade and Morden. Less disease ($P<0.01$) also developed on selfed progeny of previously selected backcross plants than on the susceptible parent Gross Lisse, and segregation for resistance occurred among nonselected backcross plants. Disease severity levels in field-grown plants were higher ($P<0.01$) in Morden than in Floradade, and fruiting was earlier and more concentrated in time in Morden. The test of seedlings in the greenhouse has potential for rapid screening of lines for partial resistance and in tomato breeding programs.

Resistance to Verticillium Wilt Race 2

A.M. Hibberd, R.G. O'Brien

Tolerance to Verticillium dahliae (race 2) was identified in glasshouse and field trials in several sources of *Lycopersicon esculentum*. Backcross 3-S5 lines from a cross of Heinz 2990 and Grosse Lisse appeared to have an adequate level of tolerance in both seedling and field trials. The ability to discriminate effectively in both tests was compromised by weak penetrance of the genes conferring tolerance. Some BC2-5-5 progeny were crossed to Fusarium resistant inbred lines for further selection. The most advanced progeny in this material were determinate and moderate yielding. No commercial lines were developed at the close of the project.

Resistance to Bacterial Leaf Spot

A.M. Hibberd

It was determined that the line Hawaii 7998 is susceptible to the strain of *Xanthomonas campertris* in Queensland and is of little use here, despite its resistance in Florida.

Rate reducing resistance in Heinz 2990 and in its BC3 F_4 progeny was identified in glasshouse experiments. Resistance appeared to be greater in two of the BC3 F_4 lines than in Heinz 2990.

Substantial but incomplete resistance, superior to that of Heinz 2990 was also detected in one breeding line. No progress beyond the production of Backcross 3 F_4 was obtained.

Extension Outcomes

- Field days at Applethorpe and Redlands Research Stations were conducted to demonstrate the effectiveness of breeding lines with resistance to bacterial canker. Producers and seed industry representatives attended.

- Determinate F_1 hybrids with bacterial canker resistance were produced for demonstration on properties at Ballandean and Helidon in South East Queensland in 1994.

- Bacterial wilt resistant lines were inspected in 1992 and 1993 by individual growers and seed company personnel at an infested trial site in Bundaberg. Several seed companies are interested in commercialising one or more lines.

- Field days were conducted at Bowen Research Station in 1993 and 1994, where F_1 hybrids from the program were displayed. All days were open to the public.

- A review of tomato breeding research in the Queensland Department of Primary Industries was conducted in August 1992. A copy of the proceedings is available from the project leader.

- Reports on various aspects of the project have been published in the Bundaberg Region Horticultural Magazine.

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