Know-how for Horticulture™

# Queensland fresh market tomato breeding

Des McGrath
QLD Department of Primary Industries

Project Number: VG97025

**VG97025**

This report is published by Horticulture Australia Ltd to pass on information concerning horticultural research and development undertaken for the fresh tomato industry.

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of Queensland Fruit and Vegetables.



ISBN 0 7341 0733 1

Published and distributed by:
Horticultural Australia Ltd
Level 1
50 Carrington Street
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399
E-Mail: horticulture@horticulture.com.au



Horticulture Australia

# QUEENSLAND FRESH MARKET TOMATO BREEDING

**D. J. McGrath *et al***

**Project Number: VG97025**

## Horticulture Australia Project Number: VG97025

Program Leader: Des McGrath, Queensland Department of Primary Industries, PO Box 538, Bowen, Queensland 4805.

Key Personnel: **Dr. C. Akem**, Department of Primary Industries, PO Box 591, Ayr, Qld, 4807

**Dr. S. Garland, Mr. I. O. Walker**, Department of Primary Industries, PO Box 538, Bowen, Qld 4805.

**Ms. M. Kelly**, Department of Primary Industries, Bundaberg Research Station, Ashfield Road, Kalkie, Queensland 4670.

**Mr. D. Persley, Mr. M. Sharman, Ms. L. McMichael**, Department of Primary Industries, 80 Meiers Road, Indooroopilly 4068.

**Dr. G. Gurr**, Pest Management Group, The University of Sydney – Orange, PO Box 883, Orange, NSW 2800.

Purpose of report: Report of fresh market tomato genetic improvement for the Queensland industry.

Acknowledgement of funding sources:

Queensland Fruit and Vegetable Growers.

Horticulture Australia Ltd.

Queensland Department of Primary Industries

Date of report: June 2003

# CONTENTS

## Media Summary

A series of 70 indeterminate tomato lines with various combinations of improved fruit quality, field production characteristics and disease resistance were developed in an applied breeding program. The source materials for the program were diverse 'gourmet' style varieties, disease resistant lines from Queensland Department of Primary Industries (QDPI) germplasm and non-commercial breeding lines developed from QDPI's resources. A large number of hybrid combinations between lines were made and at least six hybrids have been identified as potential candidates for release. An appropriate range of lines and hybrids will be offered to a commercialisation partner.

Advances in biotechnology allowed the discovery of a DNA marker for resistance to Tomato Spotted Wilt Virus and the application of another marker for resistance to Fusarium wilt. A series of tests for resistance to Fusarium wilt in plant-house conditions demonstrated how environmental factors can vary plant reactions and cause errors in breeding programs. The use of DNA markers as a reliable alternative to such tests contributed to significant advances in breeding efficiency and successful outcomes.

Breeding lines with higher soluble solids content, developed in a previous project, were used in hybrid combinations with more advanced parent lines to provide material with improved flavour. The new hybrids demonstrated the usefulness of solids content as an indicator of flavour and its application in a breeding program.

The role of host plant resistance to potato tuber moth, an important pest of tomato, was determined in a green-fruited species of tomato, *Lycopersicon hirsutum.* Two accessions of this species expressed high levels of resistance compared with the cultivated species. In a second study of eleven accessions of *L. hirsutum,* differences in resistance were related to the densities of specific types of leaf hairs. In both cases, these classes of leaf hairs were shown to be an important measure of resistance in a breeding program. One of the accessions was selected for breeding resistance but was incompatible with the cultivated species. A search for DNA markers linked to resistance in this accession was not successful.

## Technical Summary

A series of 70 indeterminate inbred breeding lines with combinations of improved agronomic performance, fruit quality and disease resistance characteristics was developed in an applied breeding program. Diverse parents with specific fruit qualities, growth habit and disease resistance were combined through two cycles of hybridisation and inbreeding. The lines were advanced by single plant selection in field trials at Gatton and Bowen during the four-year period to 2002. A total of 310 different test hybrids between these lines were generated and at least six hybrids have been selected as potential candidates for release.

Fourteen determinate lines with improved fruit quality characteristics were also developed by selection from similar tall lines segregating for growth habit. The fruit had similar firmness but generally less uniform shape.

DNA markers for resistance to two diseases increased the efficiency of selection in breeding lines. A CAPS marker linked to the *I-3* gene for resistance to Fusarium wilt was routinely applied in the development of resistant indeterminate and determinate lines. The marker allowed the identification of homozygous and heterozygous resistant genotypes and reduced the errors common in conventional plant-house assays. In a major advance, a dominant marker linked to the *Sw-5b* gene for resistance to tomato spotted wilt virus was identified and applied to breeding populations. The maker is now used routinely and will substantially reduce the extent of glasshouse screening for this disease. The application of both markers to breeding lines will allow the rapid identification segregant genotypes with both resistance genes.

Parent lines with higher soluble solids content and better flavour were crossed to determinate lines with good agronomic adaptation and inferior flavour. The resulting hybrids developed higher total soluble solids and in most cases were judged by a taste panel to have improved flavour. The high solids lines were shown to contribute better flavour when used in combination with standard parent lines.

Resistance to potato tuber moth, *Phthorimaea operculella,* was shown to be significantly higher for two accessions of *L. hirsutum* than for the cultivated species of tomato, with no effect of day length or plant age. Stepwise multiple regression using variety as the sole factor was significant ($P<0.001$) in accounting for 61.4% of the variation in larval survival. Additional factors were not significant when added to the regression model. Densities of type I, IV, VI and VII glandular trichomes were lowest in the cultivated species. Day length interacted significantly with varieties for type VII trichome density only.

A comparison of pubescence characteristics for eleven accessions of *Lycopersicon hirsutum* and the cultivated species *Lycopersicon esculentum* showed the former had denser glandular (type I, IV, VI and VII) trichomes. The mortality of neonate larvae placed on the abaxial surface of leaves was greater on seven accessions of *L. hirsutum* than on *L. esculentum*. Eleven days after inoculation, four accessions of *L. hirsutum* supported no live larvae whereas other PI lines of the same species showed reduced numbers compared with *L. esculentum.* A multiple regression model indicated a significant positive correlation between density of type IV and VI trichomes and neonate mortality, decreased larval development and decreased adult emergence. Though factors other than glandular trichomes are likely to be important, increased density of type IV and VI, along with decreased type V, were important selection criteria in breeding for resistance.

# 1. Introduction

The introduction of indeterminate fresh market tomato cultivars to the Australian market in recent years has seen a decisive change in the attributes of most fruit offered to retail consumers. The major market segment of round tomato is now largely comprised of medium-sized fruit with greater shelf life, firmness, uniformity of shape and size and aesthetic qualities than the typical beefsteak cultivars common five years ago. The quality characteristics of these firmer cultivars allow consumers to enjoy a longer post-harvest period when fruit is at maximum firmness and quality. Because fruit can be harvested at the correct maturity and then held for a longer period in the post-harvest chain before purchase, these varieties offer consumers a standard of quality superior in some respects to that of the older beefsteak types.

Since the widespread introduction of indeterminate hybrids producers have tested many varieties to maximise performance. There have been significant agronomic problems of small fruit size and reduced marketable yields, fruit hollowness, poor internal fruit quality and lack of disease resistance. The most serious diseases have been Fusarium wilt and tomato spotted wilt virus, although some secondary foliar pathogens have also damaged crops. The report identifies progress in developing indeterminate and determinate varieties with improvements in these agronomic and disease resistance attributes in Section 2.

There are continuing complaints of flavour and eating quality for many tomatoes. Work in a previous project developed better flavour in round beefsteak tomatoes by transferring higher soluble solids from a sweet Asian variety. Further progress in enhancing flavour in a wider range of lines is now reported in Section 3.

The concept of genetic resistance to insect pests as a component of an Integrated Pest Management program is attractive in terms of reduced production costs and environmental benefits. Periodic outbreaks of serious damage from potato tuber moth, *P. operculella,* occur in tomato crops so the potential of genetic resistance reported in wild species was investigated. The first objective in Section 4.1 was to confirm genetic resistance in LA1777 of *L. hirsutum,* and to study the effects of plant type, age and daylength on foliar pubescence and resistance. Secondly, additional accessions of *L. hirsutum* were studied for resistance (Section 4.2). In the last part of the study (Section 4.3), the potential for breeding through DNA markers was investigated.

# 2 Breeding for Agronomic Improvement

## 2.1 Applied Breeding – Indeterminate Lines

**Des McGrath and Ian Walker**

### Materials, Methods and Results

The objectives were to develop indeterminate inbred lines and $F_1$ hybrids with disease resistance, larger fruit size and improved internal and external fruit quality. The intention was to address these defects, characteristic of many indeterminate varieties currently available in Australia.

*Development of first-cycle inbred lines*

A series of experimental and commercial indeterminate $F_1$ hybrids were evaluated for specific attributes to be included in breeding populations. From the $F_1$ hybrids identified as superior for particular characteristic, a range of $F_5$ or $F_6$ inbred lines was developed by single plant selections in winter trials conducted at Bowen. The lines from any single $F_1$ hybrid were selected with one predominant characteristic in a background generally unimproved for other attributes. The initial $F_5/F_6$ selections with their improved performance characteristics were as follows:

Larger fruit size to 180g:

| | |
|---|---|
| $F_6$ lines from Hybrid K : | K21111, 21112, 21113, 21114 |
| | K21121, 21211, 21213, 21215, 21216 |
| | K23111, 23221, 23222, 23223, 23224, 23231, 23241, |
| | K42111, 42121, 42213, 42214 |

Superior fruit size to 160g, gel cohesion and internal fruit quality:

| | |
|---|---|
| $F_5$ lines from Hybrid R: | R2211, 2221, 2222 |
| | R3212, 3213, 3222 |

Improved external skin quality, smaller stem-end and blossom-end scars:

$F_5$ lines from Hybrid A: A 3111, 3121

*Development of short, Fusarium-resistant lines with concentrated maturity*

Fusarium race 3 resistance was introduced to indeterminate breeding lines by hybridising a determinate beefsteak resistant genotype with Hybrid R above. Segregating lines were initially evaluated in the field for agronomic performance at Bowen, then screened for disease resistance using a plant-house assay and evaluated subsequently at Bundaberg and Bowen in successive generations of inbreeding. Single plant selection for resistance in the field was followed by plant house bioassays to confirm resistance.

As expected, there was regular segregation in inbred generations for indeterminate (tall) and determinate (short) growth habit, fruit firmness and disease resistance. A significant gain at this stage of the program was the selection of extremely short plants in the equivalent of an $F_3$ generation. These were significantly smaller in plant size and stature than the original short parent in the cross, but retained fruit size of about 140g and superior fruit qualities. The short lines selected were unexpected transgressive segregants from the cross and the foundation for a second cycle of improvement. The key characteristics of these short lines are indicated in Table 1.

*Table 1* Key attributes of short statured breeding lines selected from first cross of indeterminate x determinate hybrids.

| Line 2 x Hybrid R | Fusarium Status | Agronomic Description |
|---|---|---|
| $F_4$ 1-7 | Segregating | Short, strongly determinate, fruit size to 140g, small recessed stem-end, blossom- end scars |
| $F_4$ 1-9 | Resistant | Short, fruit size to 150g, jointed fruit, some green shoulder, flat shape. |
| $F_4$ 2-4 | Susceptible | Short, compact bush shape, good quality fruit with fine appearance, medium size to 130g. |
| $F_4$ 9-1 | Resistant | Short, strongly determinate, smaller fruit 120g, good internal fruit quality. |

*Development of second-cycle inbred lines*

Three Fusarium-resistant, strongly determinate $F_6$ lines, 1721, 1911 and 1961, were derived from their parents described in Table 1. Each line was hybridised with six indeterminate hybrids: A, F, K and R above and additional two hybrids, M1 and M2. The hybrids selected for crossing to these short lines were derived from commercial or near-commercial sources and provided many of the features of 'gourmet' fruit quality. The broad objectives were disease resistance in an enhanced background of superior fruit quality, size and more manageable plant growth characteristics for trellis production. The material was also the source of superior determinate plant types.

Initial field evaluations of progeny from each hybridisation above comprised populations of 150 to 200 plants in single plots. Single plant selections based on fruit quality (size, shape, external appearance, internal structure, gel cohesion, hollowness) and field performance characteristics (fruit set, numbers of clusters, later fruit size and quality, plant vigour) were made from the crosses involving R, M1 and M2, but the best inbred lines were ultimately obtained from M1 and M2. The number of lines selected and advanced for field evaluation at

June 2002 and their details are presented in Table 2. Thereafter the numbers were reduced as more rigorous selection was applied as $F_1$ hybrids were generated. The development of these lines occurred during summer field trials at Gatton 2001 and 2002 and winter trials at Bowen 2000, 2001 and 2002.

A number of the 146 selections presented in Table 2 were assessed for Fusarium resistance using a conventional root-dip plant house assay and the results for these and additional lines are presented in Table 3. In conjunction with the field assessments made in the five trials above 70 sub-lines with significantly improved performance were selected at Gatton 2002 as the basis for hybrid construction.

*Table 2.* Indeterminate breeding lines developed at June 2002

| Pedigree | Generation of Progeny | Number of Selections |
|---|---|---|
| (1911 x M1)-1 | $F_4$ | 2 |
| (1911 x M1)-3 | $F_4$ | 22 |
| (1911 x M1)-5 | $F_4$ | 3 |
| (1911 x M1)-6 | $F_4$ | 4 |
| (1911 x M1)-7 | $F_4$ | 5 |
| (1911 x M2)-2 | $F_4$ | 1 |
| (1911 x M2)-3 | $F_4$ | 5 |
| (1911 x M2)-4 | $F_4$ | 7 |
| (1911 x M2)-5 | $F_4$ | 5 |
| (1911 x M2)-11 | $F_4$ | 1 |
| (1721 x M2)-1 | $F_4$ | 22 |
| (1721 x M2)-2 | $F_4$ | 6 |
| (9161 x M2)-1 | $F_4$ | 15 |
| (9161 x M2)-3 | $F_4$ | 3 |
| (9161 x M2)-6 | $F_4$ | 1 |
| (9161 x M2)-1-4 | $F_5$ | 2 |
| (9161 x M2)-1-8 | $F_5$ | 9 |
| (1911 x M1)-3-2 | $F_5$ | 1 |
| (1911 x M1)-3-8 | $F_5$ | 22 |
| (1911 x M1)-3-14 | $F_5$ | 10 |
| Total | | 146 |

*Table 3.* Status of selected indeterminate breeding lines evaluated for resistance to Fusarium race 3.lines in Indeterminate Breeding Lines Evaluated August 2002

| **Resistant** | **Segregating** | **Susceptible** |
|---|---|---|
| $F_3$ 82-3 | $F_4$(1911x M2) 2-6 | $F_4$(1721 x M)2-5, 8, 18 |
| $F_5$(1911x M1) 3-2-8 | $F_4$(1911x M2) 4-2, 4-3, 4-5 | $F_4$(1911 x M2) 4-4, 7 |
| $F_4$(1911x M2) 3-5 | $F_4$(1911x M2) 3-6 | $F_5$(9161 x M2) 1-8-3, 1-8-6 |
| $F_4$(1911x M2) 4-6 | $F_4$(1911x M2) 5-3, 5-4, 5-5 | $F_5$(1911 x M1) 3-8-10, 3- 8-19 |
| | $F_5$(1911x M1) 3-2-5 | $F_4$(1911 x M2) 3-2, 3-3 |
| | $F_5$(9161 x M2) 1-4-2, -1-8-5 | $F_4$(1911 x M2) 3-1 |
| | $F_5$(1911 x M1) 3-8-15, 3-8-17 | |
| | $F_5$(1911 x M11) 3-2-11 | |
| | $F_4$(1911 x M2)11-1 | |
| | $F_4$(1911 x M1) 1 -1 | |

*Descriptions of indeterminate inbred parent lines*

A description of key inbred lines selected on the basis of their performance in five consecutive trials at Gatton and Bowen is provided below. The lines represent the best useful variations of improved agronomic characteristics, disease resistance, fruit appearance and quality which provided parental combinations suitable for superior hybrid performance.

**$F_4$ (1911 x M2) –1-1-1**

Susceptible to Fusarium 3 (F3); fruit medium-large to large size to 180g, extremely firm at full red stage and 5 days later, globe shape, small, tidy stem-end and blossom-end scars. Later maturity. Phenotypic score 3.5/5.

**$F_4$ (1911 x M2)-1-1-2**

Similar to selection –1-1-1 above with a regular multi-locular internal fruit structure of approximately 5 locules.

**$F_6$ (1911 x M1)-3-2-8-1-2**

F3 resistant; medium-large flat fruit to 160g with fine features and small scars. Stem-end scar is recessed. Extremely firm. Clustered in hands of 4-5 fruit. Internal fruit shape is multilocular with good gel cohesion. Later maturity. Score 3.5/5

**$F_6$ (1911 x M1)-3-2-8-5-2**

F3 resistant: similar to selection –3-2-8-1-2 above except fruit are mostly medium size to 140g maximum. Flat uniform shape with regular 3-4 locules, good internal quality. External appearance of fruit is free of blemishes. Fruit have excellent stem-end, blossom-end scars. Score 4/5

**$F_6$ (1911 x M1)-3-2-11-8-1**

Segregating for F3; similar to selection –3-2-8-1-2- above except fruit are a little smaller in size to a maximum of 150g. Shape is more uniform and stem-end scar is extremely small and without corky appearance. Score 3/5

**$F_5$ (1911 x M1) –3-8-22-1**

Segregating for F3; medium size to 140g. Regular flat shape with four regular locules and good internal fruit quality. Blossom-end scar has a small dimple. Very firm fruit with fine features. Score 4/5

**$F_5$ (1911 x M2)-3-5-1-2**

Resistant to F3; tall, vigorous bush with later maturity. Yield is produced on widely separated clusters. Fruit size medium to medium-large 180g. Fruit quality excellent with 3 to 6 locules, good gel cohesion and no hollowness. External appearance has relatively small scars and is free of blemishes. Fruit shape is a globe. Score 3/5

**$F_5$ (1911 x M2)-4-6-1-3**

Resistant to F3; tall, vigorous bush. Fruit medium-large to 160g, flat shape with moderately uniform appearance. Later in maturity but has good yield. Fruit have 3 to 5 locules and good internal quality. Score 3/5

**$F_5$ (1911 x M2)-4-7-6-1**

Susceptible to F3; Vigorous, late, line. Fruit have medium-large size and moderately uniform shape. External appearance enhanced by attractive scars and absence of major blemishes. Internal quality is acceptable, with nearly all fruit producing at least 3 locules with good gel cohesion. Its outstanding feature is a high proportion of clusters of about 8 fruit of similar maturity produced in a split truss. This tendency to concentrated maturity within clusters has great potential. A photograph of the fruit is presented in Figure 1 below. Score 4/5

**$F_5$ (1911 x M2)-4-7-8-1**

Susceptible to F3; Vigorous, high-yielding line. Fruit have medium to medium-large fruit size to 180g and deeper globe shape. Fruit are exceptionally firm at full red stage and at 5 days later. Fruit are held in tight clusters of 5 or more, with short, prominent pedicels. External

appearance is free of most blemishes. Internally fruit have up to 5 locules, producing an acceptable multi-locular structure with wide radial walls. Score 3.5/5

**$F_5$ (1911 x M2) –5-5-1-3**

Segregating for resistance to F3; bushes have semi-determinate growth habit and reduced vigour. Fruit size is medium to medium-large, to 160g. Fruit shape is a mid-depth globe with good internal quality. Fruit have up to 6 locules, a true-mutilocular internal structure and good gel cohesion. Some minor defects in external fine cracks and minor blossom-end scars. Score 3/5

**$F_5$ (1911 x M2)- 5-5-3-2**

Segregating for F3 resistance; bushes are semi-determinate and have moderate vigour. High yield of flat fruit in clusters with consistently large size to 180g. External appearance of fruit is excellent with a high proportion showing small scars. Good internal quality, 3-4 locules. Some minor concentric cracking after rain. Score 4/5

*Indeterminate F1 hybrid performance*

A large number of $F_1$ hybrids or earlier test crosses from partially inbred lines were constructed in the 2001 and 2002 seasons using those lines displaying excellent performance. The earlier progenitors of the lines described above were included among a larger number of lines. The 310 crosses constructed in 2002 are included in Figure 4 for reference. A subset of 26 hybrids was evaluated in Gatton 2003, using hybrids derived from parent lines which had shown good combining ability for production performance, fruit quality and disease resistance.

The best performing hybrids from this evaluation involved parent lines (1911 x M1)-3-2-5, (1911 x M1)-3-2-9, (1911 x M1)-3-2-11, (1911 x M2)-4-6, (1911 x M2)-5-5 and (1911 x M2)-3-1. The first three of these lines, derived from a common parent, produced a class of medium to medium-large fruit with very fine attractive external features such as small tidy scars and up to 6 locules per fruit. The fruit was particularly uniform in shape and size. The

second class of fruit involved parent line (1911 x M2)-4-6 and (1911 x M2)-5-5; the fruit from these parents was more robust in appearance, medium-large to large, deeper in shape but with good multilocular structure and prolific yield. A larger number of hybrids to these parent lines have yet to assessed. Further combinations to other excellent parents described above have also to be assessed. Six hybrids were identified as candidates for pre-commercial testing in a wider range of locations and more will certainly be identified in the remaining hybrids to be assessed.

A more advanced group of hybrids from more highly selected and inbred genotypes of these same lines has also been constructed and preliminary indications suggest very good performance. The hybrid performance assessed so far suggests that releases will be pursued as soon as possible. The range of parent lines and the large number of potentially good hybrid combinations available should provide excellent opportunities to fully exploit the material.

*Figure 1* Fruit of $F_5$ (1911 x M2)-4-7-6-1 indicating concentration of fruit in split truss.

*Figure 4* Matrix of 310 cross combination between near-inbred indeterminate parent lines. Crossing undertaken in September/October, 2002

| **Bowen HRS Crossing - Late 2002 - September/October** | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | | | | | | | | | | | |
| 10 | 11 | 12 | 13 | 14 | 50 | 17 | 18 | 19 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 4 | 5 | 51 | 6 | 15 | 52 | 53 | 0.5 | 55 | |
| **85-1** | **87-1** | **89-1** | **102-5** | **102-13** | **884** | **886** | **B3-2** | **B3-3** | **B4-3** | **B4-5** | **B4-6** | **B5-1** | **B5-3** | **B5-5** | **A7-1** | **A7-3** | **92-11** | **98-10** | **98-22** | **562** | **842** | **R1** | **R5** | **R7** | **R12** | **Crosses** |
| X | X | X | X | X | | X | X | X | X | X | X | X | X | X | X | X | X | | X | | | | | | | 18 |
| X | X | X | X | X | | X | X | X | X | X | X | X | X | X | X | X | X | | X | | | | | | | 18 |
| X | X | X | X | X | | X | X | X | X | X | X | X | X | X | X | X | X | | X | | | | | | | 18 |
| X | X | X | X | X | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | | | | | | 20 |
| X | X | X | X | | | X | X | X | X | X | X | X | X | X | X | X | | | X | X | X | | | | | 18 |
| X | X | X | | X | X | X | | | | | | | | | | | | | | | | | | | | 6 |
| X | X | X | | | | X | | | X | X | X | X | X | X | X | X | X | | X | | X | | | | | 15 |
| X | X | X | | | | X | | | X | X | X | | | | X | X | X | | X | | | | | | | 11 |
| X | X | X | | | | X | | | X | X | X | X | X | X | X | X | X | | X | | | | | | | 14 |
| | X | X | X | X | | | X | X | X | X | X | X | X | X | X | X | | | | | | | | | | 14 |
| | | X | X | X | | X | X | X | X | X | X | X | X | X | X | X | | | X | | | | | | | 15 |
| | | | X | X | | | X | X | X | X | X | X | X | X | X | X | | | X | | | | | | | 13 |
| | | | | | | X | | | | | | X | X | X | X | X | | | | | | | | | | 6 |
| | | | X | | | | | | | | | X | X | X | X | X | | | | | | | | | | 6 |
| | | | | | | | | | | | | | | | | | | | | | | X | X | X | X | 4 |
| | | | | | X | X | | | | | | | | | | | | | | | | X | X | X | X | 6 |
| | | | | | | | | | | | | | | | | | | | | | | X | X | X | X | 4 |
| | | | X | X | | X | | X | X | X | X | X | X | X | X | X | | X | X | | | | | | | 14 |
| | | | X | X | X | | | | X | X | X | X | X | X | X | X | | X | X | | | | | | | 13 |
| | | | | | | | | | | | | | | | | | | | | | | X | X | X | X | 4 |
| | | | X | X | | X | | | | X | X | X | X | X | X | X | | X | X | | | | | | | 12 |
| | | | X | X | | X | | | | | X | X | X | X | X | X | | X | X | | | | | | | 11 |
| | | | X | | | X | | | | | | X | X | X | X | X | | X | X | | | X | X | X | X | 13 |
| | | | | | | X | | | | | | | X | X | X | X | | X | X | | | X | X | X | X | 11 |
| | | | | | | X | | | | | | | | X | X | X | | X | X | | | X | X | X | X | 10 |
| | | | X | X | | X | | | | | | | | | X | X | | X | X | | | | | | | 7 |
| | | | | | | | | | | | | | | | | X | | | | | | X | X | X | X | 5 |
| | | | | | | | | | | | | | | | | | | | | | | X | X | X | X | 4 |
| **85-1** | **87-1** | **89-1** | **102-5** | **102-13** | **884** | **886** | **B3-2** | **B3-3** | **B4-3** | **B4-5** | **B4-6** | **B5-1** | **B5-3** | **B5-5** | **A7-1** | **A7-3** | **92-11** | **98-10** | **98-22** | **562** | **842** | **R1** | **R5** | **R7** | **R12** | 310 |
| 9 | 10 | 11 | 15 | 13 | 3 | 19 | 8 | 9 | 13 | 14 | 15 | 17 | 18 | 19 | 21 | 22 | 7 | 9 | 18 | 2 | 2 | 9 | 9 | 9 | 9 | 310 |

## 2.2 Development of Resistance to Fusarium Wilt

### 2.2.1 Identification of Fusarium Resistance with a DNA Marker

**Stephen Garland**

**Materials, Methods and Results**

The assay for Fusarium 3 resistance used for the development of most progenies in Section 1.1 was a conventional root-dip screening conducted in the plant house. It is relatively simple in terms of technology but requires up to six weeks before results are obtained. The second major disadvantage with the assay is the potential for misclassification of phenotypes. The resistance gene initially transferred to new lines is widely used and effective but can be poorly expressed in some screening conditions, leading to symptom development in resistant genotypes. The resulting confusion may reduce efficiency and warrants the use of an alternative resistance gene, *I-3*.

Although *I-3* may also express disease symptoms in assays, it is more robust with fewer errors of classification. A DNA marker has been developed for this gene by Dr. David Jones, Australian National University, allowing for routine use in screening breeding lines and populations with minimal errors. The marker is demonstrated in Figure 1 below.

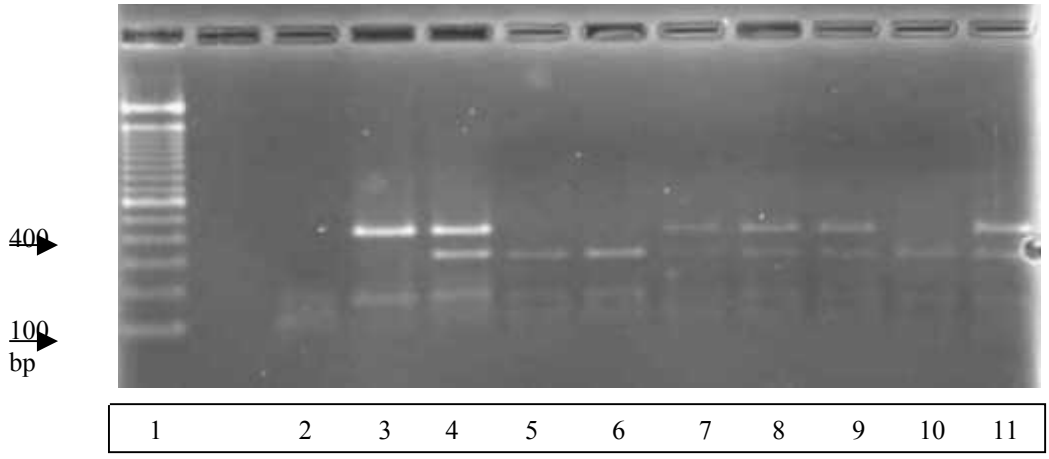



*Figure 2*. The analysis of tomato breeding lines at Bowen with a CAPS marker linked to the I-3 disease resistance gene (Fusarium race 3) after agarose gel electrophoresis and ethidium bromide staining. Lane 1: 100 bp ladder. Lane 2: PCR blank. Lane 3: homozygous resistant. Lanes 4, 7-9, 11: heterozygous. Lane 5,6,10: homozygous susceptible. The fragment of approximately 400 bp represents the marker linked to the resistant allele. The fragment at approximately 300 bp represents the marker linked to the susceptible allele. Contact Dr David

Jones, Australian National University concerning marker details and authority to use the marker.

To allow easier use of *I-3* as an effective source of resistance, 17 partially inbred indeterminate lines were crossed initially with a source of *I-3* and subsequently backcrossed to similar and additional lines, using the DNA marker to select homozygous resistant genotypes at each backcross cycle. The recurrent parents varied at each cycle in accordance with the most recent selections. Six resistant second-backcross genotypes representing a range of the best indeterminate types have been selected with the marker and are being crossed to approximately 20 new susceptible parents. The process is similar to the production of isolines which vary at only one locus, but in this case they constitute a broader range of germplasm from diverse sources. The resistant *I-3* lines now available as backcross 2 genotypes complement similar lines described in Section 1.1 and are currently useful as potential parents in $F_1$ hybrids. To ensure the best possible outcomes, these lines will be developed further through a third backcross cycle to elite parents.

### 2.2.2 The Effect of Environmental Conditions on Fusarium Screening Outcomes

**Ian Walker Des McGrath Chrys Akem**

#### Introduction

Breeding populations and tomato varieties are regularly screened for resistance to *Fusarium oxysporum* f. sp. *lycopersici* race 3 at the QDPI Bowen as part of the our breeding programs and also for third parties including universities and seed companies both here and overseas. The methodology used was developed in the early stages of the QDPI tomato breeding program to develop Fusarium Race 3 resistant tomato varieties.

In the absence of large controlled temperature growing facilities screenings are conducted in plant houses under ambient conditions of temperature and humidity. This has caused non-uniformity of results from different times of year with the pathogen being much more aggressive in the warmer months. Lines with good field resistance often suffer 50% to 85% death in screenings during these warmer months. The large portion of deaths in known resistant lines used as standards has made classification of test lines difficult and unreliable.

QDPI screenings are confined to the cooler months that give more reliable results. To try and quantify the effect of these ambient temperatures on screening results a series of nine experiments was planned to span the summer to winter period in the first half of 2003.

During the work an unusual expression of the disease emerged with resistant plants showing minimal but definite leaf yellowing and vascular staining. To confirm that these symptoms were not misclassified as Fusarium an extra experiment was conducted using reisolated inoculum from the two resistant lines and the isolated pathogen was identified by a plant pathologist.

## Methods and Materials

### *Time Series Experiments*

*Times*

A series of nine screening experiments was conducted in the first half of 2003 (Table 5). At the time of writing the last three experiments were still running.

*Table 5*. Experimental Timetable

| Trial No. | Sown | Thinned | Inoculated | Rated | Days to Inoc. | Days to Rating |
|---|---|---|---|---|---|---|
| 1 | 17 Feb | 28 Feb | 6 Mar | 28 Mar | 17 | 22 |
| 2 | 28 Feb | 19 Mar | 21 Mar | 11 Apr | 21 | 21 |
| 3 | 19 Mar | 1 Apr | 7 Apr | 29 Apr | 19 | 22 |
| 4 | 2 Apr | 14 Apr | 23 Apr | 19 May | 21 | 26 |
| 5 | 15 Apr | 23 Apr | 7 May | 30 May | 22 | 23 |
| 6 | 1 May | 14 May | 22 May | 18 Jun | 21 | 27 |
| 7 | 15 May | 27 May | 6 Jun | | 22 | |
| Reinoculation | 15 May | 27 May | 6 Jun | | 22 | |
| 8 | 30 May | 10 Jun | 18 Jun | | 19 | |
| 9 | 12 Jun | 27 Jun | | | | |

Screenings were sown approximately 2 weeks apart to cover the period from hot humid conditions of the wet season to the drier cooler winter months. The time from sowing to

inoculation was approximately 3 weeks and the time from inoculation to rating was approximately 3 weeks. This varied due to more rapid plant and disease development in warmer temperatures.

*Varieties*

Three varieties were chosen for the work:

Tristar – resistance derived from *L. pennellii*
Guardian – resistance gene *I-3* from LA716 of *L. pennellii*
Walter PF – susceptible cultivar

These varieties are routinely used as standards in screening work.

*Inoculum*

The inoculum was derived from *Fusarium oxysporum* f.sp *lycopersici* race 3 isolate 1943 maintained as lyophilised cultures by QDPI Plant Pathology. This was cultured and then subcultured onto enough plates to provide enough concentrate to be made to supply inoculum for the whole experiment. Superficial sporulating hyphae were scraped and washed from the plates, homogenised and the spore concentration enumerated on 6 March 2003. The resulting concentrate was stored in a 4ºC cold room and sub samples taken and diluted to appropriate concentrations on the day of each inoculation.

Each experiment used three inoculum strengths – 1, 0.5 and 0.1 million spores per mL. One million spores per mL is the standard strength used in screening work and the lower strengths were trialled to see if they would give a better result in the warmer months when the pathogen appears more aggressive. Previously $0.5 \times 10^6$ $mL^{-1}$ had given promising results at this time.

*Seedlings*

Seed was sown into 7" community pots of pasteurised UC (University of California – a mix of sand, peat and fertiliser) mix, seedlings were thinned at about 10 days to remove weak seedling and to reduce pot populations to give stouter seedlings.

*Inoculation*

About 21 days after sowing seedlings were dug up, the potting mix shaken and washed from the roots, excess water shaken from the roots, the roots immersed in the inoculum for several seconds and then the plants were replanted in 7" pots of fresh pasteurised UC mix 10 per pot.

*Experimental Design*

A randomised block design was used with 3 varieties x 3 spore concentrations x 3 replicates x 9 times. In the first two experiments the replicates were not completely randomised.

*Rating*

About three weeks after inoculation the plants were rated using the following 0 to 5 rating system:-

0 – completely healthy plant
1 – staining at the very tip of the main root
2 – staining to the cotyledonary node
3 – staining beyond the cotyledonary node but no external symptoms (external symptoms are yellowing of the leaves and wilting)
4 – staining beyond the cotyledonary node and external symptoms
5 – total plant collapse.

Scores 0, 1 and 2 are considered resistant and 3, 4 and 5 susceptible.

There should have been 10 plants rated to a pot but occasionally there were plant losses due to rots (mainly *Rhizoctonia*).

*Meteorological Data*

Meteorological data was collected from a Davis Weather Wizard III automatic weather station (temperatures), rainfall from a standard rain gauge. All instruments were in the meteorological area of QDPI CDTA Bowen, about 100m from the experimental area.

*Reinoculation Experiment*

A single reinoculation experiment using inoculum isolated from plants from screening 3 was inoculated on 6 June 2003.

Methodology was the same as above except for a few points.

The design was randomised blocks with 3 varieties x 3 inoculum sources x 2 inoculum strengths x 3 replicates with one uninucleate control of each variety in each replicate.

The inoculum strengths were 1 and 0.5 x $10^6$ $mL^{-1}$.

The inoculum sources were the original 1943 inoculum and reisolated inoculum derived from minimally infected plants of Tristar and Guardian from Screening 3.

A seedling shortage meant that most pots had less than 10 plants.

Isolation and identification of the pathogen was done by Dr Chrys Akem, Plant Pathologist, QDPI Ayr.

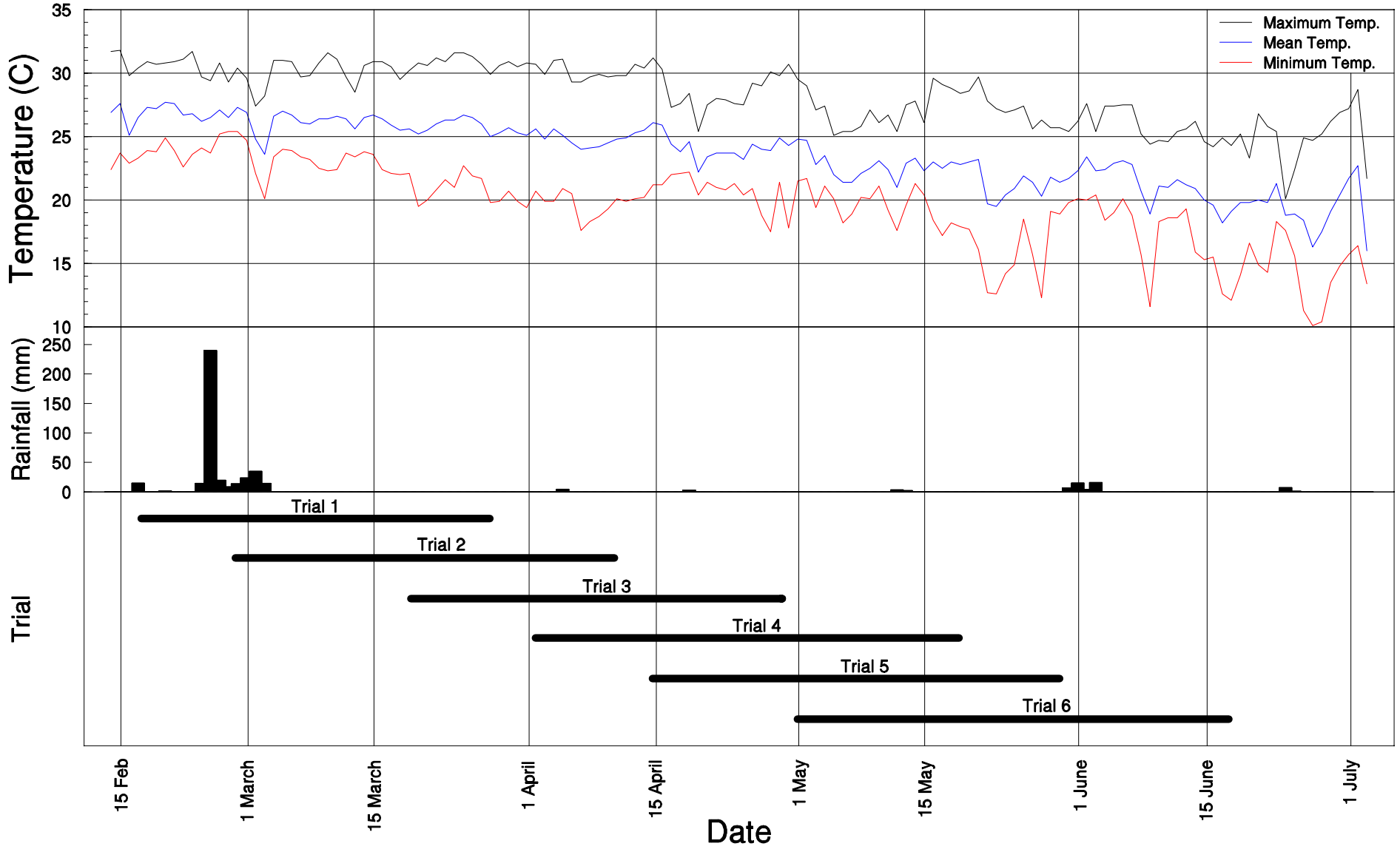
## Results

*Table 6*. Time Series Experiments Results sowing Percentage of Susceptible Plants

| | | Rating Date | | | | | |
|---|---|---|---|---|---|---|---|
| | **Inoc. Conc. spores x $10^6$/mL** | 28 March | 11 April | 29 April | 19 May | 20 May | 18 June |
| **Line** | | **Trial 1** | **Trial 2** | **Trial 3** | **Trial 4** | **Trial 5** | **Trial 6** |
| | | | | | | | |
| Guardian | 0.1 | 4 | 7 | 0 | 3 | 0 | 0 |
| Guardian | 0.5 | 0 | 37 | 67 | 13 | 4 | 0 |
| Guardian | 1.0 | 29 | 50 | 77 | 20 | 0 | 0 |
| | | | | | | | |
| Tristar | 0.1 | 21 | 59 | 3 | 10 | 0 | 0 |
| Tristar | 0.5 | 60 | 76 | 73 | 57 | 17 | 0 |
| Tristar | 1.0 | 48 | 67 | 96 | 86 | 27 | 0 |
| | | | | | | | |
| Walter PF | 0.1 | 92 | 78 | 33 | 50 | 17 | 23 |
| Walter PF | 0.5 | 96 | 100 | 89 | 97 | 77 | 77 |
| Walter PF | 1.0 | 100 | 71 | 100 | 100 | 100 | 86 |

# *Figure 3.* 2003 QDPI Bowen Fusarium Screening Trials

## Prevailing Temperatures and Rainfall and Timing of Experiments



***Reinoculation Experiment***

The pathogen isolated from both the Tristar and Guardian plants was identified as *Fusarium oxysporum* f. sp. *lycopersici*. There were very clear visual differences between the inoculums (see Figures 4 and 5).


*Figure 4*. Comparison of three isolates at 1 x $10^6$ spores $mL^{-1}$ on Walter PF


*Figure 5*. Comparison of the Tristar isolate at 1 x $10^6$ spores $mL^{-1}$ on three varieties

## Discussion

*Time Series Experiments*

Table 6 shows clearly the large percentage of apparently susceptible plants in standards with known resistance in the field (Tristar and Guardian) at the standard inoculum strength of 1 x $10^6$ spores $mL^{-1}$ in the first four trials. In Trial 5 Guardian was fully resistant but Tristar was was 27% susceptible. The lower inoculum concentrations gave better results for these resistant lines but there was very much less susceptibility in the known susceptible line (Walter PF) which would rule these out as useful concentrations for routine screenings.

In Trial 6 Tristar and Guardian displayed complete resistance and Walter PF produced almost complete (86%) infection at 1 x $10^6$ spores $mL^{-1}$. This would indicate that this inoculum strength and this time of year (see Table 5) offer the most reliable screening conditions. Figure 3 shows the prevailing temperatures and rainfall during the experiments. The steady decline in temperatures and rainfall as the year progressed can be seen. The temperatures that prevailed during the inoculation period in Trial 6 (22 May to 18 June, 2003) can be taken as optimal for providing reliable results of Fusarium screening. These temperatures would be used in a controlled temperature facility to do this work. The remaining three trials should add to the data and show how wide the window is for doing reliable trails at ambient temperatures. Once a temperature band is determined that offers reliable results this can be compared with long term temperature data held at QDPI Bowen to determine time periods (one in autumn and one in spring) when best results can be routinely obtained.

*Reinoculation Experiment*

The confirmation of the reisolated pathogen as *Fusarium oxysporum* f.sp *lycopersici* gives confidence in the rating of less severe symptoms observed in the resistant lines in the earlier trails as Fusarium.

Figure 4 shows that there has been some selection pressure in the screening trial that the reisolations were obtained from. The Tristar isolate was very much more aggressive than 1943. It also appeared to be a little more severe than the Guardian isolate although this was not certain. Formerly 1943 had been regarded as the most severe isolate available but both

these new isolates are much more aggressive and will now be lyophilised and kept in the QDPI pathology reference collection for future work.

Figure 5 shows the differential reactions to the Tristar isolate on the three test varieties. Even though this is much more aggressive than the 1943 (as seen in Figure 4) it can be seen that both Tristar and Guardian showed good resistance to it. This isolate may have a use in the colder part of the year when 1943 often takes a long time to express symptoms in susceptible lines. The longer period needed to conclude screenings in the colder part of the year often leads to confounding of symptoms with deficiencies and insect infestations and the progression of plants into flowering and fruit set. This more aggressive isolate may also be useful to shorten screening times in the optimum times of year for screening.

**Conclusion**

The first five trials in a series of nine showed poor results. Trial 6 showed expected results. The three remaining trials yet to be rated may widen the window when reliable results at ambient temperatures can be expected. Optimal temperatures will be determined and these results will be compared with long term temperature data to determine the best time of year to conduct Fusarium screenings at Bowen.

The reinoculation experiment confirmed that the mild symptoms in the resistant lines were indicative of Fusarium. Two new isolates proved to be more aggressive than the 1943 which was formerly regarded as the most aggressive. These isolates will be added to the QDPI collection and they may have a useful role in future screening work.

## 2.3 Development of Resistance to Tomato Spotted Wilt Virus

### 2.3.1 The development of a PCR based marker-system for the TSWV resistance-gene, *Sw*-5.

**Stephen Garland**

**Introduction**

Folkertsma *et al* (1999) developed a CAPS marker linked to *Sw-5* and the *Sw-5* gene has been characterised. Spassova *et al* (2001) demonstrated that the *Sw-5*b gene sequence (GenBank accession AY007366) was 'necessary and sufficient' to produce resistance to TSWV.

We have developed a PCR based marker-system that consists of a dominant marker representing the *Sw-5*b gene sequence and incorporates the CAPS marker as a positive control.

**Methodology and Results**

*Sw-5* analogues (GenBank accessions *Sw-5* a and b - AY007366; *Sw-5* c,d and e - AY007367) were aligned using ClustalW (WWW Service at the European Bioinformatics Institute http://www.ebi.ac.uk/clustalw; Higgins *et al*, 1994) in order to identify polymorphic sections, and sequence specific to *Sw-5*b. The Leucine-rich-repeat (LRR) domain was targeted for the production of *Sw-5*b sequence-specific, PCR primers. The LRR region was selected due to high levels of polymorphism and being a characteristic feature of the NBS-LRR class of disease resistance genes. *Sw-5*a has been identified as the most similar analogue to *Sw-5*b (Spassova *et al*, 2001), with only 2.2% of bases differing. 5.6% of bases were found to differ for the LRR domain and 1.1% of bases were different between *Sw-5*a and *Sw-5*b for the remainder of the gene sequence.

PCR primers were designed for a 305 bp section of the LRR domain of *Sw-5*b utilizing the computer program Primer3 (Rozen and Skaletsky, 2000). This DNA fragment had 7.9% of bases different between *Sw-5*b and *Sw-5*a. The sequence differences included a 3 bp deletion in *Sw-5*a. Primer details are provided in Table 7. Both primers were polymorphic for the 3′base when compared to the *Sw-5*a sequence. The eight base, from the 5′ end of the forward primer (Sw5b-LRR-F), and the seventh base of the reverse primer (Sw5b-LRR-R), were also

polymorphic for the same comparison. Initial testing with this primer pair indicated that specificity was obtained at a relatively low annealing temperature of 50$^{o}$C and therefore it was not necessary to incorporate artificial mismatches near the 3′ end of the primers. Specificity was tested by achieving positive amplification in resistant lines and no amplification from a susceptible cultivar.

*Table 7*: Primers specific to the LRR domain of the *Sw-5*b gene; and primer details for the CT220 fragment (Folkertsma *et al* 1999)

| **Primer Name** | **Oligonucleotide sequence** |
|---|---|
| Sw5b-LRR-F | TCTTATATTGTGGAGTTTTTGTCG |
| Sw5b-LRR-R | TCCACCCTATCAAATCCAAC |
| ZUP641 | AAGCCGAATTATCTGTCAAC |
| ZUP642 | GTTCCTGACCATTACAAAAGTAC |

Folkertsma *et al* (1999) provided primer details for the CT220 fragment, a locus within 25 kb of *Sw-5*b, and indicated that a MseI digestion was suitable for the production of a CAPS based marker for the identification of *Sw-5* (recognition sequence T▼TAA). MseI digestion of the fragment from *L.esculentum* produces five small fragments and a fragment equivalent to 94 bp. MseI digestion of a fragment from a resistant line (assuming linkage has been maintained) produces four small fragments and a fragment equivalent to 119 bp. As *Sw-5*b contains a MseI restriction site, and to facilitate co-amplification of the CT220 fragment and the *Sw5*b-LRR fragment, followed by a restriction digest for genotyping of the CT220 locus, the AseI enzyme was used. The recognition sequence for AseI (AT▼TAAT) is not contained within the *Sw-5*b fragment.

The CT220 fragment from *L. esculentum* (sequence ID `cTOF-14-J16.TH.B` - Solanaceae Genomics Network http://www.sgn.cornell.edu/cgi-bin/SGN/) possessed one restriction site for AseI and would be expected to produce one fragment equivalent to 94 bp and one equivalent to 119 bp, after digestion. The 213 bp fragment from a resistant line (assuming

linkage has been maintained) would not be cut by AseI. Primer details for the CT220 fragment are provided in Table 7.

The CT220 and *Sw-5*b fragments were found to co-amplify and digestion with AseI produced the expected results (see Figure 6). AseI (NEB) digestion followed the manufacturers instructions.

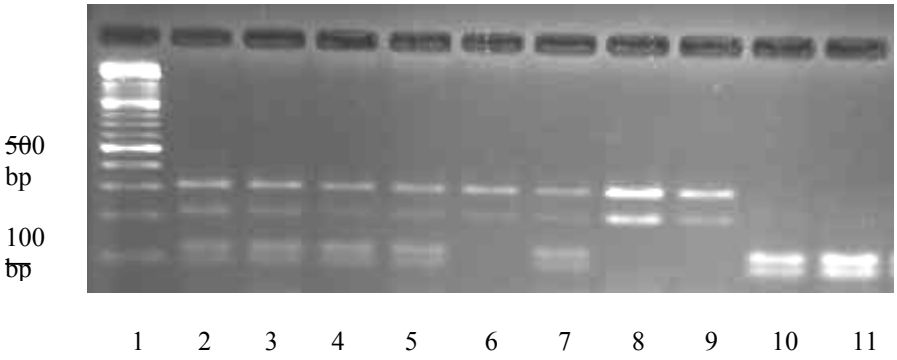



*Figure 6*. Results of co-amplification of CT220 and Sw5b-LRR fragments after digestion with the AseI restriction enzyme, agarose gel electrophoresis, ethidium bromide staining, and excitation with UV light. Notice the Sw5b-LRR fragment at approximately 300 bp, the uncut CT220 fragment at approximately 200 bp, and the two fragments at approximately 100 bp produced by the AseI restriction of the CT220 fragment. Lane 1; 100 bp size ladder. Lanes 2 to 5 and 7; individuals from a population segregating for *Sw*-5 resistance and susceptibility, showing positive amplification of the *Sw*-5b product, and heterozygous genotype for the CT220 CAPS marker. Lane 6, an individual from a population segregating for *Sw*-5 resistance and susceptibility, showing positive amplification of the *Sw*-5b product, and homozygous resistant genotype for the CT220 CAPS marker. Lane 7 and 8, separate PCR amplifications for *Lycopersicon esculentum* accession LA3667 (known to possess *Sw*-5) showing positive amplification of the *Sw*-5b product, and homozygous resistant genotype for the CT220 CAPS marker. Lane 10, *Lycopersicon pennellii* LA716 showing no amplification of the *Sw*-5b product and homozygous susceptible genotype for the CT220 CAPS marker. Lane 11, *Lycopersicon esculentum* susceptible line, showing no amplification of the *Sw*-5b product, and homozygous susceptible genotype for the CT220 CAPS marker.

50 $F_2$ individuals from a mapping-population segregating for resistance to TSWV were genotyped by screening $F_3$ plants for susceptibility or resistivity to TSWV inoculation (refer to Table 8). The same 50 $F_2$ individuals were genotyped using the marker system (see Figure

6). Marker and *Sw-5* genotypes agreed for 47 of the $F_2$ plants. For one $F_2$ plant the marker system indicated a heterozygous individual, and resistance screening indicated it to be homozygous susceptible. For two $F_2$ individuals the marker system indicated them to be homozygous susceptible and the resistance screening was inconclusive and suggested them to be homozygous susceptible or heterozygous.

*Table 8.* Glasshouse assay of random $F_3$ lines segregating for resistance to Tomato Spotted Wilt Virus. Assay conducted by Denis Persley and Murray Sharman. ELISA conducted by Lee McMichael.

| Line | Pot Rep. | Symptoms | Systemic / total plants | Line Totals | Apparent resistance | ELISA results |
|---|---|---|---|---|---|---|
| 5 | A | obvious sys.sympt., stunted | 12/14 | | | |
| 5 | B | obvious sys.sympt., stunted | 13/14 | 25/28 | Susceptible | |
| 13 | A | A few plants with hyp.l.l. | 0/13 | | | |
| 13 | B | 3 syst.sympt., several with hyp.l.l. | 3/16 | 3/29 | Segregating | |
| 16 | A | couple with fungal wilt | 0/19 | | | |
| 16 | B | A few plants with hyp.l.l. | 0/18 | 0/37 | Resistant | ELISA done (negative) |
| 17 | A | several with hyp.l.l. | 0/17 | | | ELISA done (negative) |
| 17 | B | no symptoms | 0/14 | 0/31 | Resistant | |
| 22 | A | no symptoms | 0/18 | | | |
| 22 | B | no symptoms | 0/15 | 0/33 | Resistant | |
| 23 | A | 16 with syst.sympt. | 16/20 | | | |
| 23 | B | stunted plants | 10/18 | 26/38 | Susceptible | |
| 24 | A | 1 obvious sys.sympt., few with hyp.l.l. | 1/15 | | | |
| 24 | B | obvious sys.sympt. | 6/18 | 7/33 | Segregating | |
| 29 | A | no symptoms | 0/16 | | | ELISA done (negative) |
| 29 | B | no symptoms | 0/10 | 0/26 | Resistant | |
| 32 | A | obvious sys.sympt. | 5/22 | | | |
| 32 | B | mild symptoms | 4/21 | 9/41 | Segregating | |
| 33 | A | obvious sys.sympt. | 4/15 (Segregating) | | | |
| 33 | B | obvious sys.sympt., stunted | 18/18 (Susceptible) | ?? | ?? | |
| 35 | A | no symptoms | 0/20 | | | |
| 35 | B | some with hyp.l.l., plants a bit weak | 0/22 | 0/42 | Resistant | ELISA done (negative) |
| 36 | A | obvious sys.symptoms | 11/16 | | | |
| 36 | B | severe sys.sypmtoms | 15/15 | 26/31 | Susceptible | |
| 37 | A | obvious sys.symptoms on most | 9/13 (not sure if Sus. / Seg.) | | | |
| 37 | B | obvious sys.symptoms, couple with hyp.l.l. | 5/18 (Segregating) | | ?? | |
| 41 | A | obvious systemic symptoms | 14/14 | | | |
| 41 | B | 4 with fungal disease, others obvious virus | 8/13 | 22/27 | Susceptible | |
| 43 | A | several with hyp.l.l., others systemic | 5/18 | | | |
| 43 | B | few with hyp.l.l., others systemic | 5/16 | 10/34 | Segregating | |
| 44 | A | mild symptoms, some with hyp.l.l. | 5/20 | | | |
| 44 | B | several with hyp.l.l. | 2/18 | 7/38 | Segregating | |
| 45 | A | obvious systemic symptoms | 16/21 | | | |
| 45 | B | obvious systemic symptoms, stunted | 16/18 | 32/39 | Susceptible | |
| 46 | A | several with hyp.l.l. | 0/16 | | | ELISA done (negative) |
| 46 | B | some with hyp.l.l., vigorous | 0/16 | 0/32 | Resistant | |
| 47 | A | 2 with possible mild mottle | 2/20 | | | ELISA done (positive) |
| 47 | B | 1 with possible mild mottle | 1/19 | 3/39 | Segregating | |
| 48 | A | obvious sys.sympt., few with hyp.l.l. | 6/19 | | | |
| 48 | B | obvious sys.sympt. | 4/23 | 10/42 | Segregating | |
| 49 | A | no symptoms | 0/17 | 0/17 | Resistant | |
| GL | A | mild sys.sympt. | 10/14 | | | |
| GL | B | mild sys.sympt. | 9/14 | 19/28 | Susceptible | |

*DNA extraction*

DNA was extracted from approximately 0.02g of fresh leaf tissue and ground with 1mL of extraction buffer at 60$^{o}$C (Edwards *et a*l,1991). The extract was added to 700 µL of chloroform/isoamyl alcohol (24:1), mixed, left on ice for at least half an hour, and centrifuged at 11000 rpm for three minutes. DNA was precipitated from the supernatant after adding 1 volume of isopropanol followed by five minutes centrifugation at full speed. The pellet was washed twice with 70% ethanol and resuspended in 100 µL of Tris-EDTA (TE) buffer.

*PCR*

Primers were synthesized by Proligo Pty Ltd, SCU, Lismore, NSW, Australia. PCR reactions were carried out on a Perkin Elmer, Gene Amp PCR System 9700. The reaction volume was 20µL containing 1X Roche PCR Buffer, approximately 50 ng of genomic DNA and 200µM dNTPs. The PCR reaction mix also included 100 nM of each of the four primers, 5.75 mM total $MgCl_2$, and 0.1 units of Taq DNA Polymerase (Roche). The temperature cycling conditions were 3 minutes at 94° C; followed by 35 cycles of 94° C for 30 seconds, 56° C for 30 seconds and 72° C for 1 minute; with a final hold at 72 ° C for 1 minute.

**Discussion**

The new marker system offers the ability to detect *Sw-5* without the need to perform a restriction digest, by the amplification of the Sw5b-LRR fragment. The system incorporates a positive control for the PCR in the form of the linked CT220 fragment, and therefore greatly reduces the chance of a false negative. The marker system also has the ability to predict the genotype of individuals possessing *Sw-5* (heterozygous or homozygous resistant) by performing a restriction digestion following PCR.

Genetic mapping indicated up to three recombination events between the marker and *Sw-5* (3 cM). This is not likely to be the case as CT220 is known to be within 25 kb and linked within 0.15 cM (Brommonschenkel and Tanksley (1997) of *Sw-5*. The apparent recombinations are more likely to be a function of incorrect genotyping of the *Sw-5* locus. *Sw-5* is not completely dominant (heterozygotes are less resistant than homozygotes) and therefore it may be difficult to detect a resistant response in some cases. (reviewed by Brommonschenkel *et al*,

2000). *Sw-5* does not produce resistance 100% of the time (98.7% penetrance, reviewed by Stevens et al, 1996) and further complicates genotyping.

**References**

Brommonschenkel S.H., Frary A., Frary A., Tanksley S.D., (2000). The broad-spectrum tospovirus resistance gene *Sw-5* of tomato is a homolog of the root-knot nematode resistance gene *M*i. Molecular Plant-Microbe Interactions, 13,10: 1130-1138.

Brommonschenkel S.H., Tanksley S.D., (1997). Map-based cloning of the tomato genomic region that spans the *Sw-5* tospovirus resistance gene in tomato, Mol Gen Genet, 256: 121-126.

Edwards K., Johnstone C., Thompson C., (1991). A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Research, 19, 6: 1349.

Folkertsma R.T., Spassova M.I., Prins M., Stevens M.R., Hille J., Goldbach R.W., (1999). Construction of a bacterial artificial chromosome (BAC) library of *Lycopersicon esculentum* cv Stevens and its application to physically map the Sw-5 locus. Molecular Breeding, 5: 197-207.

Higgins D., Thompson J., Gibson T.Thompson J.D., Higgins D.G., Gibson T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673-4680.

Rozen Steve and Helen J. Skaletsky (2000). Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) *Bioinformatics Methods and Protocols: Methods in Molecular Biology.* Humana Press, Totowa, NJ: 365-386

Spassova M.I., Prins T.W., Folkertsma R.T., Klein-Lankhorst R.M., Hille J., Goldbach R.W., Prins M., (2001). The tomato gene *Sw-5* is a member of the coiled coil, nucleotide binding, leucine-rich repeat class of plant resistance genes and confers resistance to TSWV in tobacco. Molecular Breeding, 7:151-161.

Stevens M.R., Heiny D.K., Rhoads D.D., Griffiths P.D., Scott J.W. (1996). A linkage map of the tomato spotted wilt virus resistance gene *Sw-5* using near isogenic lines and an interspecific cross. Acta Horticulturae, 431: 385-392

### 2.3.2 Incorporation of Resistance to Tomato Spotted Wilt Virus with *Sw-5* in indeterminate lines

**Des McGrath and Ian Walker**

$F_3$ indeterminate lines identified in Table 5 above as resistant lines were subsequently inbred to the $F_3$ generation. The $F_3$ lines were derived from an indeterminate resistant hybrid with good fruit quality but relatively poor agronomic adaptation. Following identification of their resistance status through a glasshouse bioassay, two resistant lines were crossed to 12 superior susceptible lines described in Section 1.1 above. The derived lines then segregated for *Sw-5* in the $F_5$ which is now ready for selection by the CAPS marker described in Section 1.3.1 above. The *Sw-5* lines will be complementary to the Fusarium-resistant lines indicated above so that the appropriate combinations of the two disease resistance genes can be made from the lines forming an $F_1$ hybrid. The development of these lines is now proceeding routinely and will be an essential part of continuing work towards commercialisation of multiple disease-resistant hybrids.

## 2.4 Applied Breeding – Determinate Lines

A small number of producers have continued to grow traditional determinate round varieties, largely for reasons of cost. The major disadvantage of the fruit type is firmness at full maturity and reduced post-harvest life. The introduction of firmer fruit quality, typical of indeterminate 'gourmet' fruit, to a determinate plant type would be beneficial for these growers. This objective was pursued initially through the same material outlined in Section 1.1.

The strongly determinate plant types indicated in Table 1 above were obtained by crosses of determinate x indeterminate genotypes. $F_4$ 1-9 from this series possessed many superior fruit qualities in a determinate growth habit but suffered from excessively small bush size and yield as well as some irregular fruit shape. An additional cycle of crossing to superior quality indeterminate varieties generated tall and short types. The tall genotypes were described in

Section 1.1; the following short determinate lines with good agronomic attributes and desirable fruit qualities were selected:

(1911 x M1)-6-1, 2

(1911 x M1)-7-1, 2

(1911 x M2)-4-1, 2

(1911 x M2)-5-1, 2

(9161 x M2)-1-4-1-1, 2

(9161 x M2)-1-4-2-1, 2, 3, 4

The lines from (1911 x M2)-4 and (1911 x M2)-5 were the best for agronomic performance and segregated for disease resistance. Most fruit of these lines were of similar quality and standard to the indeterminate lines described in 1.1. The plant size is somewhat smaller than conventional determinate round varieties and needs to be assessed in commercial conditions.

# 3. Flavour Improvement

A series of high solids breeding lines was developed previously from the Japanese variety Momataro, using total soluble solids (TSS) as the selection criterion in several cycles of a backcross program. Individual families were field-selected for suitable plant and fruit characteristics and single plants within lines were then assessed for TSS content.

The current project developed this material further by generating true-breeding high-solids lines from the last hybridisation and evaluating a small number of elite lines and their inter-crosses for eating quality.

**Panel Evaluations**

**Trial 1**
**Materials and Methods**

Three high TSS inbred lines, 6-46, 6-51 and 8-13, and a standard low TSS commercial cultivar were field grown at Bowen. The high TSS lines were derived from two cycles of crossing and inbreeding from the parent Momataro.

The trial was planted using a conventional spacing for ground crops of 75cm between plants, and grown according to normal commercial practice. Fruit were harvested at full colour (USDA 6) at 8am from a randomised block field design of six replications. A panel of six tasters evaluated the fruit four hours later.

The fruit was randomised across replications and presented to each panellist who scored samples for sweetness and overall acceptability according to the following scale:
Sweetness: 1 very low; 4 average; 7 very high.
Overall score: 1 very poor; 4 average; 7 excellent quality.

Whereas the scores for sweetness addressed this specific component of flavour, the overall score integrated all flavour, texture and eating quality attributes which contributed to perception of total quality. Panellists scored fruit without seeing samples because the pink

skin colour of the high TSS lines could contribute to bias. Measurements of total soluble solids (% Brix) were made on samples of harvested fruit from each plot and the data was analysed with the panel scores.

**Results**

The three high solids lines produced Brix content ranging from 5.0% to 5.2%, compared with 4.5% for the standard variety. The differences between the standard and each of the improved lines were significant (P< .05, Table 1).

The sweetness score for each of the improved lines (4.0 to 4.4) was higher than for the standard (3.5), (P < .05, Table 1), and this was reflected in a similar pattern of overall acceptability scores (P < .05, Table 1). There was a clear association of Brix content, sweetness and overall acceptability scores for these lines.

| Line | Sweetness Score | Brix | Acceptability Score |
|---|---|---|---|
| 6-46-1 | 4.0 | 5.0 | 4.3 |
| 6-51-1 | 4.4 | 5.1 | 4.5 |
| 8-13-1 | 4.0 | 5.2 | 4.4 |
| **Standard unimproved** | 3.5 | 4.5 | 3.8 |
| **LSD P<.05** | 0.5 | 0.2 | 0.4 |

*Table 1.* Panel scores for improved breeding lines and a standard cultivar.

**Trial 2**

Four hybrids, a related high-solids inbred parent line and a standard check hybrid were grown in the field at Bowen. The genotypes were designated as:

1. Hybrid 6-21-5 x 8-13-5
2 Hybrid 6-51-1- x 6-21-1
3. Hybrid 6-51-1- x 8-13-5
4. Hybrid 6-51-1 x Rin 5-2
5. Parent line 6-51-1
6. Check hybrid 9 x 2

Parent lines 6-21-5, 8-13-5 and 6-51-1 are inbred genotypes developed previously by two cycles of crossing and inbreeding from the source of high TSS, Momataro.
Rin 5-2 is an inbred line developed from a conventional bush cultivar with large, globe fruit and extended shelf life. Check hybrid 9 x 2 is a commercial cultivar with unimproved flavour characteristics.

The trial was planted using a conventional spacing within rows of 75cm for ground-grown crops. Plants were grown according to normal commercial practice and fruit were subsequently harvested for evaluation.

A panel comprising eleven experienced tasters was conducted. Fruit of each variety were harvested at full red colour (USDA 6) in the morning and presented to the panellists at 11am for scoring. The fruit were randomised over varieties and allocated as two replications in time to each of the panel who rated sliced fruit for three attributes of eating quality according to the following hedonic scale:

Sweetness: 1 very low; 4 average; 7 very high.
Acidity: 1 very bland; 4 average; 7 very acid
Overall score: 1 very poor; 4 average; 7 excellent quality.

As with the previous evaluation, panellists scored fruit without seeing skin colour so as to avoid bias.

**Results**

*Sweetness*

Differences in sweetness among the six genotypes were recognised by the panel. Hybrid 6-51-1 x Rin 2 and hybrid 9 x 2 were scored as significantly inferior to the other lines ($P < .05$, Table 2). The other hybrids with two high TSS parents and line 6-51-1 performed similarly and were rated as sweeter than other lines ($P < .05$).

*Acidity*

Hybrids 6-51-1 x 8-13-5, 6-51-1 x Rin 5-2 and 9 x 2 were rated as less acidic than the other genotypes. The differences were significant between some of this group and the most acidic hybrid, 6-51-1 x 6-21-1 ($P < .05$, Table 2).

*Overall Acceptability*

The most acceptable hybrids were 6-51-1 x 8-13-5 and 6-51-1 x 6-21-1, both of which were similar to the parent line 6-51-1. Hybrids 6-51-1 x Rin 5-2 and 9 x 2 were significantly less acceptable than the best hybrids ($P < .05$, Table 2).

| Line/Hybrid | Sweetness Score | Acidity score | Acceptability Score |
|---|---|---|---|
| 6-21-5 x 8-13-5 | 4.3 | 4.0 | 4.5 |
| 6-51-1- x 6-21-1 | 4.2 | 4.4 | 4.4 |
| 6-51-1 x 8-13-5 | 4.0 | 3.6 | 3.9 |
| 6-51-1 x Rin 5-2 | 3.6 | 3.3 | 3.6 |
| 6-51-1 | 4.3 | 4.0 | 4.4 |
| 9 x 2 | 3.5 | 3.4 | 3.7 |
| LSD P<0.05 | 0.7 | 0.7 | 0.9 |

*Table 2.* Panel scores for $F_1$ hybrids between high-solids and standard parent lines.

**Trial 3**

The following genotypes were grown in a field trial and evaluated using the methodology adopted previously**.**

1. Hybrid 6-21-1-1 x 6-51-1-1
2. Hybrid 8-13-5-2 x 6-51-1-1
3. Parent line 8-13-5-2
4. Parent line 6-51-1-1
5. Hybrid 9 x 2

Genotypes 1 to 4 comprised hybrids or inbred lines with improved TSS parents whereas genotype 5 (9 x 2) was an unimproved commercial cultivar.
The field trial was conducted as a randomised block experiment with 3 replications and fruit was harvested at the USDA 6 maturity stage as in previous years.

Five panellists evaluated mature fruit for sweetness, acidity and overall acceptability under the conditions indicated for earlier experiments.

**Results**

Panel scores for eating quality attributes are presented in Table 3.

| Hybrid/Line | Sweetness Score | Acidity score | Acceptability Score |
|---|---|---|---|
| 6-21-1 x 6-51-1 | 3.9 | 3.3 | 4.1 |
| 8-13-5-2 x 6-51-1-1 | 4.1 | 3.4 | 4.2 |
| 8-13-5-2 | 3.4 | 3.5 | 3.7 |
| 6-51-1-1 | 3.8 | 3.3 | 3.9 |
| 9 x 2 | 3.2 | 3.1 | 3.5 |
| LSD P<0.05 | 0.6 | 0.6 | 0.6 |

*Table 3.* Panel scores for high TSS $F_1$ hybrids and high and low TSS parent lines.

## Discussion

The parent lines with high total soluble solids (TSS) evaluated here were shown to develop significantly greater TSS content than the standard unimproved genotype. The improvement was generally 0.5 Brix units (Table 1). The panel recognised the higher TSS genotypes as sweeter and better overall. In $F_1$ hybrids the same pattern of higher TSS, greater sweetness and acceptability was evident when both parents displayed high TSS (Table 2). When only one parent had high TSS the hybrid appeared to produce poorer solids content and associated quality scores, similar to the standard parent line. The evidence suggested that better taste and acceptability was achieved when both parent lines had high TSS.

The use of Brix content to assess TSS in breeding lines was successful in raising the level of eating quality in several lines. The panel scores were sometimes variable between panellists but on average the assessments of individual genotypes reflected their TSS content. The knowledge of TSS in hybrid combinations and the confidence in using TSS as a selection parameter is now the basis for improvements in a range of lines in both determinate and indeterminate breeding lines.

# 4 Genetic resistance to potato tuber moth, *Phthorimaea operculella*

## Introduction

Potato tuber moth (PTM) causes significant periodic damage to tomato crops in Australia, particularly those in major Queensland production centres. Its history in tomato crops is characterised by episodes of major infestations lasting two or three years, followed by longer intervals of relatively little activity. By contrast, the other major lepidopteran pest of tomato, tomato budworm, *Helicoverpa armigera*, is a constant pest of crops in Queensland. In recent outbreaks, effective control of potato tuber moth has been possible by rigorous industry-wide pest management practices, although this has been slow and difficult.

The use of genetic resistance to arthropod pests has been widely investigated in tomato. Accessions of *Lycopersicon hirsutum*, a green-fruited, non-cultivated species, have demonstrated resistance to many species including *Helicoverpa armigera* and *Phthorimaea operculella* (Juvik *et al*., 1982). Studies of resistance to insect pests in a wide range of plants have implicated secretions from glandular trichomes as a defence mechanism and recent work has suggested a role for allelochemicals from trichomes in resistance to *P. operculella* (Ventura and Vendramim, 1995, 1996).

The concept of genetic resistance to pests as a component of an Integrated Pest Management program is attractive in terms of reduced production costs and environmental benefits. The first objective of this work (4.1) was therefore to confirm genetic resistance to *P. operculella* in LA1777 of *L. hirsutum,* identified earlier by Juvik *et al.* (1992) and to study the effects of plant type, age and daylength on foliar pubescence and resistance. Secondly, additional accessions of *L. hirsutum* were studied for resistance (4.2). In the last part of the study (4.3), bioassays of breeding populations were undertaken to analyse segregation and identify potential DNA markers.

## 4.1 Effect of plant variety, plant age and photoperiod on glandular pubescence and host-plant resistance to potato moth (*Phthorimaea operculella*) in *Lycopersicon* spp.

**G M Gurr**

**Summary**

The effect of plant age and daylength on glandular pubescence was determined for two lines of tomato derived from *Lycopersicon hirsutum* (BTN 979 and LA 1777A) and a variety of *L. esculentum* (N 91-1-1-1-1). Densities of type I, IV, VI and VII glandular trichomes were lowest in N 91-1-1-1-1 and, over all varieties, were more dense on plants aged greater than 6 weeks. Daylength interacted with variety to significantly affect densities of type VII trichomes only. Host-plant resistance to *Phthorimaea operculella*, was determined in preliminary tests using insects cultured from founders from a potato crop and in confirmatory tests using (less readily available) insects recovered from foliage of a tomato crop. Mortality of ex-potato neonates on LA 1777 A and BTN 979 foliage was higher 18 hr after placement than for N 91-1-1-1-1, with no effect of day length or plant age. Mortality for ex-tomato neonates followed a similar trend. Ten days later, two-thirds of ex-tomato larvae had established mines on N 91-1-1-1-1 but fewer (16.7 %) were live on other varieties. Stepwise multiple regression using variety as the sole factor was significant ($P < 0.001$) in accounting 61.4 % of the variation in ex-tomato larval survival but addition of other factors to the model was not significant. BTN 979 supported fewer, smaller adults to develop than did N 91-1-1-1-1, whilst no adults developed on LA 1777 A. In a non-choice test using ex-potato adults, significantly more eggs were laid on N 91-1-1-1-1 than on *L. hirsutum* varieties and 9 wk-old plants were preferred over plants three weeks older or younger. The same variety and plant age trends were evident in a free-choice test using ex-tomato adults.

### Introduction

Host-plant resistance to arthropods has been investigated as a potential solution to various pests of tomato (*Lycopersicon esculentum* Mill.). One of the most intensively researched sources of resistance is *L. hirsutum* Humb. & Bonpl. Accessions of this wild relative of the cultivated tomato have been shown to exhibit resistance to a wide range of arthropods. These include the whiteflies, *Bemisia argentifolia* Bellows and Perring (Snyder *et al*. 1998) and *B.*

*vaporariorum* (Westwood) (Gentile *et al.*, 1968); two-spotted spider mite, *Tetranychus urticae* Koch (Chatzivasileiadis and Sabelis, 1997); pink potato aphid, *Macrosiphum euphorbiae* (Thomas) (Gentile and Stoner, 1968a; Musetti and Neal, 1997); tobacco flea beetle, *Epitrix hirtipennis* (Melsheimer) (Gentile and Stoner, 1968b); and Colorado potato beetle, *Leptinotarsa decemlineata* (Carter *et al.*, 1989);

Amongst lepidopterans, *Manduca sexta* (Farrar and Kennedy, 1987); beet armyworm *Spodoptera exigua* (Hübner) (Eigenbrode and Trumble, 1993); *Spodoptera littoralis* (Boisd.), *Plusia chalcites* (Esp.), and *Heliothis* (*Helicoverpa*) *armigera* (Hübner.) (Juvik *et al.,* 1982) have all been shown to be affected by aspects of *L. hirsutum*. Two gelechiids, *Keifera lycopersiconella* and *Phthorimaea operculella* (Zeller) are also affected (Lin and Trumble, 1986 and Juvik *et al.,* 1982, respectively). Recent South American work has indicated the importance of allelochemicals from glandular trichomes in resistance to *P. operculella* (Ventura and Vendramim, 1995, 1996). This is consistent with the many other studies of resistance to other pests that have repeatedly been associated with glandular trichome densities and their secretions (Duffey, 1986).

The densities of glandular trichomes on *Lycopersicon* spp may be affected by photoperiod (Snyder *et al.,* 1998) and by plant age (Juvik *et al.,* 1982) with potential to affect resistance to arthropod pests. The aim of this study was to determine levels of glandular pubescence for a line of cultivated tomato, *Lycopersicon esculentum* (N 91-1-1-1-1), and of two breeder's lines developed from *L. hirsutum* (LA 1777 A and BTN 979) and to measure oviposition and development of *P. operculella* on plants subject to different treatments.

## Materials and Methods

### *Plant Growth*

Seed of N 91-1-1-1-1, LA 1777 A and BTN 979 was sown into moist proprietary seed raising mix (Debco 'Professional', Tyabb, Victoria, Australia) in pots of 200 ml capacity. Three sequential sowings were made at 3 wk intervals in each of two plant growth cabinets (Conviron, EF7H, Winnipeg, Canada). Cabinets were set with either a 12-hour or 16-hour day but did not differ in other respects. Cabinets shared a common cooling system providing a diurnal temperature range between 20-35$^{O}$C except for short periods of up to 38$^{O}$C resulting

from high ambient temperature. Humidity was unregulated. Plants were watered at 2-3 day intervals and fed with soluble fertiliser (Yates 'All Purpose', Milperra, New South Wales, Australia).

When plants reached the ages of 6, 9 and 12 weeks, four uniformly vigorous specimens of each variety and daylength were selected from at least double this number of plants for each of the three plant ages. One plant of each type was placed on each of four greenhouse tables in a randomised split-plot design. Plots consisted the plants of a given age and prior daylength regime and each was split by plant type to include a plant of each variety.

*Pubescence Assessment*

One of the leaflets from the terminal pair of the third youngest leaf was removed from each plant by holding the tip between finger and thumb and cutting the petiolate with scissors. Whilst still holding the tip, the leaflet was then cut into two approximately equal segments perpendicularly to the midrib. The excised portion was allowed to fall onto a plastic cutting board randomly on either its abaxial (facing away from the plant stem/ 'lower') or adaxial (facing the plant stem/ 'upper') surface. The remaining portion was then placed onto the board in the opposite orientation. Each of the two portions of leaflet was then sampled; the separate portions being used for examination of the pubescence characters of the surface that had remained out of contact with any substrate.

Leaflet samples consisted of a 1mm wide strip cut perpendicularly to the midrib and from close to the previously cut central portion. Strips of this width were achieved using a pair of scalpel blades separated by a 0.63mm thick metal spacer from an engineer’s feeler gauge. This assembly was mounted in the locked jaws of a pair of artery forceps. Leaflet strips had a slightly curved form so could be stood on one edge. Strips were positioned one at a time over a 1mm wide arena marked on microscope slide. The glandular trichomes present on either the abaxial or adaxial foliar surface were then examined by placing the slide on the stage of a compound microscope (Olympus CH2) at 100x magnification. This procedure allowed trichomes to be visualised over the 1 mm width of the leaflet strip within a small depth of field. By gradually adjusting the focus setting of the microscope, trichomes closer to the lens were brought into focus. Moving the focus setting over its full effective range progressively allowed all trichomes in the delineated 1mm x 1mm sections of leaflet to be counted for either

the adaxial or abaxial leaflet surface. This procedure was carried out on each 1mm$^2$ area four consecutive times, each allowing a count to be made of one of the four glandular trichome types defined for *Lycopersicon* spp. by Luckwill (1943). No other types of glandular trichomes were observed. A further two 1mm$^2$ areas were then inspected on each leaflet strip and values for the three observations pooled. A strip was then cut from the second portion of leaflet and the opposite (abaxial or adaxial) surface similarly examined.

*Insect Preparation*

Preliminary experiments were conducted using *P. operculella* obtained from a colony founded by individuals collected from a potato crop and maintained using methods based on Etzel (1985). As adults emerged from infested tubers, they were released into small flight cages to give mixed-sex batches. Cages comprised 700 ml plastic tubs each covered with nylon mesh held in place with rubber bands. Adults were fed by wetting small cotton wool swabs with a 5 % honey solution and placing one directly on the mesh lid of each cage. Eggs were collected by placing a Whatman 42 filter paper on the outside surface of each mesh lid and forcing it into close contact with the substrate by placing a glass Petri dish on top of each paper. This provided a favourable oviposition substrate. Egg-bearing papers were collected at 2-3 day intervals until all adults had died. Neonates, or adults reared from them, (hereafter 'ex-potato' insects) were used in experiments.

Confirmatory experiments were conducted with 'ex-tomato' insects. Tomato foliage bearing mines of *P. operculella* was collected from an infested tomato crop growing near Bundaberg, Queensland. Material was packed in paper bags and couriered to Orange, New South Wales inside insulated packaging. Two such collections were made in mid- and late November 1999. On receipt, infested foliage was placed in large plastic trays. A thin layer of sand covered the base of each tray to provide a substrate for pupation of mature larvae. Food was supplied to younger larvae in the form of tomato fruits and fresh foliage. The sand and foliage in each tray was thereafter checked twice weekly and pupae removed. As adults emerged they were handled as described for 'ex-potato' insects.

*Casual Observations and Photo-microscopy*

Small numbers (<8) of ex-tomato neonate larvae were placed on excised leaflets each variety and placed on moistened tissue paper in the base of Petri dishes. Behaviour was observed at irregular intervals over the course of 24 hr using a 9x – 55x binocular microscope. Larvae were photographed with an Olympus SC35 microscope-mounted camera using 200 ASA Fuji film.

*Experiment 1: Survival of Ex-potato Neonates.*

This experiment used three of the four batches of plants prepared as described above. Each batch constituted a block laid out in a glasshouse in a randomised-block split-plot design with three replicates. Each plot consisted of plants of uniform age and daylength regime and was split for plant type. A fine (neonate-proof) nylon mesh sleeve was secured with a rubber band around the base of each plant pot and carefully drawn up a supporting bamboo cane, leaving only the top three leaves exposed. One of the terminal pair of leaflets on the third youngest leaf of each plant was then marked with a small spot of ink from a waterproof pen. The other leaflet from this pair was previously removed for assessment of trichome densities. Five (four in replicate three) ex-potato *P. operculella* neonates were then transferred onto the adaxial surface of the marked leaflet using a fine camel hair brush. Neonates were drawn randomly from a batch that had eclosed over the preceding 48 h and only those that were conspicuously live used. Immediately after inoculation, the mesh sleeve was drawn over the top of the plant and tied closed, taking care that it did not contact the marked leaf and dislodge neonates. Insects were left undisturbed overnight and 18 ± 2 h later the mesh sleeve partially removed to allow the inoculated leaflet to be inspected with a magnifying glass. A count was made of the number of live and dead larvae on or within the marked leaflet, from which numbers of larvae elsewhere on the plant could be derived. Mortality was assumed if a larva did not respond to gentle prodding with a fine camel hair brush.

*Experiment 2:* Survival and Development of Ex-tomato Insects

This experiment used one of the four batches of plants prepared as described above. Accordingly, it contained one plant of each genotype, age and daylength regime; 18 plants in total in a randomised split-plot design with one replicate. Five ex-tomato *P. operculella*

neonates were transferred onto the adaxial surface of marked leaflets and survival assessed as described above. A second inspection took place 10 d after inoculation. On this occasion the mesh sleeve was removed and the entire plant inspected. Mesh sleeves were replaced and, 30 d after plants had been inoculated with neonates, adults began to eclose. These were removed and cages subsequently checked at 2 d intervals and additional adults similarly removed. Total length (head - wing tip) and body length (head - abdomen tip) were measured using a 10x binocular microscope fitted with an eyepiece graticule.

*Experiment 3: Non-choice Oviposition by Ex-potato Adults.*

This experiment used 7-, 10- and 13-wk old plants of each of the three varieties grown under differential daylengths as described for experiments one and two. The terminal leaflet and at least 20 mm of petiolate were removed from one young, fully expanded leaf for one plant of each type. Each of the 18 leaflets was placed in a 3 ml glass vial containing tap water. Vials were attached with double-sided tape to the base of separate, aerated 1 l plastic containers. An unmated male and two unmated female ex-potato *P. operculella* adults were than released into each. These flight cages were then laid out on a laboratory bench in a randomised split-plot design with one replicate. Plots consisted leaflets from plants of a given age and daylength origin and with each split by plant type to include a leaflet of each variety. Numbers of eggs laid on the adaxial and abaxial surface of each leaflet were subsequently recorded daily for four days.

*Experiment 4: Free-choice Oviposition by Ex-tomato Adults*.

This experiment used sequentially sown plants grown under natural lighting in a glasshouse. When plants reached the ages of 9, 12 and 15 weeks, the terminal leaflet and at least 20 mm of petiolate was removed from one young, fully expanded leaf for four plants of each age and variety. Each leaflet was placed in an individual 22 ml glass vial containing tap water. Vials were attached with double-sided tape to the base of a 180 mm-deep plastic tray measuring 480 mm by 300 mm. Experimental design was a randomised split-plot with four replicates. Plots consisting leaflets from plants of a given age with each split by plant type to include a leaflet of each variety. Six male and six female, unmated adult ex-tomato P. operculella that had eclosed less than 48 hours earlier were added to the tray and the opening covered with a nylon mesh held in place with an elastic band.

*Data Analysis*

Count data for each of the four trichome types were analysed with Gens tat 5 software (Gens tat 5 Committee, 1993) using analysis of variance to test for the effects of leaflet surface, plant type, plant age and daylength regime. The block structure was a split-split-plot with plant age/daylength as main plot, plant type as sub-plot and leaflet surface as sub-sub-plot. Inspection of residuals indicated no need for data to be transformed.

Host-plant resistance data from all four experiments were subject to analysis of variance and square root transformation was used where inspection of plots of residuals indicated this to be necessary. Data from experiment two for numbers of larvae living ten days after inoculation were analysed using stepwise multiple regression.

**Results**

*Pubescence*

*Type I trichomes*

Both the adaxial and abaxial surfaces of LA 1777 A and BTN 979 bore type I trichomes but these were rare on the adaxial surface of N 91-1-1-1-1 and absent from the abaxial surface (Fig. 1). On the former varieties, type I trichomes were more dense on the adaxial surface than abaxial. There was a significant interaction between varieties and leaflet surfaces ($P$ = 0.004). The interaction between daylength and surface was on the threshold of significance ($P$ = 0.05) with density on the abaxial surface tending to be greater for plants grown under 16-hour days (0.33 tricomes/mm$^2$) than those with 12-hour days (0.11 tricomes/mm$^2$). On the adaxial surface equivalent values were 0.60 and 0.70 tricomes/mm$^2$ respectively.

*Type IV trichomes*

Thought present on N 91-1-1-1-1, type IV trichomes were far less dense than on other varieties (Fig. 2). On BTN 979 and LA 1777 A type IV trichomes were more dense on the abaxial surface than adaxial. There was a significant interaction between varieties and surfaces ($P$ < 0.001). There was also a significant ($P$ = 0.008) overall effect of plant age on

type IV trichome density such that young plants were less pubescent than older ones, an effect particularly strong for the abaxial leaflet surface (Fig. 3).

*Type VI trichomes*

Varieties differed significantly ($P = 0.007$) in density of type VI trichomes with N 91-1-1-1-1 being markedly less pubescent (0.71 trichomes/mm$^2$) than was BTN 979 (1.86 trichomes/mm$^2$) or LA 1777 A (2.39 trichomes/mm$^2$). There was also a significant interaction between plant age and leaflet surface ($P = 0.025$) such that 6-wk old plants had less dense trichomes than did older plants (Fig. 4). 9-wk old plants age tended to have more dense type VI trichomes on the adaxial surface whilst this trend was reversed for plants of other ages. Plants of 12-wk age tended to be less pubescent than 9-wk old plants, an effect particularly marked for the adaxial leaflet surface.

*Type VII Trichomes*

All three varieties bore low densities of this small trichome. There was a significant overall effect of age ($P = 0.038$) such that 6-wk old plants were less pubescent (0.26 trichomes/mm$^2$) than were 9-wk old plants (0.81 trichomes/mm$^2$) or 12-wk old plants (0.59 trichomes/mm$^2$). There was also a significant interaction between variety, day length and leaflet surface ($P = 0.035$). On the abaxial leaflet surface type VII trichome density was greatest in LA 1777 A and lowest in N 91-1-1-1-1 with densities greater for plants grown under 12-hour days (Fig. 5 A). This effect of daylength was also strongly apparent for the adaxial surface of N 91-1-1-1-1 but was reversed for the other two varieties (Fig. 5 B).

*Host-Plant Resistance*

*Casual observations and photo-microscopy*

Neonate *P. operculella* placed on leaflets of N 91-1-1-1-1 moved readily over the substrate and rapidly commenced feeding. Most were within the foliar tissue, usually close to the midrib, within one hour of placement. Larvae placed on LA 177 A or BTN 979 moved less rapidly over the substrate. Such was the density of type IV and VI trichomes on these leaflets, neonates were largely held above, and unable to contact, the laminar surface. When

neonates first ruptured the glandular heads of these trichomes, they immediately responded by vigorous 'head wagging'. Rupturing of a trichome did not consistently lead to entrapment of the larva and, after several hours of exposure, leaflets bearing <8 neonates had ruptured trichomes widely scattered over their surface. Frequently, however, rupturing of one trichome led to the larva rupturing further nearby trichomes in its struggle to escape the exudates. This led to it becoming entrapped by the exudates at a number of points of contact (Fig. 6).

*Experiment 1: survival of ex-potato neonates*

Of the ex-potato neonates placed on the susceptible control variety, N 91-1-1-1-1, 43.3 % were live 18 hours after placement but very few of these had caused conspicuous feeding damage or established mines within the leaflet. Few of the larvae on this variety (5.6 %) had died, but a large proportion (51.1 %) was no longer on the inoculated leaflet . For varieties LA 1777 A and BTN 979 the majority (82.5 and 74.7 %, respectively) were dead on the leaflet surface. The proportions of live, and dead neonates differed between varieties ($P <$ 0.001) but there was no significant effect of plant age or pre-exposure photoperiod.

*Experiment 2: survival and development of ex-tomato insects*

Of the neonates placed on the susceptible control variety, N 91-1-1-1-1, 93.3 % were live 18 hours after placement and all of these had established mines. Very small proportions were dead on the leaflet surface or unaccounted for. In contrast, less than half of the neonates inoculated onto varieties LA 1777 A or BTN 979 were live at this time and 53.3 and 40.0 %, respectively, were dead on the leaflet surface. The proportions of live, dead and unaccounted for neonates differed between varieties ($P < 0.001$) but there was no significant effect of plant age or pre-exposure photoperiod.

Ten days after inoculation, 66.6 % of the larvae inoculated onto N 91-1-1-1-1 were live within mines whilst significantly ($P < 0.001$) fewer had survived on LA 1777 A (16.7 %) or BTN 979 (16.7 %). Stepwise multiple regression using variety as the sole factor was significant ($P < 0.001$) accounting for 61.4 per cent of the variation in larval survival data. The addition of other factors, including trichome type, was not significant.

Significantly more of the neonates inoculated onto N 91-1-1-1-1 completed development than was the case for BTN 979 (Fig. 7). The temporal trend in cumulative adult eclosion suggested that development of insects was slower in the latter variety. No adults developed from neonates inoculated onto LA 1777 A.

Mean total and body lengths did not differ between sexes for emerging adults ($P > 0.05$) so data were pooled. Total length of the three adults that developed on variety BTN 979 was 5.47 mm, significantly ($P < 0.001$) shorter than for adults that developed on variety N 91-1-1-1-1 (7.41 mm). Body length too was significantly ($P < 0.003$) shorter in BTN 979, 3.93 mm, than in N 91-1-1-1-1, 5.53 mm.

*Experiment 3: non-choice oviposition by ex-potato adults*

Eggs were deposited on both the adaxial and abaxial leaflet surfaces of all three varieties from day two of the experiment but there was no significant preference for either leaflet surface. Numbers of eggs laid on the three varieties differed significantly ($P < 0.05$) on all three recording dates, with N 91-1-1-1-1 consistently receiving the largest numbers of eggs (Fig. 8). By the end of the 4-day observation period, more eggs had been laid on LA 1777 A than on BTN 979.

Oviposition was affected also by plant age with 10-wk old plants significantly preferred over older and younger plants on days two and three of the experiment (Fig. 9). By day four, however, numbers of eggs laid on the 13-wk old plants increased such that only the 7-wk treatment differed from the 10-wk old treatment.

*Experiment 4: free-choice oviposition by ex-tomato adults*

Eggs were deposited on both the adaxial and abaxial leaflet surfaces of all three varieties but there was no significant preference for either surface. Significantly ($P = 0.012$) more eggs were laid on leaflets of variety N 91-1-1-1-1 (5.25) than on LA 177 A (0.58). No eggs were laid on BTN 979. Leaflets from 12 wk old plants received significantly ($P = 0.044$) more eggs (4.58) than did those three weeks older or younger (0.75 and 0.50, respectively).

## Discussion

*Pubescence*

Varieties differed markedly in pubescence characteristics. The densities of glandular trichome types I, IV, VI were greater on foliage of BTN 979 and LA 1777A than on N 91-1-1-1-1. Densities of smaller type VII trichome were more variable between varieties and were affected by leaflet surface and day length. Density increased under 16-hour days for the adaxial surfaces of BTN 979 and LA 1777 A but declined under this daylength for other combinations of variety and surface. Results also suggested an effect of daylength on density of type I trichomes. For this trichome type 16-hour days increased pubescence for abaxial surfaces but there was a slight reduction observed for the adaxial surface. The lack of effect of daylength on types IV and VI trichomes contrast with the work by Snyder et al., (1998) in which short days reduced the density of type VI and increased the density of type IV trichomes. Though both studies used a 12 hr day, the present study used a contrasting treatment of 16 hr rather than the shorter 8 hr used by Snyder et al (1998). The lack of a significant difference in densities of type IV and VI trichomes between 12 and 16 hour days suggests that this character is relatively consistent over daylength ranges typical of commercial growing conditions. Snyder et al (1998) do not present data for type I and VII trichomes for which effects were evident in the present study.

Foliar pubescence was affected also by plant age. Type IV, VI and VII trichome densities were lowest on 6-week old plants but tended to be no greater on 12-week old plants than those of 9 weeks age.

Given the widely reported importance of glandular trichomes in conferring resistance to arthropods in *Lycopersicon* spp. it is likely that the differences in pubescence reported herein will lead to these varieties expressing differing levels of host-plant resistance to pests such as potato moth (*Phthorimaea operculella*, Zeller).

*Host-plant Resistance*

The most consistent effect evident in this series of experiments was for variety, with insect oviposition and subsequent development being significantly depressed on LA 177 A and BTN

979 compared with N 91-1-1-1-1. Varieties such as these have scope as potato moth-resistant cultivars because high levels of resistance are expressed in a robust fashion in relation to variations in daylength and plant age.

Daylength had no significant effect on insect performance in any experiment. This is despite the fact that density of type VII trichomes was affected by daylength. This type of trichome is, however, relatively rare on the foliage of *Lycopersicon* spp and it is generally assumed that the commoner type IV and VI trichomes are more important in conferring arthropod resistance (Snyder et al., 1998). Densities of these types of trichomes were not affected by daylength.

Plant age affected oviposition preference (but not development of *P. operculella*) with medium aged plants preferred over younger and older plants by both ex-potato and ex-tomato females. Explanations for this effect are not clear from pubescence data.

There was no significant effect of foliar surface (i.e., adaxial vs. abaxial) in either oviposition experiment. This is despite that fact that densities of type I, IV, VI and VII trichomes varied between surfaces or interacted with other factors such as plant age. It is not possible to conclude that resistance to *P. operculella* is correlated with the density of any one type of glandular trichome. The dominance of variety effect in multiple regression of larval mortality against pubescence supports this. The fact that adding trichome type as a factor to the regression model was not significant suggests that no one trichome type was responsible for larval mortality and all four types may have contributed additively and possibly in concert with other plant factors.

Overall, despite the sometimes significant effects of leaflet surface, plant age and daylength on glandular trichome density and insect performance, there was a consistent and very strong effect of variety. Glandular trichomes were denser on the foliage of LA 177 A and BTN 979 than on N 91-1-1-1-1. Oviposition onto the foliage of pubescent varieties was suppressed and in separate experiments in which consistent numbers of neonate larvae were placed onto leaflets, mortality was greatest in the two pubescent varieties. Direct observations of *P. operculella* neonate larvae on the foliage of LA 177 A and BTN 979 show that exudates from glandular trichomes (especially types IV and VI) are irritant/toxic to and entrap neonates. This is consistent with the previously reported presence of the compounds 2-tridecanone, 2-

undecanone and zingiberene in the glandular trichomes of *L. hirsutum* (Carter et al., 1989; Ventura and Vendramin, 1995), that are known to be toxic to herbivorous arthropods (Chatzivasileiadis and Sabelis, 1997). Trichome exudates were also observed to entrap neonate *P. operculella* and, thought such a phenomenon has previously been reported for larvae of *Helicoverpa zea* (Hubner) (Juvik et al., 1982), this is the first report of lethal entrapment for larvae of any gelechiid. There is clear scope for this antixenosis resistance mechanism to be used in commercial varieties and contribute to the integrated management of this pest.

**Acknowledgments**

We thank H.I. Nicol (NSW Agriculture) for analysing data; M. Kelly and Z. Hossain for help with insect material and I. Zahid, D.M.Y. Read, S.F.D. Gurr and N.M.J. Gurr for technical assistance.

**References**

Carter C D, Gianfagna T J and Sacalis J N. 1989. Sesquiterpenes in glandular trichomes of a wild tomato species and toxicity to the Colorado potato beetle. Journal of Agricultural and Food Chemistry 37:1425-1428.

Chatzivasileiadis E A, Sabelis M W. 1997. Toxicity of methyl ketones from tomato trichomes to Tetranychus urticae Koch. Experimental and Applied Acarology 21:437-484.

Duffey S S. 1986. Plant glandular trichomes: their partial role in defence against insects. In Insects and the Plant Surface, pp. 151-172. Eds B Juniper and R Southwood. London: Edward Arnold.

Eigenbrode S D, Trumble J T. 1993. Antibiosis to beet armyworm (Spodoptera exigua) in Lycopersicon accessions. HortScience 28:932-934.

Etzel LK. 1985. Phthorimaea operculella. In Handbook of insect rearing Vol. 2, pp.431-442. Eds. P Singh and RF Moore, Amsterdam: Elsevier.

Farrar R R, Kennedy G G. 1987. 2-undecanone, a constituent of the glandular trichomes of Lycopersicon hirsutum f. glabratum: effects on Heliothis zea and Manduca sexta growth and survival. Entomologia Experimentalis et Applicata 43:17-23.

Gens tat 5 Committee 1993. Gens tat 5 Release 3 Manual. Oxford: Clarendon Press.

Gentile A G, Stoner A K. 1968a. Resistance in Lycopersicon and Solanum species to the potato aphid. Journal of Economic Entomology 61:1152- 1154.

Gentile A G, Stoner A K 1968b. Resistance in Lycopersicon spp to the tobacco flea beetle. Journal of Economic Entomology, 61:1347-1349.

Gentile A G, Webb R E, Stoner A K. 1968. Resistance in Lycopersicon and Solanum species to the greenhouse whiteflies. Journal of Economic Entomology 61:1355-1357.

Juvik J A, Berlinger M J, Ben-David T, Rudich J. 1982. Resistance amongst accessions of the genera Lycopersicon and Solanum to four of the main insect pests in Israel. Phytoparasitica 10:145-156.

Lin S Y H, Trumble J T. 1986. Resistance in wild tomatoes to larvae of a specialist herbivore, Keiferia lycopersicella. Entomologia Experimentalis et Applicata 41:53-60.

Luckwill L C. 1943. The Genus Lycopersicon: historical, biological, taxonomic survey of the wild and cultivated tomatoes. Scotland: Aberdeen University Press. 44 pp.

Musetti L, Neal J J. 1997. Resistance to the pink potato aphid Macrosiphum euphorbiae in two accessions of Lycopersicon hirsutum f. glabratum. Entomologia Experimentalis et Applicata 84:137-146.

Snyder J C, Simmons A M, Thacker R R. 1998. Attractancy and ovipositional response of adult Bemisia argentifolii (Hompotera: Aleyrodidae to type IV trichomes density on leaves of Lycopersicon hirsutum grown in three day-length regimes. Journal of Entomological Science 33:270-281.

Ventura M U, Vendramim J D 1995. Toxicity to larvae of Phthorimaea operculella (Zell.) of the allelochemicals 2-tridecanone and 2-undecanone present in tomato. Scientia Agricola 52:438-461.

Ventura M U, Vendramim J D 1996. Effects of genotypes of Lycopersicon spp. with different amounts of allelochemicals on Phthorimaea operculella (Zell.) (Portuguese). Pesquisa Agropecuaria Brasileria. 31:835-842.

*Figure 1*. Densities of type I trichomes on leaflet surfaces of *Lycopersicon spp*. varieties (mean and se)

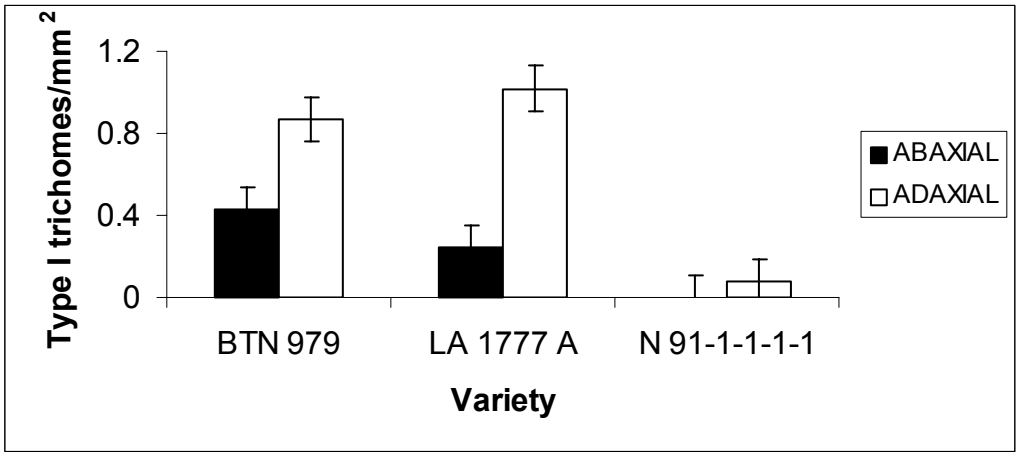


*Figure 2.* Density of type IV trichomes on leaflet surfaces of *Lycopersicon spp*. varieties (mean and se)

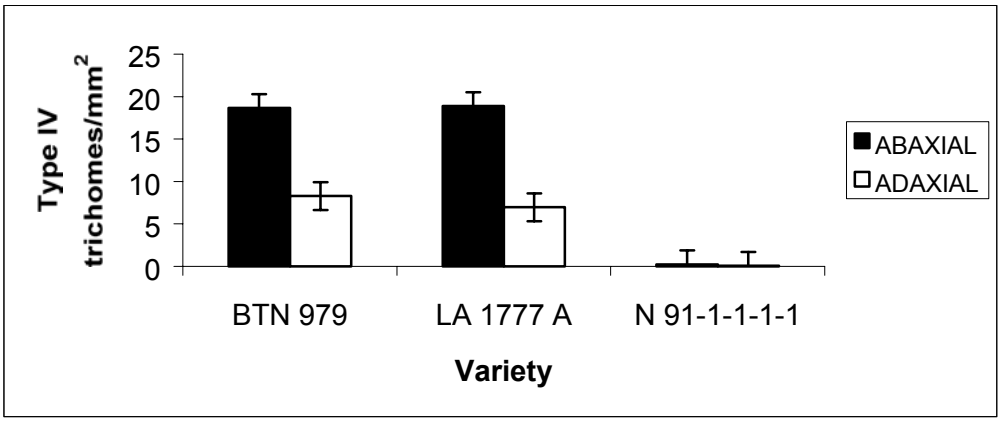


*Figure 3*. Effect of plant age on density of type IV trichomes on *Lycopersicon spp*. (mean and se)

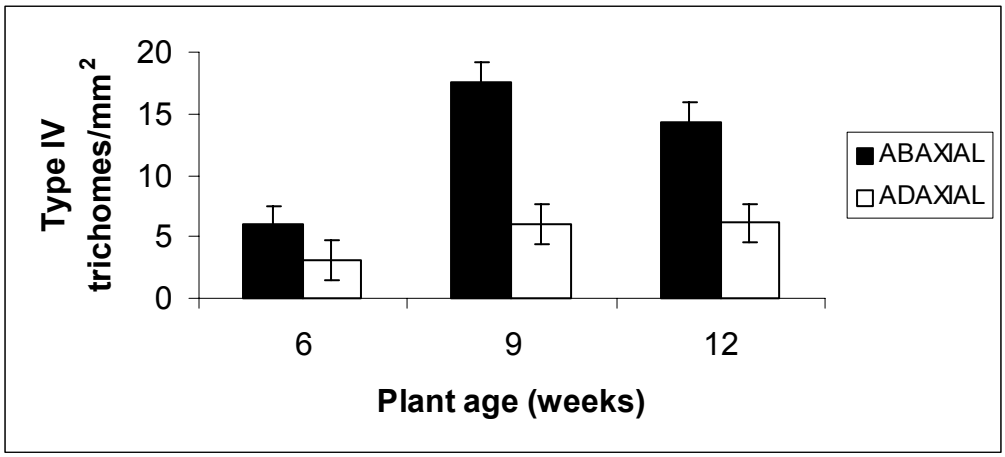


*Figure 4.* Effect of plant age on density of type VI trichomes in *Lycopersicon spp*. (mean and se)

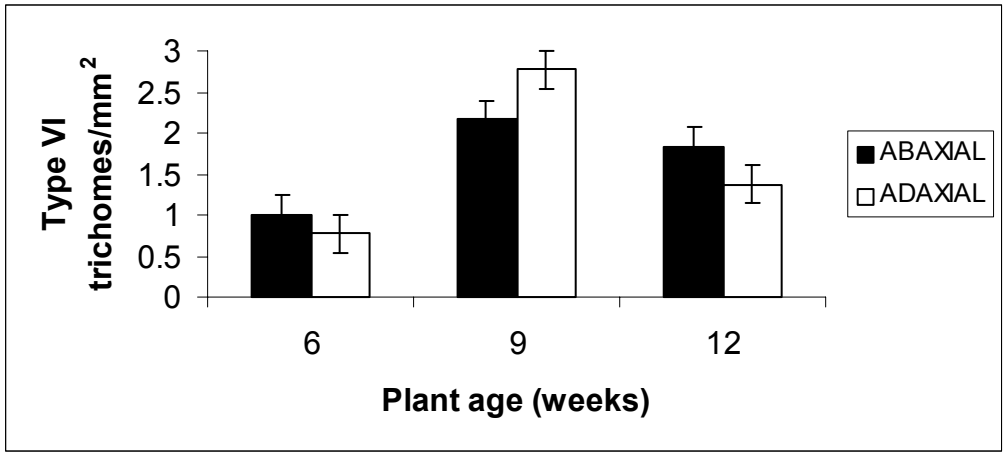
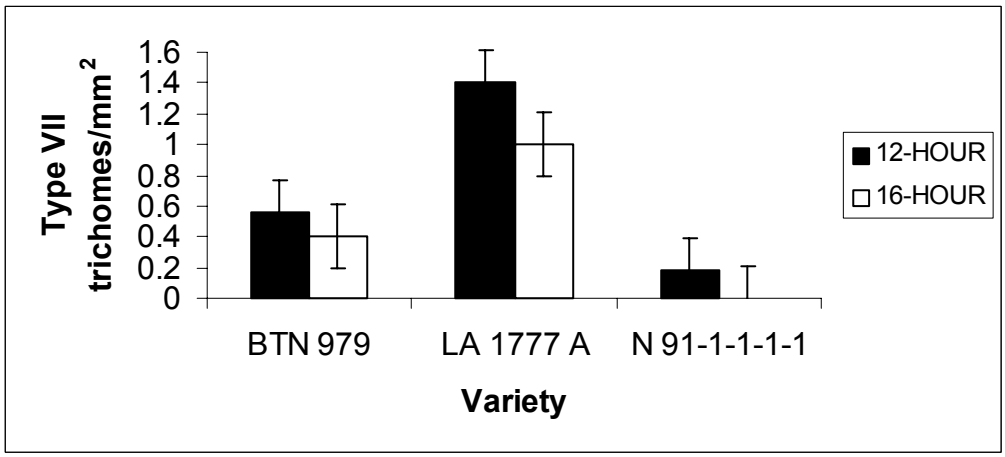


*Figure 5.* Effect of daylength on density of type VII trichomes in *Lycopersicon spp*. varieties: A, abaxial leaflet surface; B, adaxial leaflet surface (mean and se)

**A**




**B**

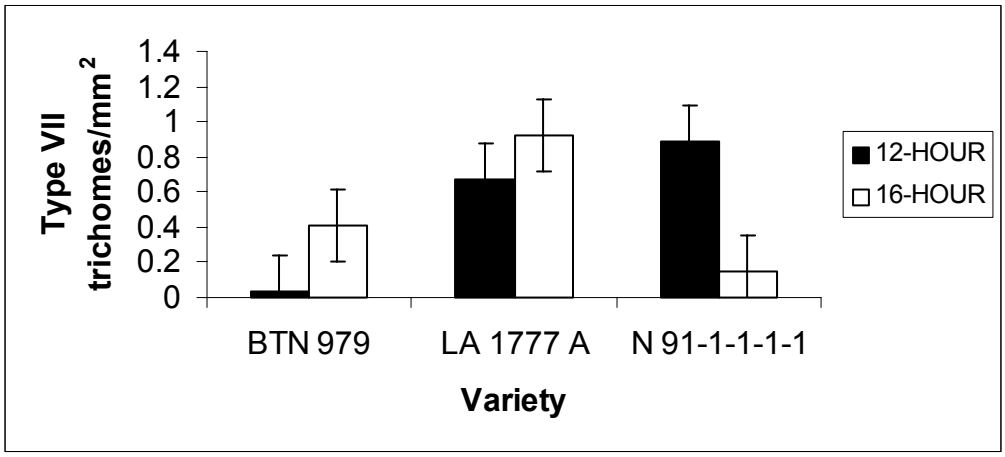


*Figure 7.* Temporal pattern of *Phthorimaea operculella* development on three varieties of tomato (mean and se) (varieties differ P = 0.07 on day 32 and at P < 0.001 on subsequent days).

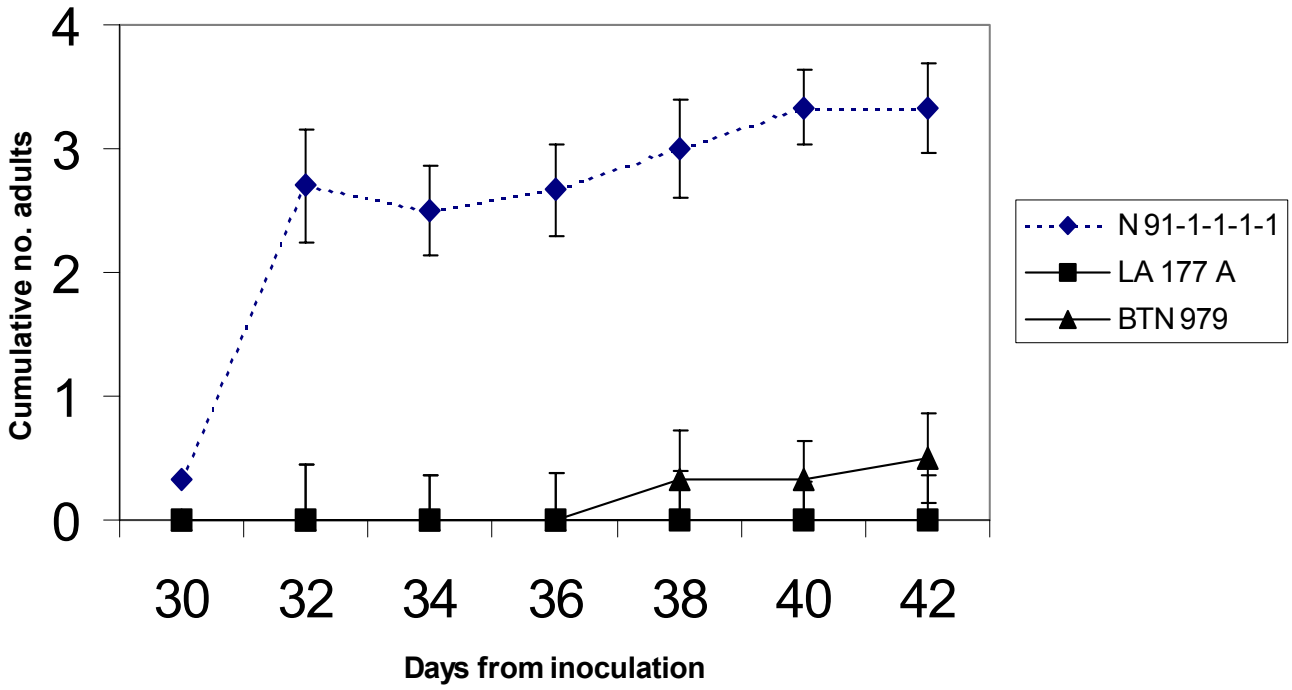


*Figure 8*. Cumulative numbers of eggs (square root transformed) laid by ex-potato *Phthorimaea operculella* on foliage of three tomato varieties under non-choice conditions (mean and se)

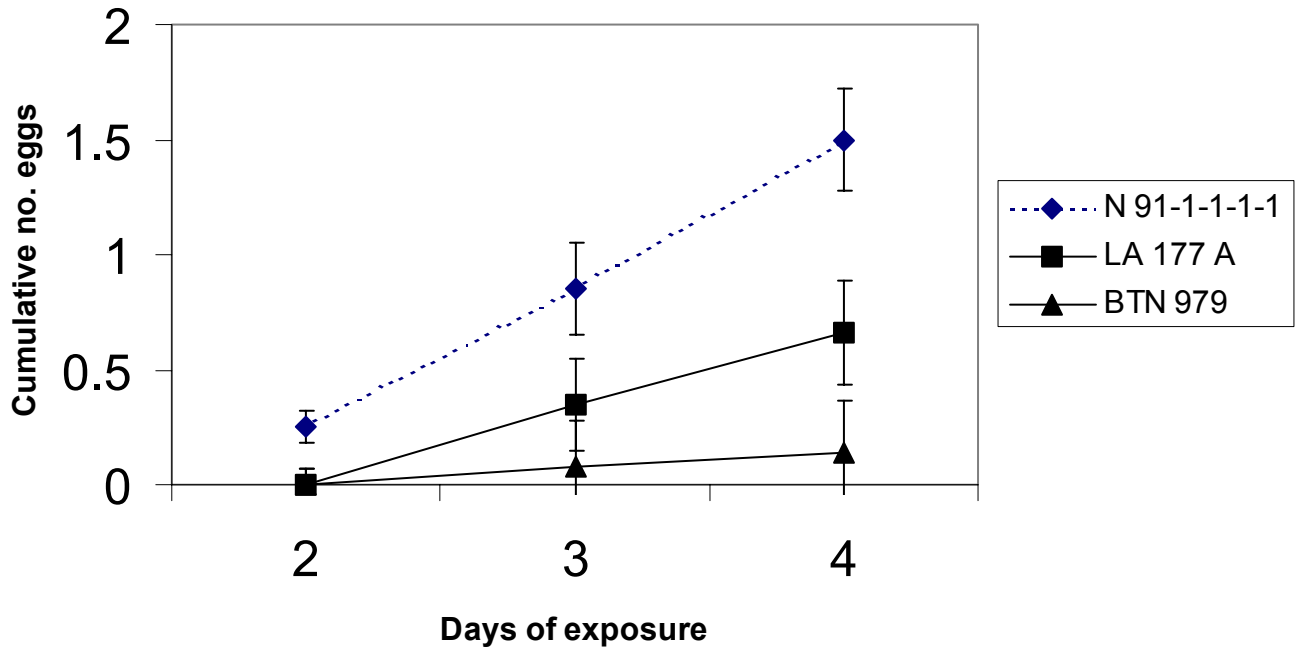
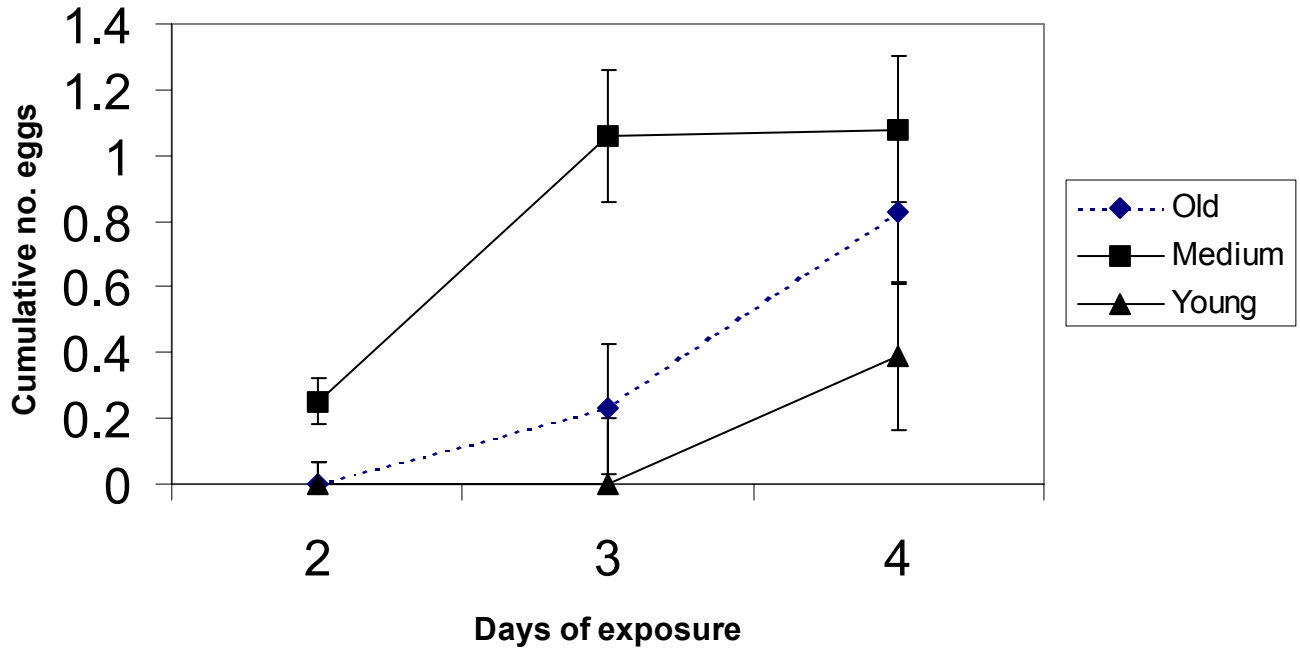


*Figure 9.* Cumulative numbers of eggs (square root transformed) laid by ex-potato *Phthorimaea operculella* on foliage of tomato plants of three ages (7, 10 and 13 weeks) under non-choice conditions (mean and se)



## 4.2 Foliar pubescence and resistance to potato moth, *Phthorimaea operculella*, in *Lycopersicon hirsutum*.

G M Gurr

**Abstract**

Pubescence characteristics for 11 accessions of *Lycopersicon hirsutum* Humb. & Bonpl. were compared with an accession of cultivated tomato (*L. esculentum* Mill.). *L. hirsutum* accessions tended to have dense glandular (type I, IV, VI and VII) trichomes but few or rare non-glandular (type V) trichomes compared with *L. esculentum.* Type IV trichomes were significantly less common on the adaxial foliar surface than the abaxial surface for most *L. hirsutum* accessions. Types I and VII trichomes were denser on the adaxial surface for small numbers of accessions. No such surface effects occurred for type VI trichomes. Removal of trichome exudates from excised leaflets using ethanol solution resulted in a reduced mortality and increased survival of neonates for the *L. hirsutum* accessions that were most lethal when not treated with ethanol solution. No such treatment effect was evident for *L. esculentum* or for the *L. hirsutum* accession with least effect on neonates when its trichomes were intact. In a glasshouse experiment with caged intact plants, mortality of neonate *Phthorimaea operculella* (Zeller) placed on the abaxial surface was greater on seven accessions of *L. hirsutum* than for *L. esculentum.* Neonates were less severely affected on the adaxial surface. Eleven days after inoculation, no live larvae were found on LA 1927, PI 127827, PI 134418 and PI 134428 and numbers on other accessions of *L. hirsutum* were lower than for *L. esculentum.* Eventual emergence of adults followed a similar trend. Multiple regression of insect data against pubescence indicated a significant positive correlation between density of type IV and VI trichomes and neonate mortality, decreased larval development and decreased adult emergence. Non-glandular type V trichomes were positively correlated with high survival of insects to 11 days and to adult. Though factors other than glandular trichomes are likely to be important, increased density of type IV and VI, along with reduced type V, are shown to be important to select in breeding for *P. operculella* resistance.

## Introduction

*Lycopersicon hirsutum* is one of the most intensively researched potential sources of resistance to pests of cultivated tomato (*L. esculentum* Mill.). Resistance has been demonstrated to at least 13 arthropod species (Gurr & McGrath, 2001) but only two studies have explored effects on the potato moth (*Phthorimaea operculella* (Zeller)). Juvik et al., (1982) reported resistance to this pest in various *Lycopersicon* accessions but did not identify the mechanism(s) of resistance. A more recent study reported irritation and entrapment of *P. operculella* neonates following rupturing of the glands of type IV and VI trichomes (Gurr & McGrath, 2001). However, an attempt to use multiple regression in that study to determine the relative impact of different types of trichomes was unsuccessful because only two *L. hirsutum* accessions were available.

Foliage of *L. hirsutum* bears type I, IV, VI and VII glandular trichomes (Luckwill, 1943). Though resistance has generally been associated with types IV and VI, a recent study of resistance to pink potato aphid (*Macrosiphum euphorbiae* (Thomas)) suggested that type I trichomes (as well as types IV and VI) may play a significant role (Musetti & Neal, 1997). A contrasting finding was forthcoming in a study with the whitefly, *Bemisia argentifolii* (Bellows & Perring) (Snyder et al., 1998). This used differing daylengths to give genetically identical plants with a range of densities of type IV and VI trichomes. Elevated levels of type IV, and depressed levels of type VI trichomes, were associated with reduced attractiveness.

The aim of the present study was to examine a larger number of *L. hirsutum* accessions than had any previously published work and thereby elucidate the extent and mechanism(s) of resistance to *P. operculella.* This involved (a) measuring the degree to which removal of trichome exudates compromised resistance and (b) testing for correlations between trichomes of different types and the operation of host plant resistance at various stages insect development.

## Materials and methods

*Plants growth.* Seed of 11 accessions of *L. hirsutum*. (Table 1) and of N 91-1-1-1-1, tomato (*L. esculentum* Mill.), were sown into moist proprietary seed raising mix (Debco 'Professional', Tyabb, Victoria, Australia) in 200 ml pots. Plants were grown in a growth

cabinet (Conviron, EF7H, Winnipeg, Canada) set with a 14-hour day and a diurnal temperature range of 20-35$^{O}$C . Relative humidity was unregulated and ranged between 40 and 100 %. Plants were watered at 2−3 day intervals and fed with soluble fertiliser (Yates 'All Purpose', Milperra, New South Wales, Australia). Seed of some accessions had poor viability and some seedlings died. Thus, 12 weeks after emergence, only 4 lines of *L. hirsutum* and N 91-1-1-1-1 were numerous enough to allow six replicates. Two accessions had three replicates, three accessions had two replicates and two accessions just one replicate.

*Pubescence assessment.* When plants reached the age of 12 weeks, pubescence of the abaxial and adaxial surfaces of the terminal pair of the third youngest leaf was assessed using the method described by Gurr & McGrath (2001). Densities were recorded for type I, IV, VI and VII glandular trichomes as well as non-glandular, type V trichomes (Luckwill, 1943).

*Insect preparation.* Experiments were conducted using *P. operculella* neonates obtained from a colony founded by individuals collected from an infested tomato crop growing near Bundaberg, Australia and maintained using methods based on Etzel (1985) as adapted by Gurr & McGrath (2001).

*Experiment 1: effect of trichome removal on neonates.* Leaflets were removed from young, fully-expanded leaves of N 91-1-1-1-1 and three accessions of *L. hirsutum* (Table 1). Half of the leaflets were randomly allocated to a treatment in which glandular trichome exudate was removed by brushing with a soft paint brush wetted with 50% ethanol in distilled water. Prior testing had suggested that 95% ethanol, as used by Dimock and Kennedy (1983), caused some phytotoxicity. Leaflets were then gently agitated in distilled water and squirted with distilled water from a wash bottle. Preliminary microscopic examination showed this removed glandular trichome exudates and did not subsequently lead to phytotoxicity. Control leaflets were treated with distilled water as described above.

Individual leaflets were then placed adaxial surface down on a filter paper, wetted with distilled water, inside Petri dishes. The tip of the petiolate was smeared with a small amount of petroleum jelly to prevent neonate entry into the leaflet via the cut surface. Five *P. operculella* neonates were then drawn randomly from a batch that has eclosed in the preceding 24 hr and placed gently on the abaxial surface of each leaflet. Lids were then added and the Petri dishes were laid out in a randomised block design with three replicates. A

further 7 blocks were similarly set up as additional, freshly-eclosed neonates became available over the next four days. Neonates were inspected and classified as 'dead' (or moribund on the laminar surface), 'live' (usually within a mine) or entrapped within the petroleum jelly 24 ± 2 hr after each block was set-up.

*Experiment 2: neonate-adult survival.* Plants were arranged on a glasshouse bench in a randomised block design with six replicates though, as outlined above, not all accessions were represented in all blocks. Individual plants stood in 9.5 L plastic buckets in which free water was continuously present for irrigation as well as to avoid insect movement into or from experimental units. A fine (neonate-proof) nylon mesh sleeve was secured with a rubber band around the base of each plant pot and drawn up a supporting bamboo cane, leaving only the top three leaves exposed. The oldest of these leaves was approximately the size as the next oldest leaf had been four days previously when pubescence densities were determined. Using a fine camel hair brush, ten neonates were then placed gently on the adaxial surface of one of the leaflets in the terminal pair of this leaf. Another 10 neonates were similarly placed on the abaxial surface of the other leaflet in the same pair. Neonates were drawn randomly from a batch that had eclosed over the preceding 24 hr. Immediately after inoculation, the mesh sleeve was drawn over the top of the plant and tied closed, taking care that the sleeve did not contact the marked leaf and dislodge neonates. The insects were left undisturbed overnight and 24 ± 2 hr later the mesh sleeve partially removed to allow the inoculated leaflets to be inspected with a magnifying glass. The number of live and dead larvae on or within the marked leaflet were counted. Death was assumed if a larva did not respond to gentle prodding with a fine camel hair brush. After this inspection, the mesh sleeve was secured over each plant. Eleven days later, sleeves were gently drawn down and the number of live *P. operculella* larvae (all within mines) recorded. Sleeves were then replaced and cages were inspected at 2-3 day intervals for presence of adults, ceasing 13 days after the last adult emerged.

*Statistical analysis*. All data were analysed with Gens tat 5 software (Gens tat 5 Committee, 1993). Inspection of trichome data residuals indicated $\sqrt{(x + 0.5)}$ transformation of the data to be appropriate. Analysis of variance was used to test for differences between accessions and between the adaxial and abaxial foliar surfaces within each accession. Insect survival and development data were subject to analysis of variance and $\sqrt{(x + 0.5)}$ transformation was used where inspection of plots of residuals indicated this to be necessary. Stepwise multiple

regression was used to investigate the relationship between neonate survival to 24 hr, numbers of mines at 11 days and total number of emerged adults (in turn) and the density of each trichome type and accession. This process involved using 5 factors (i.e., trichome types) to model insect performance. The least significant term was then deleted from the model and it was re-run. This process was continued until the most parsimonious model was derived in which all remaining factors were significant ($P < 0.05$). The percentage variance accounted for by accession as the sole factor was separately calculated.

**Results**

*Pubescence*. Type I glandular trichomes were uncommon (<1 $mm^2$) on *L. esculentum* (N 91-1-1-1-1) and significantly more dense on the adaxial surface of *L. hirsutum* accessions LA 0386, and PI 134418 (Table 1). The adaxial leaflet surface of these accessions, as well as those of PI 127826 and PI 134417, was more densely covered with trichomes of this type than were the abaxial surfaces of these accessions.

Type IV trichomes were generally the most common, though densities were affected by foliar surface and differed significantly between accessions (Table 1). With the exception of LA 2155, which had densities no greater than those observed in N 91-1-1-1-1, all *L. hirsutum* accessions bore dense type IV trichomes on their abaxial surfaces. For N 91-1-1-1-1 and 6 accessions of *L. hirsutum,* densities of type IV trichomes were lower on the adaxial surface than abaxial surface.

Densities of type VI trichomes differed significantly between accessions but were consistent between leaflet surfaces within all accessions (Table 1). Accessions LA 2155, PI 126445, PI 134417, PI 134418, PI 134428 and PI 390513 were significantly more pubescent on one or more foliar surfaces than was N 91-1-1-1-1.

*Table 1*. Foliar pubescence of *Lycopersicon esculentum* (N 91-1-1-1-1) and accessions of *L. hirsutum.*

| Accession | No. reps | Trichome density (count/mm2)[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Type I | | Type IV | | Type VI | | Type VII | |
| | | Abaxial | Adaxial | Abaxial | Adaxial | Abaxial | Adaxial | Abaxial | Adaxial |
| N 91-1-1-1-1 | 6 | 0.27 | 0.89 | 4.93 | 1.27* | 0.87 | 1.43 | 0.00 | 0.64 |
| LA 0386 | 6 | 0.38 | 2.60+* | 30.64+ | 24.50+ | 3.07 | 2.29 | 0.67 | 2.09 |
| LA 1927 | 2 | 1.49 | 1.46 | 29.97+ | 12.46+* | 3.07 | 2.00 | 3.74+ | 2.92+ |
| LA 2155 | 3 | 0.00 | 0.60 | 7.12 | 1.93 | 10.13+ | 10.39+ | 0.71 | 1.11 |
| PI 126445 | 3 | 0.27 | 1.69 | 22.93+ | 13.86+ | 5.55+ | 1.43 | 0.27 | 3.83+* |
| PI 127826 | 6 | 0.56 | 2.00* | 30.86+ | 22.64+ | 3.00 | 2.70 | 0.27 | 0.78 |
| PI 127827 | 2 | 0.44 | 2.00 | 38.94+ | 16.97+* | 3.00 | 4.47 | 3.34+ | 3.07+ |
| PI 134417 | 6 | 0.12 | 2.26* | 29.97+ | 2.36* | 6.05+ | 6.68+ | 0.54 | 0.44 |
| PI 134418 | 6 | 0.50 | 2.60+* | 35.86+ | 3.87* | 4.65+ | 7.79+ | 0.38 | 0.85 |
| PI 134428 | 1 | 0.00 | 2.00 | 72.94+ | 3.00* | 12.03+ | 15.98+ | 3.00+ | 0.99 |
| PI 251305 | 2 | 0.44 | 1.46 | 24.50+ | 6.42+* | 0.99 | 0.00 | 2.67+ | 1.49 |
| PI 390513 | 1 | 0.00 | 0.00 | 24.00+ | 21.87+ | 3.00 | 8.99+ | 0.00 | 2.00 |
| P abaxial vs adaxial | | <0.001 | | <0.001 | | ns | | 0.015 | |
| P accessions | | ns | | <0.001 | | <0.001 | | <0.001 | |
| P interaction | | ns | | <0.001 | | ns | | ns | |

[1] Means are retransformed from √(x + 0.5). Asterisk denotes significant ($P < 0.05$) difference between abaxial and adaxial means within an accession and trichome type. Plus symbol denotes significant ($P < 0.05$) difference between the value and the *L. esculentum* control value within the column.

*Table 2.* Effect of removal of glandular trichome exudates on *Phthorimaea operculella* neonate survival on *Lycopersicon esculentum* (N 91-1-1-1-1) and accessions of *L. hirsutum*.

| Accession | Number of neonates: | | | |
|---|---|---|---|---|
| | Dead | | Live | |
| | Exudates Removed[1] | Control | Exudates Removed[1] | Control |
| N 91-1-1-1-1 | 0.10a | 0.60a | 3.90a | 3.80a |
| PI 126445 | 1.30b | 1.60a | 3.30a | 3.30ab |
| PI 127826 | *1.60b | 2.50ab | *3.30a | 2.20bc |
| PI 134417 | *1.40b | 3.10b | *2.90a | 1.90c |
| *P accession* | <0.001 | | 0.021 | |
| *P treatment* | <0.001 | | 0.025 | |
| *P interaction* | ns | | ns | |

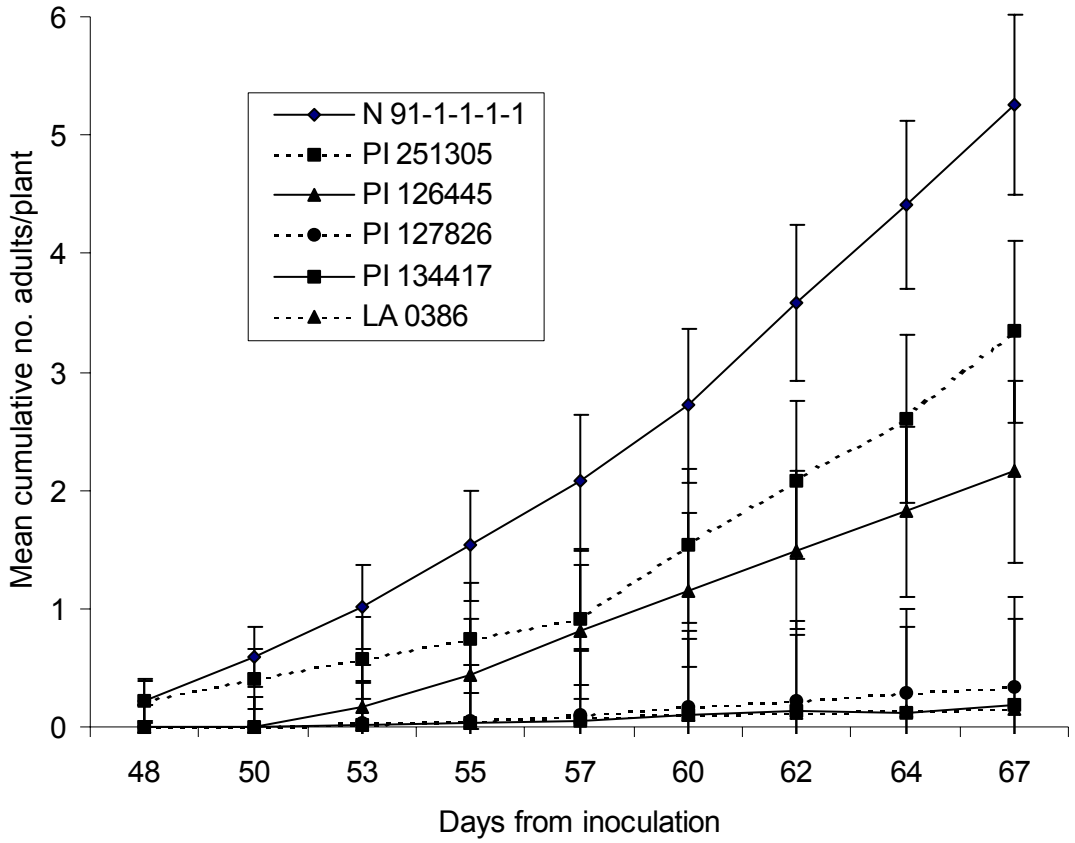
[1] Values followed by an asterisk differ significantly ($P<0.05$) from the accession's control mean. Values within a column followed by the same letter do not differ.

*Table 3.* Survival of *Phthorimaea operculella* larvae on *Lycopersicon esculentum* (N 91-1-1-1-1) and accessions of *L. hirsutum*.

| Accession | No. reps | No. Dead at 24 hrs[1] | | No. mines at 11 days[1] |
|---|---|---|---|---|
| | | Abaxial | Adaxial | |
| N 91-1-1-1-1 | 6 | 0.00 | 0.00 | 5.75 |
| LA 0386 | 6 | 0.56 | 0.22 | 0.38+ |
| LA 1927 | 2 | 0.44 | 0.00 | 0.00+ |
| LA 2155 | 3 | 2.46+ | 0.27* | 1.11+ |
| PI 126445 | 3 | 0.99 | 0.00 | 0.87+ |
| PI 127826 | 6 | 0.82+ | 0.12 | 0.44+ |
| PI 127827 | 2 | 2.29+ | 0.00* | 0.00+ |
| PI 134417 | 6 | 1.22+ | 0.27* | 0.44+ |
| PI 134418 | 6 | 3.50+ | 0.60* | 0.00+ |
| PI 134428 | 1 | 2.00+ | 3.00+ | 0.00+ |
| PI 251305 | 2 | 0.99 | 0.00 | 2.00+ |
| PI 390513 | 1 | 3.99+ | 2.00+ | 0.99+ |
| P abaxial vs adaxial | | <0.001 | | - |
| P accessions | | <0.001 | | <0.001 |
| P interaction | | ns | | - |

[1] Means are retransformed from $\sqrt{(x + 0.5)}$. Asterisk denotes significant ($P < 0.05$) difference between abaxial and adaxial means within an accession and trichome type. Plus symbol denotes significant ($P < 0.05$) difference between than value and the *L. esculentum* control value within the column.

*Figure 1.* Temporal pattern of development of adult *Phthorimaea operculella* on *Lycopersicon esculentum* (N 91-1-1-1-1) and accessions of *L. hirsutum* inoculated with neonates.




Type VII trichomes were relatively uncommon, especially on N 91-1-1-1-1 (Table 1). Accessions LA 1927, PI 126445, PI 127827, PI 134428 and PI 251305 were more pubescent on one or both foliar surfaces than was N 91-1-1-1-1. PI 126445 bore significantly more type VII trichomes on its adaxial than the abaxial leaflet surface.

Non-glandular (type V) trichomes were present on the abaxial and adaxial leaflet surfaces of N 91-1-1-1-1 (0.48 and 1.14/mm$^2$, respectively). They were significantly less dense on the adaxial surface of LA 2155 and PI 127826 (0.27 and 0.22 /mm$^2$, respectively) and absent from abaxial surfaces of these accessions. Non-glandular trichomes were not recorded from either leaflet surface of other accessions of *L. hirsutum.*

*Experiment 1*. Few neonates died on leaflets of N 91-1-1-1-1 irrespective of whether trichome exudates were removed or not (Table 2). Compared with N 91-1-1-1-1, significantly more neonates died on leaflets with exudates intact, of the pubescent accessions, PI 126445, PI 127826 and PI 134417. For the latter two accessions, removal of exudates significantly reduced mortality. Small numbers of neonates were entrapped in the petroleum jelly or left the leaflets so numbers of live neonates was not simply an inverse of the number that died. Removal of exudates had a significant effect on numbers of live neonates only for the two most pubescent accessions on which survival was otherwise significantly lower than on N 91-1-1-1-1 (Table 2).

*Experiment 2*. No neonates died on either the abaxial or the adaxial surfaces of N 91-1-1-1-1 (Table 3). Dead larvae were recorded from one or both surfaces of all *L. hirsutum* accessions. In all accessions for which there was a significant effect of foliar surface - LA 2155, PI 127827, PI 13447 and PI 134418 - mortality was greatest on the abaxial surface.

Numbers of larvae alive within mines 11 days after inoculation differed significantly between accessions (Table 3). N 91-1-1-1-1 bore an average of 5.75 larvae whilst significantly fewer were recorded on for all *L. hirsutum* accessions. No live larvae were recorded on LA 1927, PI 127827, PI 134418 and PI 134428 and no adults were subsequently recorded developing from these accessions. Total emergence of adults from N 91-1-1-1-1 was significantly greater than for all *L. hirsutum* accessions from which adults developed (Figure 1). More adults developed on PI 251305 and PI 126445 than from other *L. hirsutum* accessions.

The regression model that best fitted data on neonate mortality at 24 hr was: Y = - 0.203 + 0.0363 type IV + 0.0924 type VI. Within this, all terms were significant and the model accounted for 21% of variance. Accession as a sole factor accounted for 17.1% of variance in mortality data. The regression model that best fitted data on numbers of mines 11 days after inoculation was: Y = 2.923 - 0.0839 type IV - 0.2113 type VI + 1.217 non-glandular. Within this, all terms were significant and the model accounted for 43% of variance. Accession as a sole factor accounted for 79.4% of variance in mine count data. The regression model that best fitted data on total adult emergence was:

Y = 2.569 - 0.0766 type IV - 0.1942 type VI + 0.898 non-glandular. Within this, all terms were significant and the model accounted for 38.8% of variance. Accession as a sole factor accounted for 75.6% of variance in adult emergence data.

**Discussion**

Removal of exudates from *L. hirsutum* accessions resulted in numbers of live larvae on leaflets at 24 hr that were no lower than observed for *L. esculentum.* This suggests an important role of trichome exudates in *P. operculella* resistance of *L. hirsutum* as has recently been shown in *Solanum bethaultii* (Malakar & Tingey, 1999). However, larval mortality, though reduced by removal of exudates from *L. hirsutum* accessions, remained significantly higher than observed for *L. esculentum.* A similar incomplete negation of resistance in *L. hirsutum* by exudate removal has been reported for *Heliothis zea* (Dimock & Kennedy, 1983) and *M. euphorbiae* (Musetti & Neal, 1997). Thus, in *P. operculella* and other insect pests, factors other than glandular trichome exudates play a significant role in resistance.

The above conclusion is supported by the multiple regression models of aspects of *P. operculella* performance analysed against the pubescence data. The most parsimonious models for neonate and subsequent larval development as well as numbers of emerging adults, though containing only statistically significant terms, accounted for no more than 43% of variance.

Though this is one of the most extensive published studies of resistance in *L. hirsutum*, data exist for some of the accessions studied herein. PI 134417 was studied by Musetti and Neal (1997) who reported densities of type I, IV and VI trichomes similar to those in the present study. PI 134417 was also found to be the most resistant of two accessions to *M. euphorbiae.* The abaxial surface only of LA 1927 was studied by Snyder et al. (1998) who reported type IV and VI densities similar to those recorded in the present study. This accession was amongst the most resistant of six accessions to *B. argentifolii.* Both these accessions exhibited significant levels of resistance to *P. operculella* and this broad agreement indicates that trichome-based resistance in *L. esculentum* may be effective for a wide range of arthropod species.

Notwithstanding the likely role of other, poorly understood factors, knowledge of the important role of trichomes offers scope to develop host plant resistance to *P. operculella* in cultivated tomatoes. Early work by Juvik et al. (1982) indicated that levels of resistance to this pest in *Lycopersicon* spp. observed in the laboratory were evident also in the field. Transfer of genes for expression of trichome characters from *L. hirsutum* to hybrids with *L. esculentum* has also been shown possible (Snyder & Carter, 1985). An important caveat is that any such breeding program will need to be based on an understanding the precise role of each type of trichome in order that optimal parents are used and subsequent selection procedures are efficient.

Simple comparison of the reduced number of larvae in mines of *L. hirsutum* with trichome densities suggests the importance of type IV trichomes; for it was this type that was most consistently elevated compared with *L. esculentum*. The role of type IV trichomes is supported by the 24 hr survival data for larvae. For all four accessions in which there was a significant difference between the abaxial and adaxial surface, insect mortality was highest on the abaxial surface where type IV trichomes were denser. No effect of surface was evident for type VI trichomes and type I and VII trichomes were significantly denser only on adaxial surfaces. Multiple regressions supported the role of type IV trichomes but indicated that type VI were more important in explaining all three aspects of insect response.

These regression models therefore support the microscopic observations of *P. operculella* neonate irritation, entrapment and mortality following rupture of glands of type IV and VI trichomes on other *L. hirsutum* accessions (Gurr & McGrath, 2001). The effect of type IV and VI trichomes is associated with presence of 2-tridecanone, 2-undecanone and zingiberene in the glandular trichomes of *L. hirsutum* that are known to be toxic to herbivorous arthropods (Carter et al., 1989; Chatzivasileiadis and Sabelis, 1997; Farrar & Kennedy, 1987) including *P. operculella* (Ventura and Vendramin, 1995).

Regression models showed also that type V, non-glandular trichomes were significantly, positively correlated with increased numbers of larvae at 11 days and high total adult emergence. The positive correlation of type V trichomes with greater susceptibility to *P. operculella* is similar to the trend observed for *B. argentifolia* (Heinz and Zalom, 1995). Overall, results suggest that breeders seeking resistance to *P. operculella* can disregard type VII trichomes, that are small and difficult to count, as well as type I trichomes.

**Acknowledgments**

I thank M. Kelly for providing insect founders, H.I. Nicol for analysing data and A. Raman for reviewing a draft.

**References**

Carter, C. D., T. J. Gianfagna & J. N. Sacalis, 1989. Sesquiterpenes in glandular trichomes of a wild tomato species and toxicity to the Colorado potato beetle. Journal of Agricultural and Food Chemistry 37: 1425-1428.

Chatzivasileiadis, E. A. & M. W. Sabelis, 1997. Toxicity of methyl ketones from tomato trichomes to *Tetranychus urticae* Koch. Experimental and Applied Acarology 21: 437-484.

Dimock, M. B. & G. G. Kennedy, 1983. The role of glandular trichomes in the resistance of *Lycopersicon hirsutum* f. *glabratum* to *Heliothis zea.* Entomologia Experimentalis et Applicata 33: 263-268.

Etzel, L. K., 1985. Phthorimaea operculella. In: P. Singh and R. F. Moore (eds), Handbook of Insect Rearing. Vol. 2. Elsevier, Amsterdam, pp. 431-442

Farrar, R. R. & G. G. Kennedy, 1987. 2-undecanone, a constituent of the glandular trichomes of *Lycopersicon hirsutum* f. *glabratum*: effects on *Heliothis zea* and *Manduca sexta* growth and survival. Entomologia Experimentalis et Applicata 43: 17-23.

Gens tat 5 Committee, 1993. *Gens tat 5 Release 3 Manual*. Oxford: Clarendon Press.

Gurr, G. M. & D. McGrath, 2001. Effect of plant variety, plant age and photoperiod on glandular pubescence and host plant resistance to potato moth (*Phthorimaea operculella*) in *Lycopersicon spp.* Annals of Applied Biology 138: 221-230.

Heinz, K. M. & F. G. Zalom, 1995. Variation in trichome-based resistance to *Bemisia argentifolii* (Homoptera: Aleyrodidae). Journal of Economic Entomology 88: 1494-1502.

Juvik, J. A. M. J. Berlinger, T. Ben-David, & J. Rudich, 1982. Resistance amongst accessions of the genera *Lycopersicon* and *Solanum* to four of the main insect pests in Israel. Phytoparasitica 10: 145-156.

Luckwill L C., 1943. The Genus *Lycopersicon*: historical, biological, taxonomic survey of the wild and cultivated tomatoes. Scotland: Aberdeen University Press, 44 pp.

Malakar, R & W. M. Tingey, 2000. Glandular trichomes of *Solanum bethaultii* and its hybrids deter oviposition and impair growth of potato tuner moth. Entomologia Experimentalis et Applicata 94: 249-257.

Musetti L. & J. J. Neal, 1997. Resistance to the pink potato aphid *Macrosiphum euphorbiae* in two accessions of *Lycopersicon hirsutum* f. *glabratum.* Entomologia Experimentalis et Applicata 84: 137-146.

Snyder, J. C. & C. D. Carter, 1985. Trichomes on leaves of *Lycopersicon hirsutum, L. esculentum* and their hybrids. Euphytica 34: 53-64.

Snyder, J. C., A. M. Simmons & R. R. Thacker, 1998. Attractancy and ovipositional response of adult *Bemisia argentifolii* (Homoptera: Aleyrodidae to type IV trichomes density on leaves of *Lycopersicon hirsutum* grown in three day-length regimes. Journal of Entomological Science 33: 270-281.

Ventura M. U. & J. D. Vendramim, 1995. Toxicity to larvae of *Phthorimaea operculella* (Zell.) of the allelochemicals 2-tridecanone and 2-undecanone present in tomato. Scientia Agricola 52: 438

## 4.3 Genetic resistance to potato tuber moth in *L. hirsutum*

**Margaret Kelly and Des McGrath**

### Introduction

Resistance to potato tuber moth, *Phthorimaea operculella,* (PTM) in accession LA1777 of *L. hirsutum* was reported by Juvik et al. (1982) and confirmed in a recent study by Gurr and McGrath (2001). Several analyses of the inheritance of a major toxic compound, 2-tridecanone (2-TD), a methyl-ketone compound localised in tips of type IV and VI glandular trichomes of *L. hirsutum,* have suggested multiple gene control. Fery and Kennedy (1983) reported a minimum of three genes in PI 34417 controlling expression of 2-TD. In a cross involving PI 34417, Barbosa and Malufopod (1996) found relatively high broad sense heritability of 2-TD concentration and suggested 2-TD-based selection should be effective as an indirect selection criterion for arthropod resistance in tomato. Nienhuis et al. (1987) used molecular markers to determine loci in three linkage groups correlated with 2TD concentration in PI 134417.

The effectiveness of glandular trichomes in host plant resistance may be affected by environmental factors as well as plant attributes such as age and genotype. The influence of these non-genetic factors may reduce the heritability of host-plant resistance and therefore the effectiveness of selection in applied breeding programs. Molecular markers associated with multiple pest resistance genes are, in principle, an attractive means of increasing selection efficiency in breeding populations. The purpose of this research was to investigate the inheritance of resistance to potato tuber moth in LA1777 of *L. hirsutum* and identify the potential for selection in breeding populations using DNA markers.

### Materials and Methods

*Plant Populations*

Populations for bioassay and genetic analysis were derived from crosses of inbred parent *L. esculentum* N91 to *L. hirsutum* LA1777. Three $F_1$ hybrids were obtained by crossing N91 with three selections from LA1777 to provide $F_1$ (N91 x LA1777-1), $F_1$ (N91 x LA1777-2)

and $F_1$ (N91 x LA1777-3). Backcross generations were then generated by crossing N91 with one or more plants from each of the three F1 crosses. The F1 hybrid genotypes were designated as follows:

Hybrid 1 $F_1$ [N91 x LA1777-1] plant 1
Hybrid 2 $F_1$ [N91 x LA1777-1] plant 2
Hybrid 3 $F_1$ [N91 x LA1777-1] plant 3
Hybrid 4 $F_1$ [N91 x LA1777-2] plant 1
Hybrid 5 $F_1$ [N91 x LA1777-2] plant 2
Hybrid 6 $F_1$ [N91 x LA1777-3] plant 1

The backcrosses used in subsequent work were designated BC A (N91 x Hybrid 2) and BC B (N91 x Hybrid 4). $F_2$ generations were not obtained because $F_1$ plants failed to set seed.

*Bioassay*

An assessment of host genotype resistance was made by scoring leaflets for mine size. Seedlings of backcross populations A, B and C and control lines Floradade, N91 and LA1777-1, LA1777-2 and LA1777-3 were raised in containerised trays to provide plants for assay. Five replications of the latter lines were randomised in the trial as were three replications of rooted cuttings of hybrids 2 and 4. Only single plants of the backcrosses were available. Plants raised from seed were transferred to larger plots containing UC mix (50:50 sand:vermiculite) and marked leaves were inoculated with neonate larvae less than 24 hours old. The seedlings were raised and plants inoculated in an open plant house subject to a diurnal temperature cycle of 14 – 27 C. Scores of mine size were recorded 12 days after inoculation using the following scale: 0 = no damage, 1 = minute damage, 2 = small mine, a single line or circle with no transparency of damaged leaf, 3 = mine size up to 40 mm square with transparent leaf. First small larvae present, 4 = large mine from 40 to 100mm square. Mobile larvae in mines with significant damage, 5 = large mines 100 to 150mm square with large, active larvae, 6 = >60% leaf area mined, 7 = >90% leaf area mined. A second inoculation was undertaken at a later growth stage to provide additional data. The mean score across classes for each genotype was used as an index of resistance from which groups of lines were formed for marker analysis.

*Analysis of Segregation for Resistance*

A comparison of the distribution of resistance from the backcross generation with parent resistance scores was the only available means of evaluating segregation. The distribution of 55 lines of backcross N91 x $F_1$[N91 x LA[1777-1] and 70 lines of backcross N91 x $F_1$ [N91 x LA1777-2] were determined in relation to scores for N91 and LA1777.

*DNA Marker Analysis*

Randomly Amplified Polymorphic DNA (RAPD) analysis was used to examine the potential for identifying markers associated with resistance (Nybom, 1994). DNA was extracted according to Kang Fu et al (1993) and amplified using the protocol of Bentley and Bassam (1996). An Ericomp Delta Cycler 1 Tm system was used to amplify DNA. The resulting DNA products were separated with polyacrylamide gel electrophoresis and visualised with silver staining.

Based on bioassay results, groups of backcross lines displaying extreme susceptible and resistant phenotypes were formed to identify polymorphisms associated with resistance, using the technique of Bulk Segregant Analysis (Michelmore et al 1991). DNA pools were constructed by mixing DNA from the lines comprising each of the contrasting resistant and susceptible groups. Any polymorphisms identified between the groups were then examined for association with resistance.

**Results**

*Bioassay*

The mean mine size for LA1777 genotypes was small compared with the *L. esculentum* control, and both $F_1$ hybrids developed mines similar in size to their LA1777 parents (Table 1).

| Genotype | Index of Mine Size (0 = no damage, 7 = .90% mined leaf area) |
|---|---|
| N91 | 5.57 |
| LA1777-1 | 1.50 |
| [N91 x LA1777-1] plant 2 | 1.67 |
| [N91 x LA1777-1] plant 1 | 2.00 |

*Table 1.* Mine size of host genotypes after inoculation with neonates of potato tuber moth

The degree of resistance expressed in both backcross populations transgressed the values of their susceptible and resistant parents, ranging from 0.6 – 7.0 in N91 x Hybrid 2 and 0.2 – 5.0 in N91 x Hybrid 4 (Figure 1). In both backcrosses the frequency distribution was skewed towards resistance.

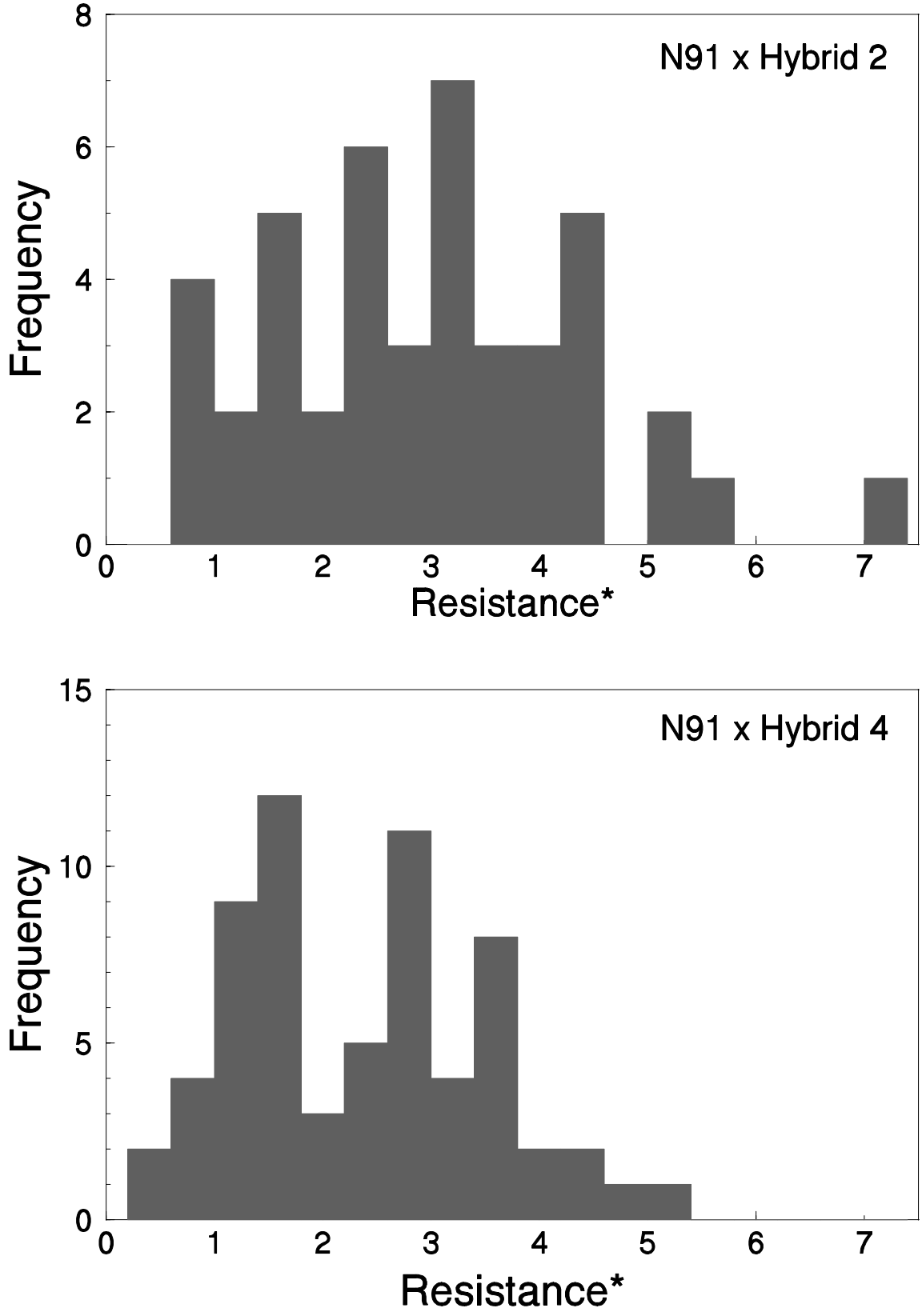
*Segregation*

Both backcrosses segregated strongly for resistance with continuous distributions skewed towards for resistance (Figure 1). Almost no backcross genotypes developed large or extensive mines typical of the susceptible parent, although mines intermediate in size were common. For both populations the most frequent class sizes were found near LA1777 and the mean of the distribution. (Figure 1).

*DNA Markers*

Resistance scores of backcross genotypes were assessed to select individuals which expressed extreme phenotypes for susceptibility and resistance. Four groups of lines, a susceptible and resistant group from each of Backcrosses A and B were formed. Bulk segregant analysis of combined DNA from these groups produced a series of differences in DNA profiles between groups. The details of primers and genotypes compared are presented in Table 2 and a gel image illustrating differences in amplified DNA products between susceptible lines 112, 113 and 135 and resistant lines 50, 99 and 100 is presented in Figure 2. Although many differences in patterns between susceptible and resistant groups were apparent, they were generally inconsistent and unreliable. Repeated attempts to reproduce particular patterns were mostly unsuccessful so that the evidence for polymorphisms was not compelling. The analysis provided no reliable polymorphisms which could be used as markers for resistance.

Figure 1. Resistance to *Phthorimaea operculella* in Two Backcross Populations




*Mean score of mine size (0 = no damaage, 7 = extensive mining)

*Figure 2.* Randomly Amplified Polymorphic DNA profile of backcross susceptible lines 112, 113, 135 and resistant lines 50, 99 and 100 using Operon primer AF20.

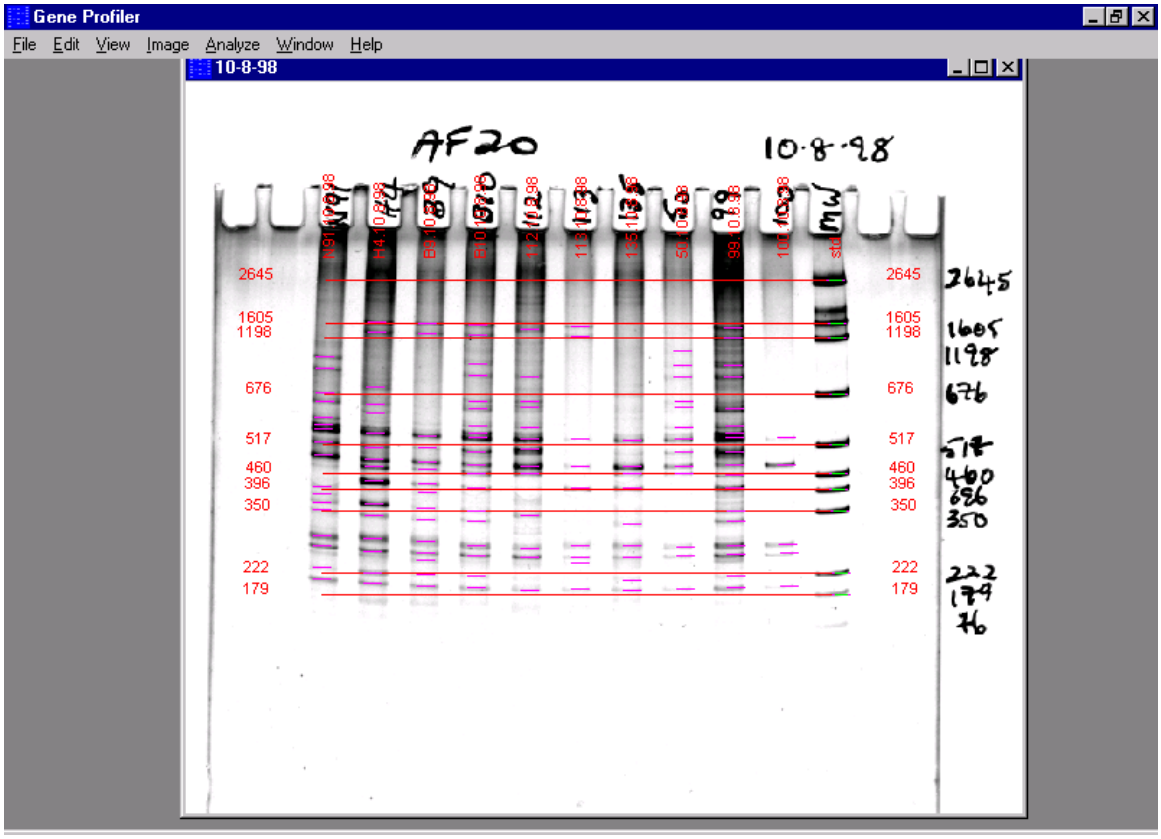


*Table2.* Primers and genotype bulks which generated differences at the fragment size indicated.

| Primer | Genotype Bulks and Fragment Size |
|---|---|
| **AM07** | Difference between line 92 (N91XH4) & bulks 3,4,5 & 6, |
| **AN16** | Bulks 5 & 6 (676bp) |
| **AI01** | Bulks 5,6 (396bp) |
| **AI13** | Bulks 3 & 4 (396bp) |
| **AI13** | Bulks 3,4,5&6(600, 500, 396, 300, bp) |
| **AI13** | Bulks 3, 4, 5&6 |
| **AI13** | Bulks 5, 6(350bp) |
| **AI13** | Bulks 9, 10(350bp) |
| **AI13** | Bulks 7, 8(396bp) |
| **AI13** | Bulks 9,10 |
| **AI13** | Bulks 7&8(396bp) |
| **AI13** | Bulks 9&10 |
| **AL13** | Bulks 5,6(396bp) |
| **AK04** | 1198bp, 800bp |
| **AM05** | Bulks 3,4(460bp) |
| **AK18** | Bulks 19,20,21,22. |
| **AL16** | Bulks 3,4. (676bp) |
| **AL16** | Bulks 3,4. |
| **AL16** | Bulks 19,20,21,22. |
| **AK11** | Bulks 3,4 (530bp) |
| **AL14** | Bulks 3,4. (676bp) |
| **AL14** | Bulks 3,4,5& 6(350bp) |
| **AK16** | Bulks 5,6 (517bp) |
| **AH14** | Bulks 3,4.(260bp) |
| **T12** | Bulks 3,4 (800bp) |
| **AD02** | Bulks 5,6. (800bp) |
| **AN04** | Bulks 23,24. (676 bp) |
| **AN04** | Bulks 19, 20, 21, 22, 23, 24. |
| **AK06(1)** | Bulks 4, 5 (460bp) |
| **AD12** | Bulks 3, 4, 5,& 6 (350bp). |
| **AF17** | Bulks 3, 4 (517bp) |
| **AF20** | Bulks 3,4,5,&6. |
| **AF20(2)** | Bulks3, 4, 5,&6. (150 bp). |
| **AF20** | Bulks 23, 24(@150bp) |
| **AF20** | Bulks 19, 20(@150bp) |
| **AF01** | Bulks 3, 4 (300bp) |
| **AC13** | Bulks5, 6 (500 bp). |
| **AC13** | Bulks 19&20 (460 bp). |
| **AC13** | Bulks 23&24 (460 bp) |
| **AC14** | Bulks 3,4,5&6 (460 bp). |
| **AC16** | Bulks 3,4, 5&6 (500bp) |
| **T17** | Bulks 23, 24 (600 bp) |
| **AI01** | Bulks 23, 24 (600 bp) |

**Discussion**

There was clear evidence from the bioassay here and the work of 4.1 above that LA1777 was highly resistant to potato moth. The analysis of inheritance and gene action for resistance was restricted by varying degrees of difficulty in obtaining hybrid or selfed progenies from the interspecific cross of *L. esculentum* and *L. hirsutum*. Because of genetic incompatibility the $F_2$ generation and selfed progenies of the first backcross could not be used, leaving only the first backcross of $F_1$ to *L. esculentum* available for a study of segregation.

Nonetheless, the continuous distribution of resistance in the available backcross populations suggested several genes determined its inheritance, and the strong expression of resistance in $F_1$ hybrids indicated completely dominant gene action. There was also evidence in the backcrosses of distorted distribution towards resistance, suggesting more complex forms of gene action. These results supported previous studies which indicated multigenic control for toxic compounds implicated in resistance.

The major difficulty in advancing progenies for applied breeding was widespread incompatibility in early backcross generations. This caused sterility in all resistant first backcross genotypes and prevented further development of the material. Many of the susceptible genotypes were quite prolific so there may have been adverse linkages with genes for resistance.

The bioassay was conducted under ambient temperatures in a plant house and was difficult to conduct. The mobility of insect larvae and the lack of replication for plants of backcross genotypes made data collection difficult. However the relative performance of the genotypes was consistent with repeated assays so the data obtained was considered reliable.

The difficulty in identifying reproducible polymorphisms in the interspecific cross may have had several causes. It was evident from the pattern of segregation of the backcross and the published literature that resistance in tomato to insect pests is likely to be determined by several genes, leading to inherently complex segregation. The work reported in section 4.2 identified two classes of trichomes as significant factors in resistance but these accounted for only 21% of total variance for resistance. Including a non-glandular term in the regression increased this statistic to 43%, suggesting there were other poorly understood processes in

resistance. Multigenic inheritance was a major difficulty in identifying useful polymorphisms. If there were several genes making cumulative additive contributions to resistance and their heritability was reduced by misclassification then the effectiveness of bulk segregant analysis would also be reduced. Because the technique relies on an accurate classification of genotypes, a larger population of lines would also be required to identify the most resistant and susceptible segregants if several genes were contributing.

The use of RAPD analysis was also potentially unreliable. Although it has been popular because of its ease and simplicity this class of polymorphisms has often been difficult to reproduce consistently. The data can therefore be difficult to interpret.

The work reported in section 4.2 demonstrated high levels of resistance to potato moth in a wider range of accessions of *L. hirsutum.* The regression models of potato moth performance against trichome density data indicated a large but not exclusive role for particular classes of trichomes in conferring resistance in this species. Snyder and Carter (1985) reported the transfer of greater trichome densities from *L. hirsutum* to *L. esculentum*, indicating a mechanism by which breeders could enhance levels of insect resistance in the cultivated species. Type IV and VI trichomes are key determinants of resistance to potato moth in *L. hirsutum* and were associated with higher levels of larval mortality, reduced development and mining activity. The heritability of trichome densities should be greater than an index score for resistance so its use in applied breeding as a correlated selection criterion for resistance could lead to gains in efficiency. The use of LA1777 for resistance was not possible because of sterility in early breeding generations but it may be possible to select equally resistant lines of *L. hirsutum* from those screened in section 4.2 and proceed without the problems encountered with LA1777. Identification of individual lines with higher trichome densities would then offer a more efficient means of developing resistant breeding lines.

The evidence of trichome-based resistance to a wider range of insect pests is well-established from the work reported in the previous section and the published literature. In particular, LA1927 of *L. hirsutum* was found to have significant resistance to *B. argentifolii*, silverleaf whitefly, now a serious pest of tomato in Queensland. There are no doubt other accessions of the same species with similar or better resistance which could serve as a basis for breeding. Appropriate use of trichomes as a source of resistance to potato moth may also confer a degree of resistance to silverleaf whitefly in cultivated tomato.

There has been little success in breeding insect resistance to commercial cultivars of tomato despite the prevalence of resistance in many accessions of uncultivated species. There are a number of reasons but the serious technical difficulties are genetic compatibility, poor heritability and the problems of applying an appropriate selection criterion for resistance. In most cases breeding programs for resistance to tomato pathogens rely on strong resistance genes and simple selection regimes whereas this is not the case for insect resistance. This research has demonstrated a major advance in understanding the specific classes of trichomes effective in conferring resistance; the use of densities of type IV and VI trichomes in identifying appropriate sources of resistance selection, although time-consuming, is likely to provide more accurate determination of resistant genotypes. Trichome density should be a key selection attribute when attempting transfer of resistance to *L. esculentum.* Although this research considered genetic resistance to potato moth in particular it provides a broader basis for successful applications in breeding resistance to other insects such as silverleaf whitefly.

**References**


Bentley, S. & B. Bassam,, 1996. A Robust DNA Amplification System Applied to Analysis of Genetic Variation Within *Fusarium oxysporum* f. sp. *cubense*. J. Phytopathology 144, pp 207-213.

Fery, R. L. & G. G. Kennedy, 1983. Inheritance of a factor in *Lycopersicon hirsutum f. glabratum* conditioning resistance to the tobacco hornworm (*Manduca Sexta)*. HortScience 18:169

Gurr, G. M. & D. McGrath, 2001. Effect of plant variety, plant age and photoperiod on glandular pubescence and host plant resistance to potato moth (*Phthorimaea operculella*) in *Lycopersicon spp.* Annals of Applied Biology 138: 221-230.

Juvik, J. A., M. J. Berlinger, T. Ben-David, & J. Rudich, 1982. Resistance amongst accessions of the genera *Lycopersicon* and *Solanum* to four of the main insect pests in Israel. Phytoparasitica 10: 145-156.

Michelmore, R.W., I. Paran & R. V. Kesseli, 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. USA. Vol. 88, pp 9828-9832, November, 1991, Genetics.

Nienhuis, J., T. Helentjaris, M. Slocum, B. Ruggero & A. Schaefer, 1987. Restriction Fragment Length Polymorphism Analysis of Loci associated with Insect Resistance in Tomato. Crop Sci. 27:797-803.

Nybom, H., 1994. DNA fingerprinting – A useful tool in fruit breeding. Euphytica 77: 59-64.

Snyder, J. C. & C. D. Carter, 1985. Trichomes on leaves of *Lycopersicon hirsutum, L. esculentum* and their hybrids. Euphytica 34: 53-64.

## 5. Technology Transfer

A large number of breeding lines and potential hybrids have resulted from substantial investment in improvement in indeterminate tomato breeding during the last four years. All parties in the project have a strong interest in commercialisation to ensure the material becomes available to Australian industry.

During the life of the project, breeding material has been developed in collaboration with significant industry personnel, particularly producers and seed industry representatives. Accordingly, project staff are confident of the merit of the best lines and hybrids and this material is now in the process of commercialisation.

A tender will be offered to established seed companies by September 2003 with the intention of releasing a defined set of inbred lines to be used in specific hybrids and in the breeding programs of the licence holder. Discussion and information about the material has occurred to make the commercialisation process as straight forward as possible. It is proposed that at least two of the hybrids developed in this project will be released from the lines available now. Seed production and marketing of the new releases will be undertaken with the expertise of the commercial partner and royalties will be distributed pro-rata among the equity partners. A further program of collaborative parent line development will be a condition of the release agreement with the commercial partner.

Future work will be conducted jointly, developing new combinations of disease resistance and fruit quality factors in the successful lines developed so far. It is expected that collaboration will continue with the partner in future years.

# 6. Recommendations

The following conclusions and recommendations are presented:

1. A series of indeterminate parent lines and at least six hybrids have been identified as significant improvements in terms of agronomic performance and fruit quality. A release of this material to a commercial alliance partner is recommended.

2. The terms of the release should specify further research collaboration involving the commercial partner. This would involve joint research and development of the indeterminate material by project members and partner. The focus of further work should be to build on improved agronomic and disease performance by developing fruit quality and colour using the crimson and high pigment genes.

3. Continued development of determinate lines with 'gourmet' fruit attributes should be supported.

4. The routine use of DNA markers in this program has delivered major gains in efficiency for the development of disease resistance. Their further use for improvement of multiple disease resistance and fruit quality attributes is recommended.

5. Resistance to insect pests such as potato tuber moth was shown to be widespread in accessions of L. hirsutum. Although genetic incompatibility prevented the use of a highly resistant accession of this species for breeding, this study demonstrated several other accessions with useful resistance. Type IV and VI trichomes were associated strongly with resistance and should be a key selection criterion in breeding programs. This is likely to offer the best advances in breeding.