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Enhancing root and soil health in tomato and melon cropping systems

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Queensland Department of Primary Industries

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VX99043 Enhancing root and soil health in tomato and melon cropping systems

- A description of work completed up to the premature termination of funding on 12 November 2001

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Media summary

This project is/ was investigating the reasons for diminished root and soil health in tomato and melon cropping systems. The project consisted of two main research themes, including (1) the effects of organic amendments on the physical, chemical, and biological properties of the soil and the interaction with root disease organisms, and (2) assessment of a broad range of commercially available bio-additive products claimed to increase yield and improve soil health.

Field trials conducted at Bundaberg and Bowen showed that a yearly input of up to 34 tonnes of carbon per ha per year (in the form of sugar cane trash, a green manure crop, and/ or molasses), greatly augmented the soil microbial populations. However, the marketable yield of the tomato and melon crops was not increased, and in some seasons, decreased slightly. Molasses was shown to have a variable benefit in stimulating soil microbes. It was suspected that the yield depression from addition of the organic amendment treatments for some crops grown in the trials was due to a nitrogen (N) draw-down effect. The N draw-down in crops grown in soil to which organic matter is applied could be overcome by applying soluble N fertiliser through the trickle irrigation tubing in the first 2 weeks after transplanting or allowing a period of at least 13 weeks from incorporation to field planting. Studies of the effects of the organic matter treatments on tomato stem pathogens *Fusarium* spp. and *Verticillium* spp. (fungi capable of vascular invasion via the roots and in some cases may be pathogenic and cause wilt diseases depending on the species and strains involved) revealed inconclusive results.

Comprehensive testing of a broad range of commercially available bio-additive products (including E2001/ Multibacter, Trichogrow/ Trichoflow, various Nutri-Tech Solutions products, Humega/ SupaHumus, and Kelpak) showed that Kelpak was ranked in the top 2 treatments for weight of marketable fruit in 5 of the 6 trials. However, differences were only significant for the 2001 tomato trial at Bowen, and then only between Kelpak and Supa Humus. This lack of difference between treatments for the yield and quality parameters that were measured indicated that growers using these products would be unlikely to see any visible effects to the crop. Despite anecdotal evidence aplenty, the absence of comprehensive field studies on bio-additive products of the kind undertaken in this project can be explained by the quantity and meticulous nature of the work required to conduct such experiments. The fact that our team conducted the work at 2 geographically diverse sites makes the work even more extraordinary.

Technical summary

This project is/ was investigating the reasons for diminished root and soil health in tomato and melon cropping systems. The project consisted of two main research themes, including (1) the effects of organic amendments on the physical, chemical, and biological properties of the soil and the interaction with root disease organisms, and (2) assessment of a broad range of commercially available bio-additive products claimed to increase yield and improve soil health.

Organic amendment trials

Field trials were conducted at each of Bundaberg and Bowen research stations to assess the effects of organic amendments on soil and root health. The Bowen research site has a history of Fusarium Wilt Race III in tomato and the sudden wilt root complex in melon. The Bundaberg site has a history of sudden wilt. The organic amendments applied (including sugarcane trash once per year and molasses applied to each crop) provided a yearly input of 0 to 34 tonnes of carbon (C) per ha per year, depending on treatment. Because the plots were permanent, the effects of the organic amendment treatments were cumulative (albeit for only 2 years of the proposed 4-year study).

We found that whereas the soil microbial populations were augmented by the addition of organic material, crop yield did not increase, and in some seasons, decreased slightly. PL-FAME data showed that addition of molasses following the planting of the crop did not increase microbial activity above that achieved with the respective pre-plant organic matter additions, and may even have had a slight negative effect. These findings concur with those at the Bundaberg site for microbial biomass C and free living nematodes, which were unaffected by molasses application. However, for the Bowen site, the stimulatory effect of molasses on free living nematodes in both years and on microbial biomass in 2001 shows that this product can have a variable effect on soil microbial populations. Therefore, there appears to be at best a variable benefit in applying molasses to the soil during crop growth in terms of stimulating soil microbial activity. Yield depression from C addition to the soil in which the tomato and melon crops were grown was probably caused from a nitrogen (N) draw-down effect. The difficulty in managing N nutrition of the crop could be overcome by applying soluble N fertiliser through the trickle irrigation tubing in the first 2 weeks after transplanting or allowing a period of at least 13 weeks to pass from incorporation of the organic material to field planting. Studies of the effects of the organic matter treatments on tomato stem pathogens *Fusarium* spp. and *Verticillium* spp. (both these fungi are capable of vascular invasion via the roots and in some cases may be pathogenic and cause wilt diseases depending on the species and strains involved) revealed inconclusive results.

The idea of this project was that we could make a 'long-term' assessment of the effects of organic amendments on soil health. With another 2 years for the project to run, it was anticipated that there would be another tomato and melon crop grown at Bowen and another 2 melon and 2 tomato crops grown at Bundaberg.

Bio-additive field trials

A broad range of commercially available products which are claimed to increase yield and improve soil health were also being investigated. Intensive field trials were well under way at both the Bowen and Bundaberg Research Stations at the time of early termination of the project funding. The products being tested were selected by a process involving expressions of interest (Queensland Fruit and Vegetable News, 16 March 2000). Due to the large number of entries received, the final selection was based on products which were deemed not to be fertilisers, since the impact of nutrition could be easily eliminated by this decision. The products that were selected included E2001/ Multibacter, Trichogrow/ Trichoflow, various Nutri-Tech Solutions products, Humega/ SupaHumus, and Kelpak. The proposed duration of study for 4 years was to allow the full effect of the bio-additive treatments to be assessed over time and for a range of environmental conditions.

Fruit yield and quality parameters such as Brix° were assessed for crops grown at Bowen (rockmelon in 2000 and tomato in 2001) and at Bundaberg (tomato in the 2000 and 2001 autumn seasons, and rockmelon in the 2000 and 2001 spring seasons). Despite the fact that Kelpak was ranked in the top 2 treatments for weight of marketable fruit in 5 of the 6 trials, differences were only significant for the 2001 tomato trial at Bowen, and then only between Kelpak and Supa Humus. This lack of difference between treatments for the yield and quality parameters that were measured indicated that growers using these products would be unlikely to see any visible effects to the crop. Despite anecdotal evidence aplenty, the absence of comprehensive field studies on bio-additive products of the kind undertaken in this project can be explained by the quantity and meticulous nature of the work required to conduct such experiments. The fact that our team conducted the work at 2 geographically diverse sites increases the relevance of this finding.

1. Introduction

This project was developed to investigate diminished root and soil health in tomato and melon cropping systems. Two main research themes were being pursued, including (1) assessing the effects of organic amendments on the physical, chemical, and biological properties of the soil and the interaction with root disease organisms, and (2) determining the merit of using commercially available bio-additive products for crop production.

The project was originally designed to run over a relatively 'long-term' (4 year) time frame. This approach was taken to maximize the effects of the treatments on the soil environment and to understand the mechanisms for these changes. The withdrawal of financial support by QFVG after less than 2 years (on 12 November 2001), and the subsequent termination of research work shortly thereafter, severely restricted the manifestation of the full treatments effects. The cumulative effects of the various treatments, particularly in the latter stages of the project, were expected to be profound.

2. Status of the project milestones at the time of termination

At the time of early termination of the project, all due milestones were achieved on time (Table 2.1), and work was progressing in accordance with the agreed research protocols and schedule of activities.

This project was set up to run for 4 years. A relatively 'long-term' approach was taken to maximise change in the soil environment. A reduction in the duration of the project severely limited the full effect of the treatments on the soil. The idea of this project was that we could make a 'long-term' assessment of the effects of organic amendments and commercial bio-additive products on soil health. With another 2 years for the project to run, it is anticipated that there would be large changes in the soil condition as a result of the treatments imposed.

Table 2.1 Milestones for VX99043 - enhancing root and soil health in tomato and melon cropping systems
A tick has been placed next to milestones which were achieved at the time of project termination.

Milestone	Date due	Description/ criteria
1 ✓	1 Feb 2000	HRDC Research, Development and Commercialisation Agreement signed and voluntary contribution received (if relevant). Intellectual Property arrangements in place
2 ✓	31 Oct 2000	Primary research sites selected and initial treatments applied. Primary research sites selected in the Bowen, Bundaberg and Gatton districts. Appropriate treatments, including commercially-available products and organic amendments applied in replicated factorial field experiments or observation trials following physical, chemical and microbial characterisation of the soils.
3 ✓	31 Mar 2001	Do our own research (DOOR) extension strategy set in place. Collaborating growers identified and assisted to establish research sites on their farms and to apply appropriate treatments.
4 ✓	31 Oct 2001	Preliminary field trials of commercial bio-additive products completed. Representative commercial products selected and applied according to the manufacturers' specifications. Yield and quality data recorded and interpreted.
5	31 Mar 2002	Preliminary assessment completed of the effects of field treatments on soil carbon dynamics Completed an initial assessment of the effect of the organic amendments used in the field trials on the carbon fractions in the soils.
6	31 Oct 2002	Field days held which successfully disseminated to a total of 150 growers the methodology for the establishment of optimum soil health

		Background information on the project circulated, demonstration plots provided and talks given to educate growers in the best practices to adopt for the establishment of healthy soil.
7	30 Mar 2003	Temporal effects of the field trial treatments on the characteristics of the soil microbial communities measured Relationships between treatments imposed at the primary research sites and the characteristics of the microbial population (total biomass, population diversity , and metabolic potential) determined.
8	31 Dec 2003	Best strategies identified to achieve healthy soils and root growth. Correlations between incidence of soil-borne disease and physical, chemical and microbial characteristics of the soils completed. Effects of organic amendments on root health, soil pathogen suppression and fruit lycopene levels determined. Recommendation of commercially-available biological products made. A report prepared on the best strategies to improve soil health by organic amendments, including a rudimentary economic analysis.
9	31 Mar 2004	Final report received by due date and all previous milestones achieved. HAL will notify acceptance or otherwise within 1 month of receipt.

3. Organic amendment trials

3.1 Introduction

The many groups of organisms which comprise a healthy soil create an interdependency for food and energy, and function together to exchange materials vital to the maintenance of the system. Collectively, the system has become known as the soil food web. Although certain species of microorganisms are harmful to crops, most are beneficial and even essential for the well-being of plants. Their value lies in the roles they play in the decomposition of organic matter, as a living reservoir of nutrients, the active cycling of nutrients in the soil, the suppression of pathogens, and the improvement of soil structure. Every organism in the soil is the source of food for other organisms. Bacteria, fungi, and root herbivores most actively utilise plant materials as available sources of food, and they provide food for other organisms within the soil food web. A complexity of interactions and interdependencies persists throughout the web and contributes to the decomposition of organic material and the supply of mineral nitrogen to the soil solution.

Many of the methodologies used in conventional vegetable production (e.g. tillage, application of inorganic fertilisers, use of plastic mulch, fumigation) lead to the destruction of the organisms that comprise the web, and therefore, the contributions these microbes make to the health of the soil resource. The concept of healthy soil as an active biological reservoir of both beneficial and antagonistic organisms is often overlooked as a contributor to failing plant health.

The primary hypothesis of this study was that labile carbon is the primary food source required to stimulate and enhance the soil food web. Without it, biological activity in the soil wanes, providing opportunities for pathogenic species. In other words, labile carbon is the primary food source which is the driving force behind a dynamic soil food web and a healthy soil. To test the hypothesis, 2 field trials were established to examine the long-term (4-year) effects of various organic treatments on the general health of soils used for growing tomato and melon crops. This study reports the initial 2 years of work up to the premature termination of funding at the end of the spring season of 2001.

3.2 Materials and methods

Field trials were conducted on a free-draining clay loam soil at Bundaberg (Haplic Mesotrophic Red Ferrosol, Isbell 1996) and on an alluvial silty clay loam soil at Bowen (Melanic Hycocalcic Black Dermosol, Isbell 1996), Queensland. The Bowen research site has a history of Fusarium Wilt Race III in tomato and the sudden wilt root complex in melon. The Bundaberg site has a history of sudden wilt.

Both soils had been used for vegetable production for many years. Before the commencement of the trials, one soil core was taken in December 1999 to a depth of 90 cm from a central position within each of the 6 designated replicates. Each core was then divided into 6 depth intervals (viz. 0-10, 10-20, 20-30, 30-50, 50-70, or 70-90 cm). For each depth interval at each trial site, soil was bulked, dried and analysed for total carbon (C) and total nitrogen (N) (Table 3.1). Additionally, for each of the 6 designated replicates per site, surface soil (0-10 cm) was bulked from 20 randomly-selected positions in December 1999, dried, and subsequently analysed for total C and total N. Means were similar for the 2 sites, and the range of values (given in brackets after the mean) indicated a uniformity among replicates: Bowen – 1.57 (1.44-1.75) %C, 0.10 (0.09-0.11) %N; Bundaberg – 1.65 (1.61-1.67) %C, 0.16 (0.16-0.16) %N.

Table 3.1. Total C and total N concentrations at 6 depth intervals for soils designated for the organic amendment field trials at Bowen and Bundaberg.

Depth	Bundaberg		Bowen	
(cm)	Total C (%)	Total N (%)	Total C (%)	Total N (%)
0-10	1.68	0.16	1.63	0.09
10-20	1.67	0.16	1.51	0.09
20-30	1.42	0.15	1.32	0.03
30-50	0.98	0.12	0.90	0.01
50-70	0.77	0.09	0.61	<0.01
70-90	0.53	0.07	0.47	<0.01

In the initial stage of the field trials, soil samples (0-15 cm) were taken on 13 April 2000 at the Bundaberg site and on 6 July 2000 at the Bowen site, air dried, and analysed for cations. The analysis showed that an adequate availability of cations was present at the sites (Table 3.2).

Table 3.2 Cation analysis of the surface (0-15 cm) soil sampled in the initial stages of the field trials conducted at Bundaberg and Bowen.
For each site, the value is the mean of all 6 replicates for plots designated for Treatments 1, 2, 4, and 5. The range of values is presented in brackets following the mean. Soil was sampled on 6 July 2000 at Bowen and on 13 April 2000 at Bundaberg.

Cation	Unit of measurement	Bundaberg	Bowen	Desirable range[A]	Method	Reference(s)
Ca	cmol(+)/ kg	7.6 (7.1-8.5)	13.0 (10.7-15.0)	> 3.0	1:20 soil:1M NH_4Cl @ pH 7.0	Tucker (1971); Loveday *et al.* (1972)
Mg	cmol(+)/ kg	2.0 (1.8-2.2)	6.4 (5.3-7.2)	> 0.4	1:20 soil:1M NH_4Cl @ pH 7.0	Tucker (1971); Loveday *et al.* (1972)
K	cmol(+)/ kg	0.77 (0.58-1.00)	0.46 (0.34-0.59)	0.37-1.5	1:20 soil:1M NH_4Cl @ pH 7.0	Tucker (1971); Loveday *et al.* (1972)
Na	cmol(+)/ kg	0.21 (0.18-0.25)	0.67 (0.47-0.82)	< 2.0	1:20 soil:1M NH_4Cl @ pH 7.0	Tucker (1971); Loveday *et al.* (1972)
CEC[B]	cmol(+)/ kg	10.6 (9.9-11.8)	20.6 (16.9-23.3)	> 4.0	1:20 soil:1M NH_4Cl @ pH 7.0	Tucker (1971); Loveday *et al.* (1972)

[A] Incitec (1989). Soil interpretation manual. Volume II. Tomatoes and capsicums - sands and sandy loam soils for south east Queensland and northern New South Wales. Interpretation Chart No. 91.
[B] Cation exchange capacity estimated as the sum of basic cations.

The Bowen and Bundaberg trials consisted of the same 6 organic amendment treatments in a randomised block design with 6 replicates. Plots (10m x 6.75m = 67.5m^2) were delineated with permanent pegs at strategic positions around the perimeter of the trials from which string lines were run for critical methodology.

The following operations occurred at both the Bowen and Bundaberg trial sites, except where otherwise stated. During December 1999 and 2000, 42 t dry sugarcane trash (20 t C/ ha – based on C concentration of 47.4% for the dried cane trash used in December 1999) was incorporated with a rotary hoe into the top 30 cm of soil of plots designated for Treatments 4-6. A crop of forage sorghum and Lablab (sown together at 5 and 25 kg/ha, respectively) was then grown in these plots (Table 3.3). At Bundaberg, 2 x 1 m^2 quadrats per plot were taken on 21/2/00 (at the end of the first year's cover crop) and on 18/1/01 (at the end of the second year's cover crop) to estimate top dry weight (5.97 and 4.43 t/ ha, respectively). At Bowen, 2 x 1 m^2 quadrats per plot were sampled on 18/4/00 and 5/2/01 (at the end of the first and second year's cover crops, respectively) to estimate top dry weight (10.25 and 7.75 t/ha, respectively). Analysis of the dried plant material sampled on 18/1/01 from the Bundaberg cover crop revealed a C concentration of 41.1%. In January-February (Bundaberg) or March-April (Bowen), the cover crop grown in Treatments 4-6 was flail mowed. For Treatments 2, 3, 5, and 6, 21 t dry sugarcane trash (10 t C/ ha) was then laid on the surface of the soil (Table 3.3). All plots where then worked with a rotary hoe twice (in opposite directions to minimise lateral movement of soil) to a depth of 25 cm to ensure that any surface material was buried

Table 3.3 Details of the organic amendment treatments used in field trials conducted at Bowen and Bundaberg.
The cane trash and green manure treatments were applied once per year, for the spring tomato crop at Bundaberg and for the winter tomato or melon crop at Bowen. Because a tomato and a melon crop were grown each year at Bundaberg, the molasses treatment was applied twice per year at that site. The approximate amount of C from each input (value in brackets) was calculated from analyses of the C concentration of the dry cane trash (47%), the dry green manure crop (41%) and the wet molasses (30%).

Treatment number	5-14 weeks pre-plant[¶] (C input t/ha)	4 weeks pre-plant[†] (C input t/ha)	First 10 wk of crop[#] (C input t/ha)	Approximate cumulative C input (t/ ha)
1	Bare fallow (0)	Nil (0)	Nil (0)	0

2	Bare fallow (0)	Cane trash (10)	Nil (0)	10
3	Bare fallow (0)	Cane trash (10)	10 weekly applications of 500 L molasses (2)	12
4	Cane trash¶ + green manure crop (20 + 2)	Nil (0)	Nil (0)	22
5	Cane trash¶ + green manure crop (20 + 2)	Cane trash (10)	Nil (0)	32
6	Cane trash¶ + green manure crop (20 + 2)	Cane trash (10)	10 weekly applications of 500 L molasses (2)	34

¶ At approximately 14 weeks before transplanting the tomato or melon crop, one round bale of cane trash was spread over the soil surface of Treatments 4,5, and 6 and rotary hoed into the soil. One round bale of dry cane trash (285 kg @ 474 gC/ kg) per plot (10m x 6.75m) is equivalent to 20 t C/ ha. Following the incorporation of the cane trash into the soil, 5 kg/ ha Jumbo sorghum seed was mixed with 25 kg/ ha Lab lab seed and then sown.
† At approximately 4 weeks before transplanting the tomato or melon crop, the green manure crop of sorghum/ lab lab grown in treatments 4, 5, and 6 was flail mown and 7.5 square bales of cane trash were spread over the soil surface of Treatments 2, 3, 5, and 6. All plots were then ploughed. Fifteen square bales of cane trash are equivalent to one round bale of cane trash. Therefore, 7.5 square bales of cane trash per plot is equivalent to 10 t C/ ha.
# From 0 to 10 weeks after transplanting the tomato or melon crop, molasses at a rate of 500L/ ha/ week for 10 weeks (total of 5,000 L/ha) was injected through the trickle irrigation system of Treatments 3 and 6.

After final incorporation of the cane trash and/ or cover crop material, raised beds (1.5 m between centres) were formed, running in a north-south direction. Plots consisted of 5 rows of length 10 m, with the centre 3 rows designated datum rows. For adjacent plots, the adjoining guard row was common to both plots. Fertilisers were incorporated into the soil beds to supply a basal supply of nutrients in accordance with standard industry practice. Polyethylene trickle irrigation tubing (wall thickness 200 µm, internal diameter 16 mm, emitter spacing 300 mm) was laid along the centre of each bed at a depth of 3 cm below the soil surface. Soil beds were covered with 25 µm polyethylene mulch film prior to planting the tomato or melon seedlings. In Bundaberg, white plastic mulch was used for the autumn season, whereas black plastic mulch was used for the spring season. Only black plastic mulch was used in Bowen for the winter season trials conducted at that site.

Tomato or rockmelon seedlings were transplanted 50 cm apart in the centre of the raised beds, resulting in a population of 13,333 plants/ ha. In Bundaberg, addition of the sugarcane trash and/or green manure was followed by an autumn tomato crop in both 2000 and 2001 (Table 3.4). However, in Bowen, incorporation of these organic amendment treatments were followed by either a tomato crop (in 2000) or a rockmelon crop (in 2001). For rockmelon crops grown in the spring season of 2000 or 2001 in Bundaberg, no new sugarcane trash and/ or cover crop materials were applied immediately prior to planting.

Table 3.4 Details of the organic amendment trials conducted at Bowen and Bundaberg.

Site	Season	Crop	Cultivar	Date of sowing	Date of field planting	Date of first harvest	Date of final harvest	Number of harvests
Bowen	Winter	Tomato (ground crop)	Tempest	20 Jun 00	18 Jul 00	28 Sep 00	20 Oct 00	3
	Winter	Rockmelon	Southern Cross	19 Apr 01	17 May 01	30 Jul 01	10 Aug 01	5
Bundaberg	Autumn	Tomato (trellised crop)	Mercedes	18 Feb 00	29 Mar 00	28 Jun 00	28 Jul 00	4
	Spring	Rockmelon	Eldorado	20 Jul 00	25 Aug 00	6 Nov 00	14 Nov 00	3
	Autumn	Tomato (trellised crop)	Queensland Red	17 Jan 01	20 Feb 01	4 May 01	13 Jun 01	3
	Spring	Rockmelon	Eastern Star	03 Jul 01	10 Aug 01	29 Oct 01	12 Nov 01	5

After transplanting the tomato or melon seedlings, molasses was injected into the trickle irrigation system of Treatments 3 and 6 (Table 3.3) at the rate of 500 L/ ha/ week for 10 weeks (total of 5,000 L/ ha for the life of the crop). Carbon analysis of fresh molasses sampled from the Bundaberg trial source in December 1999 revealed a C concentration of 27.5%. Therefore, 5,000 L molasses/ ha contributed approximately 2 t C/ ha, given that 1L molasses weighs approximately 1.4 kg at 25°C. Because a tomato and a melon crop were grown each year at Bundaberg, the molasses treatment was applied twice per year to plots designated for Treatments 3 and 6 at that site.

Soluble nutrients were administered through the trickle irrigation tubing (fertigation) in accordance with standard industry practice and the results of sap nitrate testing which was conducted on an *ad hoc* basis. Muriate fertilisers (viz. those

containing chloride) were not used in basal or fertigation applications. This approach was taken since some proponents of alternative farming systems suggest that chloride can adversely affect the potential benefit of bio-additive products. Additionally, herbicides were not applied within the trial sites for the same reason. Integrated pest management was employed during the cropping cycle, a practice which is common for the local industries in Bowen and Bundaberg.

The nitrate concentrations of sap expressed from the petiole of the youngest mature leaves from rockmelon plants grown in the outer 2 datum rows of each plot were measured with Merckoquant test strips read by a Nitracheck nitrate meter on 7 and 19 September 2001 at Bundaberg. These sample dates corresponded with early and late fruit set, respectively. Otherwise, sap nitrate testing was used throughout the trials on an informal basis to ensure nutrient applications were adequate for optimal growth.

Soon after transplanting the tomato seedlings at Bundaberg, a representative sample of soil was collected (6/4/00 or 16/3/01) at a depth of 0-15 cm from the outer 2 datum rows of each plot. These soil samples were used for the assessment of nematodes. Near the end of the harvest period of the tomato crops grown at Bundaberg (19/7/00 or 1/5/01) or the tomato (26/9/00) or melon (30/7/01) crops grown at Bowen, a representative sample of soil (0-15 cm) was again collected from the same outer 2 datum rows of each plot. These soil samples were used for the assessment of nematodes, microbial biomass, labile C, and composition of the soil microbial community.

Five stem sections were also taken between the cotyledon node and the third internode at the latter sampling time (19/7/00 or 1/5/01 for Bundaberg, or 26/9/00 for Bowen) from randomly-selected tomato plants grown in the outer datum rows for assessment of stem pathogens. Complete stem sections between the first and second nodes of the main stems of the tomato plants were removed after the final fruit harvests at both trial sites. After suitable surface sterilisation and desiccation, the sections were incubated to determine the extent of any invasion of the vascular material by the fungi *Fusarium* spp. and *Verticillium* spp. Both these fungi are capable of vascular invasion via the roots and in some cases may be pathogenic and cause wilt diseases depending on the species and strains involved. Fusarium wilt can be a major disease in both regions while the symptoms of Verticillium wilt are generally restricted to winter growth. Microscopic examination of the vascular bundles at the cut ends of the stem sections revealed whether fungal growth was present. Five stems were sampled in this manner from each plot each season. It was not appropriate to analyse the data obtained from the stem incubation procedure since the fungi present were not tested for their pathogenicity to tomato. There is a sound argument that at least some of the *Fusarium* spp. found growing from the vascular bundles may not have been pathogenic (and possibly endophytic). Since testing for fungal pathogenicity had not been attempted by the premature conclusion of the project, interpretation of the extent of vascular invasion must remain equivocal.

Nematodes were extracted from 200 mL sub-samples using the Baermann tray technique. Both free-living and plant-parasitic nematodes were counted, with free-living nematodes being separated into fungal feeders (FF), bacterial feeders (BF), and omnivores/ predators (OP – including large Dorylaims and Mononchs). Microbial biomass C was estimated by the microwave irradiation method (Wang *et al.*2001). Briefly, duplicate field moist soil samples were weighed into extraction vessels, and one duplicate was microwave irradiated to lyse microbial cells. Soluble organic carbon was then extracted by 0.5 M K_2SO_4 from both the microwaved and non-microwaved samples. The difference in soluble organic carbon levels was expressed as mg C/kg soil, and is a measure of the microbial biomass carbon.

The composition of the soil microbial community was determined by PL-FAME analysis of the profile of fatty acid methyl esters (FAMEs) chemically derived from phospholipids (PL) extracted from the soil samples taken at Bowen (26/9/00 and 30/7/01) and Bundaberg (19/7/00 and 1/5/01) (Zelles, 1999; Pankhurst *et al.*, 2001). Twelve grams of soil from each field replicate was extracted overnight in a chloroform:methanol:phosphate buffer (1:2:0.8 v/v/v) and then filtered through fast ashless filter paper into a separation funnel. Samples were shaken and the phases allowed to separate overnight. The $CHCl_3$ layer was decanted and evaporated to dryness on a rotary evaporator. The extract was resuspended in $CHCl_3$ and applied to a column of silicic acid conditioned with $CHCl_3$. Phospholipids were eluted with methanol and dried under N_2 at 30°C and then subjected to acid methanolysis. An internal standard (nonadecanoic acid methyl ester) was added to the- resulting phospholipid fatty acid methyl esters (PL-FAMEs) and then redissolved in hexane for gas chromatographic analysis. PL-FAMEs were separated by capillary by gas chromatography using a Hewlett Packard HP gas chromatograph fitted with a flame ionisation detector and a silica capillary column. The FAME peaks were identified using a computer program. The peak areas were normalized against the internal standard and expressed as $\mu g\ g\ soil^{-1}$.

Data on up to 80 different fatty acids was routinely obtained in the PL-FAME profiles of individual soil samples. PL-FAMEs of interest were those that could be used as biomarkers for different microbial functional groups. These included: total bacteria, 15:0, i15:0, a15:0, i16:0, 17:0, i17:0, a17:0, cy17:0 and cy19:0; gram negative bacteria, cy17:0 and cy19:0;

gram positive bacteria, 15:0, i15:0, a15:0, i16:0, 17:0, i17:0, a17:0; total fungi, 18:2ω6c; mycorrhizal fungi, 16:1ω5c. The ratio of 18:2ω6c: total bacterial PL-FAMEs was taken to represent the ratio of fungal:bacterial biomass in the soil.

Fruit from rockmelon and tomato plants were harvested from 10-centrally-positioned-plants grown in the centre datum row of each plot at Bundaberg. At Bowen, fruit were harvested from plants grown in the centre datum row within an 8m section for rockmelon or within a 6.75 m^2 section for tomato. Harvested fruit were graded into marketable and reject, counted and weighed. Marketability for tomato fruit was determined by Appendix 3 of the Tomato Style Allowances Tomato Quality Guide (QI97001, p.28), whereas rockmelons were deemed to be marketable if >700 grams at Bundaberg and > 500grams at Bowen, and free from cracks, blemishes, and rot. A description of the harvest information for the 6 trials conducted in this study is provided in Table 3.4.

Brix° measurements (total soluble solids) were made using a refractometer on melon fruit at peak harvest grown at the Bundaberg site and at the first and fourth harvests at the Bowen site. Five randomly-selected marketable fruit per plot were cut vertically, and a spoonful of flesh from the top (sweetest) and the bottom (blandest) of each half were combined, squeezed and read. Brix° values were also determined for the 2001 tomato trial at Bundaberg on 20 red full colour fruit sampled on 19/6/01 (at the end of the harvest period) from the outer 2 datum rows of each plot. A 4mm section was taken from the equatorial plane of each tomato fruit, and all 20 sections were then squeezed and read with a refractometer. A correction factor based on temperature was employed to derive 'true' Brix° readings for all samples.

Analysis of variance (ANOVA) was used to test the effects of treatments. Treatment means were compared using the protected l.s.d. procedure operating at P=0.05. Given that the nematode data are based on counts, a cube root transformation was performed on the data prior to ANOVA. Comparison of PL-FAME abundance was made using 2-way ANOVA. All statistical analyses were completed using Genstat™ (GenStat 2000).

3.3 Results

3.3.1 2000 Bowen trial – Tomato cv. Tempest

The weight and number of marketable fruit harvested from plants grown in Treatments 1 and 4 were higher than those of plants grown in the other treatments (Table 3.5). Marketable fruit weight and number were least in the molasses treatments (Treatments 3 and 6). In general, there appeared to be a negative correlation between organic matter input 4 weeks pre-plant and yield of marketable fruit, with molasses application further reducing yield of fruit. It is likely that a N draw-down effect from the high amounts of organic C applied prior to planting to the soil affected yield. Foliar leaf analyses confirmed this hypothesis, since the N concentration of leaves of plants grown in Treatment 6 (2.85%) were lower than that measured in the leaves of plants grown in Treatment 1 (3.57%). The organic amendment treatments had no effect on the average weight of a marketable fruit (Table 3.5).

Table 3.5 The effect of the organic amendment treatments on various yield parameters of tomato cv. Tempest grown in 2000 in the field at Bowen Research Station.
Means within columns followed by the same letter are not significantly different at P=0.05 (F-test for main effect of organic matter treatment). For the average weight of a marketable fruit, the effect of organic matter addition was not significant.

Treatment	Weight of marketable fruit (t/ ha)	Number of marketable fruit per ha	Weight of a marketable fruit (g)
1	109.5c	652,149c	168
2	80.9b	465,482b	175
3	69.9ab	412,149ab	169
4	106.3c	614,223c	173
5	80.0b	462,519b	174
6	59.0a	352,593a	167
l.s.d. (P=0.05)	11.5	73,945	9

In general, the number of fungal and bacterial-feeding nematodes and the total number of free-living nematodes at the Bowen site increased as the amount of organic matter increased, especially molasses (compare Treatments 2 versus 3, and 5

versus 6). The site was infested with reniform nematode (*Rotylenchulus reniformis*) and counts of this nematode at harvest clearly showed that its population density was reduced by organic amendments (Table 3.6). Population densities in Treatments 2 and 3 were lower than in non-amended soil (Treatment 1) A similar effect is apparent if nematode densities in Treatments 5 or 6 are compared with Treatment 4. The slightly higher population densities in Treatments 4-6 (which had a green manure crop) compared with Treatments 1-3 (bare fallow) are probably due to nematode reproduction on the green manure crop before it was incorporated into soil.

Table 3.6 Nematode counts per 200 mL of soil sampled on 26 September 2000 from the organic amendment treatments at the Bowen trial site.
The crop species grown at the time of sampling was tomato cv. Tempest. Both free-living and plant-parasitic nematodes were counted, with free-living nematodes (FLN) being separated into fungal feeders (FF) and bacterial feeders (BF).
A cube-root transformation $(X+1)^{1/3}$ was performed on the data prior to ANOVA. Transformed means within a column followed by different letters are significantly different at P=0.05 (F-test for treatments). A back-transformed mean is given after each transformed mean.

Treatment	Reniform		FF		BF		Total FLN	
	Transf	Back	Transf	Back	Transf	Back	Transf	Back
1	10.6c	1187	7.7a	461	9.0a	735	10.7a	1227
2	4.9a	115	8.8a	683	12.6b	1985	14.0b	2749
3	5.3a	146	13.3c	2362	17.1c	5026	19.9c	7868
4	11.0c	1341	8.5a	622	9.5a	843	11.5a	1508
5	8.0b	503	8.6a	633	9.6a	884	11.6a	1568
6	5.6a	176	10.7b	1231	11.6b	1572	14.3b	2948
l.s.d. (P=0.05)	1.9		1.4		2.0		2.0	

There was a strong relationship between increasing organic matter input and the various C fractions (Table 3.7).

Table 3.7 Various C fractions within soil sampled on 26 September 2000 from the organic amendment treatments at the Bowen trial site.
Means within a column followed by different letters are significantly different at P=0.05 (F-test for treatments).

Treatment	Microbial biomass C (µg/ g)	Leco C (%)	Leco N (%)	C1 (mg/ g)	C3 (mg/ g)	C1/ Leco C (%)
1	52.3a	1.41a	0.080a	1.19a	2.25a	8.45
2	63.5a	1.65bc	0.084ab	1.36ab	2.28a	8.43
3	79.6ab	1.62b	0.089bc	1.45ab	2.44a	8.94
4	63.8a	1.68bc	0.093cd	1.82c	2.56a	10.75
5	106.1bc	1.75bc	0.099de	1.72bc	3.07b	9.84
6	116.3c	1.84c	0.103e	1.84c	3.62c	10.04
l.s.d. (P=0.05)	29.2	0.18	0.008	0.37	0.42	2.00

The vascular systems of 17-23% of the stems of the tomato cv. Tempest plants were colonised with *Fusarium* spp. No *Verticillium* spp. were found associated with any of the samples (Table 3.8).

Table 3.8 Percentage stem sections sampled on 26 September 2000 with vascular fungal invasion for Tomato cv. Tempest plants at the Bowen trial site.

Treatment	*Fusarium* spp.	*Verticillium* spp.	Total stems invaded
1	23.3	0	23.3
2	16.7	0	16.7
3	26.7	0	26.7
4	20.0	0	20.0
5	20.0	0	20.0
6	20.0	0	20.0

3.3.2 2001 Bowen trial – Rockmelon cv. Southern Cross

The organic matter treatments did not have any effect on the yield or fruit quality parameters that were measured (Table 3.9). The longer period which had elapsed (13 weeks) between the final application of organic matter (Treatments 2, 3, 5, 6) and field planting the 2001 rockmelon crop, compared with the relatively short period (5 weeks) for the equivalent operation in the 2000 tomato trial at Bowen, may have aided in the dissipation of the N draw-down effect that was apparent in the Bowen tomato trial.

Table 3.9 The effect of the organic amendment treatments on various yield parameters of rockmelon cv. Southern Cross grown in 2000 in the field at Bowen Research Station.
The effect of treatments was not significant at P=0.05 for any of the measured fruit parameters (F-test for main effect of organic matter treatment).

Treatment	Weight of marketable fruit (t/ha)	Number of marketable fruit (per ha)	Total weight of all fruit (t/ha)	% Marketable fruit (by weight)	Average weight of a marketable fruit (g/ fruit)	Brix°
1	37.5	28,738	39.3	95.4	1,318	12.38
2	37.6	31,237	39.8	94.6	1,209	12.41
3	33.9	28,322	36.9	91.7	1,201	12.70
4	37.7	30,960	40.1	94.1	1,219	12.05
5	37.2	29,571	39.4	94.6	1,258	12.26
6	37.4	30,543	40.8	92.1	1,227	12.15
l.s.d. (P=0.05)	3.5	2812	4.1	5.5	99	0.53

The number of fungal-feeding nematodes increased progressively with the addition of organic C (Table 3.10). However, the number of bacterial-feeding nematodes tended to be higher in treatments to which molasses was applied (Treatments 3 and 6) than in the other treatments. As for the previous year at the Bowen site, transformed means of the number of reniform nematodes showed that its population density was reduced by organic amendments.

Table 3.10 Nematode counts per 200 mL of soil sampled in August 2001 from the organic amendment treatments at the Bowen trial site.
The crop species grown at the time of sampling was rockmelon cv. Southern Cross. Both free-living and plant-parasitic nematodes were counted, with free-living nematodes (FLN) being separated into fungal feeders (FF) and bacterial feeders (BF). A cube-root transformation $(X+1)^{1/3}$ was performed on the data prior to ANOVA. Transformed means within a column followed by different letters are significantly different at P=0.05 (F-test for treatments). A back-transformed mean is given after each transformed mean.

Treatment	Reniform		FF		BF		Total FLN	
	Transf	Back	Transf	Back	Transf	Back	Transf	Back
1	17.4c	5303	7.0a	338	8.8abc	671	10.1a	1032
2	12.8ab	2101	8.7b	653	9.8bcd	952	11.9bc	1701
3	13.5b	2481	8.4b	592	10.1cd	1029	12.1bc	1771
4	13.9b	2708	8.4b	596	8.5ab	604	10.8ab	1255
5	11.9ab	1680	10.3c	1082	7.9a	492	12.0bc	1736
6	11.0a	1319	10.1c	1029	10.4d	1137	13.3c	2373
l.s.d. (P=0.05)	2.1		1.1		1.5		1.5	

Microbial activity in soil to which the highest input of organic matter was applied (Treatment 6) was higher than for the other treatments (Table 3.11). The amount of Leco C progressively increased with successive increases in C input.

Table 3.11 Various C fractions within soil sampled in August 2001 from the organic amendment treatments at the Bowen trial site.
Means within a column followed by different letters are significantly different at P=0.05 (F-test for treatments).

Treatment	Microbial biomass C (µg/ g)	Leco C (%)	Leco N (%)	C1 (mg/ g)	C3 (mg/ g)	C1/ Leco C (%)
1	38.7a	1.43a	0.091a			
2	41.2a	1.53b	0.098b	No	No	No
3	59.2a	1.51ab	0.098b	data	data	data
4	51.3a	1.63c	0.105bc			
5	59.7a	1.69cd	0.108c			
6	84.8b	1.75d	0.115d			
l.s.d. (P=0.05)	23.1	0.09	0.007			

3.3.3 2000 Bundaberg trial – Tomato cv. Mercedes – autumn season

The weight of marketable fruit and the number of marketable fruit per ha did not differ for tomato plants grown in the various organic amendment treatments (Table 3.12). The lack of an effect of the treatments was probably due to the short time period over which the trial was conducted.

Table 3.12 The effect of the organic amendment treatments on various yield parameters of tomato cv. Mercedes grown in 2000 in the field at Bundaberg Research Station.
There were no differences between treatments for any of the parameters at P=0.05.

Treatment	Weight of marketable fruit (t/ha)	Number of marketable fruit (per ha)	Average weight of a marketable fruit (g/ fruit)	% Marketable fruit (by weight)
1	34.9	332,666	105	86.5
2	33.7	321,999	105	86.6
3	33.2	319,333	104	87.0
4	33.9	331,777	102	86.1
5	31.1	303,110	103	85.6
6	33.9	329,777	102	84.9
l.s.d. (P=0.05)	6.2	56,629	4	3.7

For both the early and late sampling times during the tomato crop, the transformed means for fungal and bacterial feeding nematodes, and total numbers of free-living nematodes increased as the amount of cane trash and green manure increased (Table 3.13). Analysis of the transformed means showed that the addition of molasses increased the number of bacterial-feeding and free-living nematodes at the late sampling time only (Treatment 2 versus Treatment 3). Otherwise, molasses had no impact on any measured parameter. The plant parasitic reniform nematode was present in low numbers, and was unaffected by the organic matter treatments.

Table 3.13 Nematode counts per 200 mL of soil sampled on 6 April 2000 and on 19 July 2000 from the organic amendment treatments at the Bundaberg trial site.
The times of sampling the soil corresponded to an early (6 April 2000) and a late (19 July 2000) stage in the development of the tomato crop (cv. Mercedes). Both free-living and plant-parasitic nematodes were counted, with free-living nematodes (FLN) being separated into fungal feeders (FF) and bacterial feeders (BF).
A cube-root transformation $(X+1)^{1/3}$ was performed on the data prior to ANOVA. Transformed means within a column followed by different letters are significantly different at P=0.05 (F-test for treatments). A back-transformed mean is given after each transformed mean.

Treatment	Reniform		FF		BF		Total FLN	
	Transf	Back	Transf	Back	Transf	Back	Transf	Back
Early sampling								
1	2.67	18	7.8a	481	9.0a	723	10.9a	1276
2	1.53	3	10.7b	1217	11.1b	1370	14.0b	2761

3	1.52	3	10.5b	1147	10.7b	1210	13.5b	2443
4	3.58	45	12.1c	1775	13.8c	2621	16.7c	4665
5	2.39	13	12.9c	2121	12.9c	2136	16.7c	4690
6	2.26	11	12.9c	2161	13.5c	2459	17.1c	4982
l.s.d. (P=0.05)	2.21		1.2		1.4		1.4	
Late sampling								
1	1.71	4	7.3a	393	9.4a	824	11.0a	1323
2	1.50	2	9.9b	963	12.3b	1846	14.7b	3195
3	1.00	0	10.2b	1070	13.8c	2616	15.9c	4019
4	3.11	29	11.5c	1500	14.2c	2868	16.9cd	4800
5	1.81	5	12.6d	2009	14.8c	3234	18.0e	5812
6	1.00	0	11.9cd	1693	14.7c	3202	17.4de	5285
l.s.d. (P=0.05)	1.40		1.0		1.2		1.1	

Labile C fractions C1 and C3 increased strongly with increasing organic matter input (Table 3.14), although microbial biomass C did not differ among treatments.

Table 3.14 Various C fractions within soil sampled on 19 July 2000 from the organic amendment treatments at the Bundaberg trial site.
Means within a column followed by different letters are significantly different at *P*=0.05 (*F*-test for treatments).

Treatment	Microbial biomass C (µg/ g)	Leco C (%)	Leco N (%)	C1 (mg/ g)	C3 (mg/ g)	C1/ Leco C (%)
1	63.9	1.64a	0.155	1.21a	2.39a	7.41a
2	62.2	1.73ab	0.158	1.32ab	2.59ab	7.63ab
3	103.5	1.79b	0.162	1.38bc	2.61ab	7.75b
4	74.1	1.81b	0.163	1.44c	2.91bc	7.91b
5	74.2	1.98c	0.215	1.67d	3.08c	8.42c
6	86.2	2.00c	0.177	1.72d	2.93bc	8.59c
l.s.d. (*P*=0.05)	28.0	0.09	0.041	0.11	0.44	0.32

The percentage of incubated stem sections colonised by *Fusarium* spp. and *Verticillium* spp. was 43 – 63%, and this result did not appear to be related to treatment (Table 3.15).

Table 3.15 Percentage tomato cv. Mercedes stem sections with vascular fungal invasion sampled from the Bundaberg trial site on 19 July 2000.

Treatment	*Fusarium* spp.	*Verticillium* spp	Total stems invaded
1	60.0	3.3	63.3
2	36.7	6.6	43.3
3	56.7	3.3	60.0
4	60.0	3.3	63.3
5	60.0	3.3	60.0
6	60.0	3.3	60.0

3.3.4 2000 Bundaberg trial – Rockmelon cv. Eldorado – spring season

Apart from the average weight of a marketable fruit, the organic amendment treatments had no effect on the yield parameters that were measured (Table 3.16). The weight of an individual marketable fruit appeared to be unrelated to the organic amendment treatments.

Table 3.16 The effect of the organic amendment treatments on various yield parameters of rockmelon cv. Eldorado grown in 2000 in the field at Bundaberg Research Station.
Means within a column followed by different letters are significantly different at P=0.05 (F-test for treatments).

Treatment	Weight of marketable fruit (t/ha)	Number of marketable fruit (per ha)	Total weight of all fruit (t/ha)	% Marketable fruit (by weight)	Average weight of a marketable fruit (g/ fruit)	Brix°
1	60.4	28,667	68.2	88.8	2,113abc	7.70
2	60.7	29,778	65.8	92.0	2,045a	7.69
3	61.4	30,667	67.1	91.3	2,001a	7.42
4	58.7	28,222	65.3	89.1	2,077ab	7.84
5	62.6	28,222	68.7	91.0	2,216c	7.79
6	57.8	26,667	66.7	87.1	2,163bc	7.99
l.s.d. (P=0.05)	9.5	4,551	8.5	8.5	113	0.58

3.3.5 2001 Bundaberg trial – Tomato cv. Queensland Red – autumn season

The total weight and number of marketable fruit was higher (P<0.05) for plants grown without a green manure crop and an initial application of cane trash (Treatments 1 and 2) than for plants grown with these materials incorporated into the soil (Treatments 4 and 5) (Table 3.17). This result was probably due to a N draw-down effect from the high amounts of organic C applied to the soil at the very early stage of seedling development. Indeed, sap nitrate testing of plants grown in Blocks 2 and 5 at bud initiation at 21 days after transplanting (DAT) showed a higher mean nitrate concentration for the zero organic matter treatment (Treatment 1 - 3,990 mg/L) than for the highest organic matter treatment (Treatment 6 - 2,810 mg/L). Despite the application of 60 kg N/ ha in the basal fertiliser and twice-weekly applications of 5 kg N/ ha as soluble fertiliser from 24 DAT, the mean petiole sap nitrate concentration of plants grown in Treatment 1 (3,970 mg/L) still remained higher than that of plants grown in Treatment 6 (3,130 mg/L) at 45 DAT.

As the crop neared the green mature stage of fruit development, the symptoms of pith necrosis (hollow stem) developed in the crop. Initially, chlorosis of the older leaves appeared, with dark brown to black necrotic on the stems of some plants accompanied by profuse development of adventitious roots. Internally, varying shades of brown discolouration preceded complete breakdown of the stem pith in some plants. Although the disease is associated with the bacterium *Pseudomonas corrugata*, its incidence is associated with succulent plant growth caused by high N fertilisation and low night temperatures. These conditions matched those experienced towards the latter half of the crop cycle.

Molasses application had no effect for any of the yield parameters measured (Table 3.17). The organic amendment treatments had no effect on the average weight of a marketable fruit, percent marketable fruit by weight, or Brix° (Table 3.17).

Table 3.17 The effect of the organic amendment treatments on various yield parameters of tomato cv. Queensland Red grown in 2001 in the field at Bundaberg Research Station.
Means within a column followed by different letters are significantly different at P=0.05 (F-test for treatments).

Treatment	Weight of marketable fruit (t/ha)	Number of marketable fruit (per ha)	Average weight of a marketable fruit (g/ fruit)	% Marketable fruit (by weight)	Brix°
1	63.9c	508,667b	126	81.4	4.9
2	67.2c	522,667b	129	85.1	4.8
3	62.2bc	485,333b	129	82.8	4.8
4	53.1a	418,444a	127	81.5	4.7
5	54.8ab	415,333a	133	81.2	5.0
6	60.9bc	470,889ab	129	83.4	4.9
l.s.d. (P=0.05)	7.5	66,074	5.7	3.3	0.3

For both the early and late sampling times, the response of fungal and bacterial feeding nematodes, and total numbers of free-living nematodes was similar to that found for the previous year at the Bundaberg site, with a progressive increase in numbers as the amount of cane trash and/ or green manure increased (Table 3.18). In general, molasses had either no impact overall, or a slight detrimental (non-statistical) effect on the number of free living nematodes. The plant parasitic reniform nematode was again present in low numbers, and was unaffected by the organic matter treatments.

Table 3.18 Nematode counts per 200 mL of soil sampled on 16 March 2001 and on 1 May 2001 from the organic amendment treatments at the Bundaberg trial site.
The times of sampling the soil corresponded to an early (16 March 2001) and a late (1 May 2001) stage in the development of the tomato crop (cv. Queensland Red). Both free-living and plant-parasitic nematodes were counted, with free-living nematodes (FLN) being separated into fungal feeders (FF) and bacterial feeders (BF).
A cube-root transformation $(X+1)^{1/3}$ was performed on the data prior to ANOVA. Transformed means within a column followed by different letters are significantly different at P=0.05 (F-test for treatments). A back-transformed mean is given after each transformed mean.

Treatment	Reniform		FF		BF		Total FLN	
	Transf	Back	Transf	Back	Transf	Back	Transf	Back
Early sampling								
1	3.29	35	7.9a	496	10.1a	1026	11.7a	1580
2	1.20	1	13.4b	2378	15.8bc	3921	18.9bc	6793
3	1.00	0	12.5b	1933	14.7b	3189	17.6b	5404
4	5.12	133	16.9c	4817	16.0bc	4072	21.2cd	9527
5	2.38	12	18.2c	5988	16.5c	4483	22.6d	11496
6	1.81	5	19.0c	6880	15.1bc	3415	22.2d	10984
l.s.d. (P=0.05)	3.02		3.5		1.6		3.1	
Late sampling								
1	11.3	1442	8.1a	525	11.4a	1496	12.8a	2081
2	4.8	110	13.5b	2432	15.5bc	3694	18.6b	6413
3	6.7	300	12.7b	2043	14.3b	2899	17.3b	5150
4	7.6	438	14.1b	2778	15.2bc	3490	18.8b	6601
5	5.9	204	19.2c	7121	18.2d	5988	24.1c	13997
6	3.6	46	19.2c	7088	16.3cd	4322	22.9c	12039
l.s.d. (P=0.05)	8.9		2.5		1.9		2.5	

Microbial activity was stimulated incrementally with progressive organic matter inputs (Table 3.19). Leco C and N also showed a similar trend.

Table 3.19 Various C fractions within soil sampled on 1 May 2001 from the organic amendment treatments at the Bundaberg trial site.
Means within a column followed by different letters are significantly different at P=0.05 (F-test for treatments).

Treatment	Microbial biomass C (µg/ g)	Leco C (%)	Leco N (%)	C1 (mg/ g)	C3 (mg/ g)	C1/ Leco C (%)
1	31.9a	1.80a	0.175a	1.64	3.22	9.11c
2	48.9ab	1.97ab	0.190b	1.64	3.33	8.31bc
3	58.0bc	1.97b	0.191b	1.68	3.12	8.53bc
4	75.6cd	2.11bc	0.198bc	1.65	2.30	7.84ab
5	80.8d	2.21cd	0.198bc	1.77	3.33	8.12b
6	80.4d	2.32d	0.209c	1.63	2.96	7.02a
l.s.d. (P=0.05)	22.3	0.17	0.012	0.12	0.77	0.88

Table 3.20 indicates the percentage of stem sections colonised by *Fusarium* spp. No *Verticillium* spp. were identified in this planting. Between 17% (Treatment 2) and 67% (Treatment 6) of the stems were colonised by *Fusarium* spp.

Table 3.20 Percentage stem sections with vascular fungal invasion sampled on 1 May 2001 from tomato cv. Queensland Red plants grown at the Bundaberg trial site.

Treatment	*Fusarium* spp	*Verticillium* spp	Total stems invaded
1	36.7	0	36.7
2	16.7	0	16.7
3	33.3	0	33.3
4	40.0	0	40.0
5	40.0	0	40.0
6	66.7	0	66.7

3.3.6 2001 Bundaberg trial – Rockmelon cv. Eastern Star – spring season

The organic matter treatments had no effect on the yield parameters that were measured (Table 3.21). The concentration of nitrate expressed from the petioles of young mature leaves showed that N nutrition was either unrelated (early fruit set) or not closely linked (late fruit set) to increasing organic matter. This result indicates that the N draw-down effect that was prevalent in crops immediately following the incorporation of organic matter into the soil (e.g. tomato crops grown in Bowen in 2000 and in Bundaberg in 2001) appeared to have dissipated after a 6-month period.

Table 3.21 The effect of the organic amendment treatments on various yield parameters and petiole sap nitrate of rockmelon cv. Eastern Star grown in 2001 in the field at Bundaberg Research Station.
Means within a column followed by different letters are significantly different at P=0.05 (F-test for treatments).

Treatment	Weight of marketable fruit (t/ha)	Number of marketable fruit (per ha)	Total weight of all fruit (t/ha)	% Marketable fruit (by weight)	Average weight of a marketable fruit (g/ fruit)	Brix°	Sap nitrate (7/9/01) (mg/L)	Sap nitrate (19/9/01) (mg/L)
1	46.2	34,000	49.2	93.5	1,342	11.1	3,927	4,060a
2	46.2	34,667	50.1	91.8	1,324	11.1	4,447	4,953b
3	42.3	31,556	45.8	92.4	1,321	11.8	4,057	4,323a
4	39.6	31,333	43.0	88.9	1,218	10.6	3,953	4,580ab
5	40.4	30,667	42.4	94.4	1,289	11.5	4,293	4,630ab
6	42.9	32,000	46.9	90.5	1,310	11.4	4,463	4,213a
l.s.d. (P=0.05)	13.3	8,043	13.0	7.9	131	0.8	448	579

3.3.7 PL-FAME analysis

There were significant treatment effects at both sites on the total amount of PL-FAMEs extracted from the soil and the amount of selected biomarker PL-FAMEs associated with different microbial functional groups (Table 3.22). The total amount of PL-FAMEs extracted is indicative of the total microbial biomass. At both sites the addition of organic matter to the soil resulted in significant ($P<0.05$) increases in total PL-FAMEs (microbial biomass). These increases ranged from 40-120% at Bundaberg and from 80-140% at Bowen. At both sites the biggest increase was with Treatments 5 and 6 which received 32 and 34 t C /ha respectively. Also at both sites there was a significant increase in the amount of PL-FAME biomarkers for fungi, mycorrhizal fungi and the ratio of fungi to bacteria with all organic matter treatments. The increases in fungi were very large (400% with Treatment 5 at Bundaberg, 600% with Treatment 6 at Bowen). At Bowen the amount of PL-FAME biomarkers for total bacteria, gram-positive bacteria (e.g. spore-forming *Bacillus* species) and gram-negative bacteria (e.g. *Pseudomonas* species) was also significantly increased by all organic matter treatments. However, at Bundaberg only Treatments 5 and 6 significantly increased each of these bacterial groups.

There was no site x treatment interaction for any of the PL-FAME measurements made, indicating that the treatments had similar impacts at both sites. There was a significant time effect, with all measurements higher on the second sampling occasion (i.e. in 2001). PL-FAMEs for fungi, gram-positive bacteria and the fungal: bacterial ratio, were higher at Bundaberg, whereas the PL-FAME for mycorrhizal fungi was higher at Bowen.

Table 3.22 Effect of different organic matter treatments on the amount (µg/g soil) of PL-FAMEs used as biomarkers for different microbial functional groups in soil from the RASH trials at Bundaberg and Bowen.
Mean treatment effects for data obtained on the two sampling occasions (2000 and 2001) is presented.

Microbial functional group	Organic matter treatment						LSD (P<0.05)
	1	2	3	4	5	6	
Bundaberg							
Total bacteria	0.98	1.30	1.42	1.35	2.07	1.78	0.39
Gram-positive bacteria	0.72	0.94	1.06	0.97	1.46	1.26	0.28
Gram-negative bacteria	0.26	0.35	0.36	0.39	0.61	0.51	0.11
Total fungi	0.05	0.14	0.15	0.13	0.25	0.22	0.07
Mycorrhizal fungi	0.06	0.11	0.13	0.13	0.21	0.16	0.04
Ratio Fungi:bacteria	0.05	0.11	0.10	0.10	0.12	0.12	0.03
Total PL-FAMEs[a]	3.62	5.09	5.45	5.21	7.99	6.68	1.67
Bowen							
Total bacteria	0.95	1.57	1.56	1.81	2.00	1.96	0.46
Gram-positive bacteria	0.72	1.17	1.40	1.33	1.46	1.41	0.35
Gram-negative bacteria	0.22	0.40	0.42	0.47	0.54	0.55	0.05
Total fungi	0.02	0.08	0.11	0.09	0.12	0.14	0.03
Mycorrhizal fungi	0.07	0.17	0.16	0.22	0.26	0.26	0.07
Ratio Fungi:bacteria	0.02	0.05	0.07	0.05	0.06	0.07	0.02
Total PL-FAMEs	2.78	5.14	5.11	5.97	6.88	6.63	1.72

3.4 Discussion

The results clearly show that there are marked changes in the soil biota when large quantities of organic matter are added to intensively cropped vegetable-growing soils. For example, after a green manure crop and two applications of cane trash (Treatment 5) in 2000, there was a fourfold increase in total numbers of free living nematodes at the Bundaberg site (Table 3.13) and a substantial increase in microbial activity at the Bowen site (Table 3.7).

The PL-FAME analysis showed that the organic matter treatments had a significant impact on the microbiology of the soils at both Bundaberg and Bowen. All treatments increased total PL-FAMEs in the soil, indicative of an increase in microbial biomass. This increase was associated with a major increase in fungi and in the ratio of fungi to bacteria in the microbial biomass. This result is not surprising, given that the bulk of the organic matter added to the soil was cane trash, which has a high C/N ratio, and would thus be expected to favour an increase in fungi. The impact of the organic matter treatments on the soil microbiology, whilst similar at both sites, was slightly greater at Bowen. This effect was possibly due to the fact that the soil at Bowen had lower starting levels of microbial biomass (total PL-FAMEs) and fungi and a much lower fungal: bacterial ratio in the microbial biomass (all indicative of a less fertile soil biologically) than the soil at Bundaberg (Table 3.22).

Increasing the organic matter input from 10 t C /ha to 34 t C /ha was associated with a progressive increase in total PL-FAMEs (microbial biomass) and in all microbial functional groups. The greatest impact was where two additions of organic matter were made pre-plant (i.e. Treatment 5).

The PL-FAME data showed that addition of molasses following the planting of the crop (Treatments 3 and 6), did not increase microbial activity above that achieved with the respective pre-plant organic matter additions, and may even have had a slight negative effect. These findings concur with those at the Bundaberg site for microbial biomass C and free living nematodes, which were unaffected by molasses application. However, for the Bowen site, the stimulatory effect of

molasses on free living nematodes in both years and on microbial biomass in 2001 shows that this product can have a variable effect on soil microbial populations. Therefore, there appears to be at best a variable benefit in applying molasses to the soil during crop growth in terms of stimulating soil microbial activity.

The PL-FAME analysis showed that all measurements were higher in 2001 than in 2000, and this finding is consistent with the counts of free-living nematodes at the Bundaberg site. Overall, however, microbial biomass C at both sites and free-living nematodes at Bowen did not increase from 2000 to 2001, indicating that data collection at a single point in time provides no more than a snapshot of the biotic differences between soils.

Whereas increasing organic C stimulated the microflora in the soil, it had little to no effect on the yield of tomato fruit. For the 2001 season at the Bundaberg site, tomato yields tended to be reduced by organic matter treatment. This effect was probably caused from a the changing status of soil N fixation and availability in a soil loaded with organic matter. The availability of soil N changed from being limited by organic matter fixation to abundant as the organic material broke down, enabling applied fertilizer became more available than before. The difficulty in managing N nutrition of the crop in this dynamic system resulted in a dearth of this element early in the life of the crop and an excess of N later in the crop's life. A combination of N excess at fruit bearing age and decreasing night temperatures led to the development of pith necrosis (also known as hollow stem) in tomato plants grown at the Bundaberg site. The bacterium *Pseudomonas corrugata* was responsible for this phenomenon.

The issue of a N draw-down effect was a recurring theme throughout these trials, with plants grown in the higher organic matter treatments producing a lower yield of marketable fruit than plants grown in the nil organic matter treatment in 2 of the 6 trials conducted. Basal applications and standard fertigations designed for optimal N management in conventionally-grown tomato and melon crops were clearly insufficient to overcome the tie-up of N from the high levels of organic material used in some treatments in these trials. The limitations to yield by N deficiency were most profound early (within the first few weeks after transplanting), when competition for N between microbes feeding on the organic material and the roots of the crop plants was high. The optimal to supra-optimal amounts of N fertiliser applied during the growth of the crops (to overcome the N draw-down effect) also caused problems. Most notably was the 2001 tomato crop at Bundaberg, in which the development of pith necrosis (and the associated bacterium *Pseudomonas corrugata*) indicated an over supply of N fertiliser late in the tomato crop.

At least 2 strategies are available to address the N management issues of organic matter amelioration of the soil. Firstly, the schedule for N applications to a crop grown in this environment needs to be shifted forward to immediately after transplanting. Fertigations supplying N towards the end of the crop cycle also need to be carefully monitored by tools such as sap testing, to ensure concentrations are not high enough to induce pith necrosis or poor fruit quality. Secondly, planting of the crop could be delayed following organic matter incorporation to allow time for soil microbes to break down the majority of material and to reduce the risk of a N draw-down. The work at Bowen showed that a period of 13 weeks from incorporation of organic matter to field planting (2001 trial) is required to avoid the N draw-down effect, whereas a period of 5 weeks (2000 trial) was insufficient to overcome this effect. The temporal effect of the organic matter treatments on N nutrition was also shown by the spring 2001 rockmelon crop at Bundaberg, which demonstrated little to no effect of these materials on sap nitrate. Whereas a 6-month period is sufficient to dissipate the N draw-down effect, it is likely that a considerably lower activity of micro-organisms would also prevail.

The single season of data from the Bowen site is not sufficient to draw any conclusions regarding the levels of *Fusarium* spp. recovered from the vascular system of the sampled stem sections. At the Bundaberg site, there is some indication that some treatments may have started to influence the colonisation of tomato stems by *Fusarium* spp., but since the varieties between seasons were different, nothing conclusive can be drawn. It could be speculated that the level of non-pathogenic *Fusarium* spp. recovered from the stems could increase as bio-activity within the plots increased, while that of pathogenic strains may decrease. Conclusive indications of this cannot be drawn since pathogenicity testing was not carried out. Most of the *Fusarium* spp. recovered were *F. oxysporum*.

A rudimentary economic analysis of the best strategies for improving soil health by organic amendments was to be conducted at the end of the project as an adjunct to the final report. However, at the time of termination of the project, the addition of the organic matter treatments either had no effect or had a negative effect on the yield of marketable fruit. The effect of organic materials on stimulating microbial activity in the soil was an interesting finding, but the direct effect of marketable yield on gross margin is the ultimate measure of success or failure of a particular technique or innovation. Therefore, an economic analysis in these circumstances would be a nonsense.

The organic amendment trials have so far shown that whereas the soil microbial populations have been stimulated by the addition of organic material, crop yield has not increased, and in some seasons, has decreased. This project was due to run

for another 2 years to more fully assess the 'long-term' implications of the work. The management of soil health by addition of organic amendments is a technique which may provide a practical alternative for growers most affected by the phase out of the soil fumigant methyl bromide in 2005.

4. Bio-additive field trials

4.1 Introduction

The bio-additives industry in Australia is thriving despite virtually no objective data. This project was assessing a broad range of commercially available products which are claimed to increase yield and improve soil health. Intensive field trials were being conducted at both Bowen and Bundaberg.

4.2 Materials and methods

Expressions of interest (advertised in the Queensland Fruit and Vegetable News, 16 March 2000) were called to ascertain the bio-additive treatments to be applied in the factorial experiments conducted at Bowen and Bundaberg Research Stations. Due to the large number of entries received, the final selection was based on products which were deemed not to be fertilisers, since the impact of nutrition could be easily eliminated by this decision. The products that were selected (Table 4.1) were considered to be adjuncts to existing farming systems, not considered to be fertilisers in their own right, and were added to the soil or crop plants in relatively small amounts. A requirement on the supplier was that inclusion of their product in the trial was dependant on the maintenance of the formulation during the trial. This continuity of treatment to the same plots at the trial sites was to allow the full effect of the bio-additive treatments to be assessed over time and for a range of environmental conditions.

Table 4.1 Commercial products which were under assessment in trials at Bowen and Bundaberg.

Product(s)	Company	Purported action(s)	Method of application	Reason(s) for inclusion
E2001/ Multibacter	Agricultural Research Technologies Pty Ltd	Free-living N fixers, promoters of fungi in soil	Injection through the trickle irrigation system	Requested by Horticulture Australia Ltd
Trichogrow and Trichoflow	Agrimm Technologies Ltd	Promotes plant growth and vigour and exclusion of some harmful microbes	Incorporation with seedling growing media, seedling drench and injection through the trickle irrigation system	Trichoderma product with good technical support and press coverage
Various Nutri-Life Products	Nutri-Tech Solutions Pty Ltd	Range of beneficial plant responses	Various, including a seedling treatment, injection through the trickle irrigation system and foliar application	Rapidly-expanding company with high profile in the bio-additives industry
Humic acid -Humega (Bundaberg) -SupaHumus (Bowen)	-BioFlora -Agrichem Manufacturing	Stimulates micro-organisms, increases soil CEC, improves soil structure	Injected through the trickle irrigation system	Humic acid was one of the first 'alternative' products to be sold commercially. Some positive anecdotal evidence
Kelpak	Cobbett Pty Ltd	Plant growth promotant	As a seedling drench and foliar spray	Good technical support. World-wide sales network

Standard cultural practices, including seedling transplants, raised soil beds, trickle irrigation tape, plastic mulch, basal fertiliser application and fertigation were used at the Bowen and Bundaberg trial sites. However, muriate fertilisers (viz. those containing chloride) were not used in basal or fertigation applications. This approach was taken since some proponents of alternative farming systems suggest that chloride can adversely affect the potential benefit of bio-additive products. Additionally, herbicides were not applied within the trial sites for the same reason. Integrated pest management was employed during the cropping cycle, a practice which is common for the local industries in Bowen and Bundaberg. Soil beds were raised and covered with 25 µm polyethylene mulch film prior to planting the tomato or melon seedlings. White plastic mulch was used for the autumn season in Bundaberg, whereas black plastic mulch was used for the spring season in Bundaberg and for the winter season at Bowen.

Prior to application of basal fertiliser, analysis of the surface 15 cm of the soil at the Bundaberg site on 28/2/00 showed it to have the following chemical characteristics: pH 6.2 (1:5 soil:water); electrical conductivity 0.5 dS/m (1:5 soil:water);

organic C 1.3% ($K_2Cr_2O_7 + H_2SO_4$); NO3-nitrogen (N) 4.8 mg/ kg (1:5 soil:water); available phosphorus (P) 83 mg/kg (1:100 soil:0.5 mol/L $NaHCO_3$); exchangeable potassium (K) 0.50 cmol(+)/kg (1:20 soil:1 mol/L NH_4Cl, pH 7.0); and effective cation capacity 8.42 cmol(+)/kg. Lime was applied to the Bundaberg trial site at 2.5 t/ ha on 29/2/00 and at 3.5 t/ha on 8/12/00. Subsequent analysis of the surface 15 cm of the soil in the E2001/Multibacter and Control treatments at the Bundaberg site on 14/2/01 showed it to have the following chemical characteristics (Table 4.2)

Table 4.2. Chemical analysis of the surface 15 cm of the soil in the Control and E2001/ Multibacter treatments at the Bundaberg bio-additives trial site for samples taken on 14/2/01.

Property	Unit of measurement	Untreated Control	E2001/ Multibacter	Desirable range[A]	Method
pH		6.40	6.30	6.0-7.0	1:5 soil:water
Electrical conductivity	dS/ m	0.6	0.7	< 0.30	1:5 soil:water
Organic C	%	1.3	1.2	> 2.0	$K_2Cr_2O_7 + H_2SO_4$
NO_3-N	mg/ kg	6.3	7.9	25-60	1:5 soil:water
P	mg/ kg	92	92	60-100	1:100 soil:0.5M $NaHCO_3$
K	cmol(+)/ kg	0.60	0.63	0.37-1.5	1:20 soil:1M NH_4Cl @ pH 7.0
Ca	cmol(+)/ kg	7.35	7.13	> 3.0	1:20 soil:1M NH_4Cl @ pH 7.0
Mg	cmol(+)/ kg	2.26	2.18	> 0.4	1:20 soil:1M NH_4Cl @ pH 7.0
Na	cmol(+)/ kg	0.15	0.15	< 2.0	1:20 soil:1M NH_4Cl @ pH 7.0
CEC[B]	cmol(+)/ kg	10.35	10.09	> 4.0	1:20 soil:1M NH_4Cl @ pH 7.0
Fe	mg/ kg	29	30	> 2.0	1:2 soil:0.005M DTPA
Cu	mg/ kg	9.2	9.1	0.3-10	1:2 soil:0.005M DTPA
Mn	mg/ kg	107	112	4-45	1:2 soil:0.005M DTPA
Zn	mg/ kg	5.2	4.8	1-10	1:2 soil:0.005M DTPA
Cl	mg/ kg	15	15	< 300	1:5 soil:water
SO_4-S	mg/ kg	56	61	20-100	1:5 soil:0.01M $Ca(H_2PO_4)_2$
B	mg/ kg	1.3	1.4	2-5	1:2 soil:hot 0.01M $CaCl_2$

[A] Incitec (1989). Soil interpretation manual. Volume II. Tomatoes and capsicums - sands and sandy loam soils for south east Queensland and northern New South Wales. Interpretation Chart No. 91.
[B] Cation exchange capacity estimated as the sum of basic cations.

A green manure crop of sorghum cv. Jumbo was sown at 15 kg/ ha during the mid-summer period of 2000/01 at both Bundaberg and Bowen trial sites.

The application rates and programs for the bio-additive treatments were provided by the suppliers. Trials at Bowen and Bundaberg included a control treatment and 5 bio-additive treatments, replicated in 4 blocks at each site. Plots consisted of 3 rows, each row 10m long with the centre row of each plot used for data collection. Permanent pegs were inserted around the perimeter of the trial sites to allow the exact positions of plots to be determined with string-lines for tomato and melon crops in subsequent years. Seed for all crops were sown into seedling trays, then transplanted into the field once the plants could be pulled easily from the cells. Details of the test crops grown at the trials sites are provided in Table 4.3.

Table 4.3 Details of the commercial bio-additive trials conducted at Bowen and Bundaberg.

Site	Crop	Cultivar	Date of sowing	Date of field planting	Date of first harvest	Date of final harvest	Number of harvests
Bowen	Rockmelon	Hammersley	30 Jun 00	31 July 00	5 Oct 00	19 Oct 00	5
	Tomato	Queensland Red	22 May 01	28 June 01	18 Sep 01	11 Oct 01	4
Bundaberg	Tomato	Mercedes	18 Feb 00	11 Apr 00	09 Aug 00	24 Aug 00	3

Rockmelon	Eldorado	22 Aug 00	26 Sep 00	22 Nov 00	04 Dec 00	5
Tomato	Queensland Red	17 Jan 01	20 Feb 01	10 May 01	14 Jun 01	3
Rockmelon	Eastern Star	03 Jul 01	13 Aug 01	29 Oct 01	12 Nov 01	5

Field treatment applications commenced for the different bio-additive products in accordance with the suppliers' recommendations. The complexity of application requirements is shown for the first tomato crop grown at Bundaberg (Appendix 1). Nitrate concentrations of sap expressed from the petiole of the youngest mature leaves from rockmelon plants grown in the 2 guard rows of each plot were measured with Merckoquant test strips read by a Nitracheck nitrate meter on 12 and 21 September 2001 at Bundaberg. These sample dates corresponded with early and late fruit set, respectively. Otherwise, sap nitrate testing was used throughout the trials on an informal basis to ensure nutrient applications were adequate for optimal growth.

Fruit from rockmelon and tomato plants were harvested from 10-centrally-positioned-plants grown in the centre row of each plot at Bundaberg. At Bowen, fruit were harvested from plants grown in the centre row within an 8m section for rockmelon or within a 6.75 m^2 section for tomato. Harvested fruit were graded into marketable and reject, counted and weighed. Marketability for tomato fruit was determined by Appendix 3 of the Tomato Style Allowances Tomato Quality Guide (QI97001, p.28), whereas rockmelons were deemed to be marketable if >700 grams and free from cracks, blemishes, and rot.

Brix° measurements (total soluble solids) were made using a refractometer. For melon grown at Bundaberg and Bowen, 5 randomly selected marketable fruit per plot at peak harvest were cut vertically, and a spoonful of flesh from the top (sweetest) and the bottom (blandest) of each half were combined, squeezed and read. Brix° values were also determined for the 2001 tomato trial at Bundaberg on 20 red full colour fruit sampled from the guard rows of the treatment plots at final harvest. A 4mm section was taken from the equatorial plane of each tomato fruit, and all 20 sections were then squeezed and read with a refractometer. A correction factor based on temperature was employed to derive 'true' Brix° readings for all samples.

Analysis of variance was used to test the effects of treatments. Treatment means were compared using the protected l.s.d. procedure operating at P=0.05.

4.3 Results

4.3.1 2000 Bowen trial – Rockmelon cv. Hammersley

Fruit yield and quality (Brix°) parameters measured for each of the treatments showed no differences at the 5% level of probability between treatments for fruit weight, fruit number, average fruit size, or % marketable fruit (Table 4.4). There was also no difference between treatments in terms of fruit quality as measured by Brix°.

Table 4.4 Bio-additive treatment effects on yield and quality parameters of rockmelon cv. Hammersley grown at Bowen Research Station in 2000.
There were no differences between treatments for any of the parameters at P=0.05.

Treatment	Marketable fruit weight (t/ha)	Marketable fruit number (per ha)	Average marketable fruit size (kg)	% Marketable fruit by weight	Average Brix°
Kelpak	44.2	26,042	1.70	93.7	9.9
Trichodry/Trichoflow'	44.9	24,792	1.80	94.5	10.5
E2001/Multibacter	45.2	27,083	1.70	96.0	10.3
Supa Humus	46.5	23,542	1.97	95.5	10.7
Nutri-Tech	47.1	27,083	1.74	92.0	9.9
Untreated Control	50.4	27,083	1.85	92.1	10.2
l.s.d. (P=0.05)	8.9	5,343	0.22	6.75	1.1

There were no visual differences observed between treatments throughout the growth of the crop. Disease problems encountered in the crop were gummy stem blight and late infections of downy mildew and mosaic virus. None of these diseases were associated with any particular treatment and were not deemed to have caused any significant yield reduction.

4.3.2 2001 Bowen trial – Tomato cv. Queensland Red

Fruit yield parameters measured for each of the treatments are shown in Table 4.5.

Table 4.5 Bio-additive treatment effects on yield parameters of tomato cv. Queensland Red at Bowen in 2001. Means within columns followed by the same letter are not significantly different at P=0.05 (F-test for treatments).

Treatment	Marketable fruit weight (t/ha)	Marketable fruit number (per ha)	Total fruit weight (t/ha)	Total fruit number (per ha)	Average marketable fruit size (g)	% Marketable fruit (by weight)
Supa Humus	84.5a	526,495a	87.1a	538,343a	158.9	96.9
Trichodry/Trichoflow	93.7ab	550,932ab	97.7ab	568,704ab	170.4	96.0
Nutri-Tech	97.9ab	570,925ab	101.9ab	589,438ab	171.6	96.0
Untreated Control	101.3ab	637,200b	104.3ab	653,121b	159.6	97.1
E2001/Multibacter	101.4ab	603,507ab	104.4ab	617,577ab	168.3	97.1
Kelpak	102.7b	603,137ab	106.1b	620,169ab	170.6	96.8
l.s.d. (P=0.05)	18.1	106,720	18.0	105,640	13.3	2.2

Marketable fruit weight for the 'Supa Humus' treatment was lowest, but significantly different from only the highest yielding treatment, 'Kelpak'. Marketable fruit number was also lowest for the 'Supa Humus' treatment but was only significantly lower than the 'Untreated Control' treatment which produced the highest marketable fruit number. Total fruit weight and number also showed this same trend with the 'Supa Humus' treatment yielding significantly less than the 'Kelpak' and 'Untreated Control' respectively. There were no significant treatment differences for the average marketable fruit size or % marketable fruit. It was planned that the trial at Bowen would be continued over the next two seasons with treatments being applied to the same plots to allow for any longer term effects to show. It was planned that the test crops would continue to be alternated between rockmelon and tomato crop species.

4.3.3 2000 Bundaberg trial – Tomato cv. Mercedes – autumn season

Despite the fact that the weight of marketable fruit produced by tomato plants grown with the Trichogrow/ Trichoflow, Kelpak, and E2001/ Multibacter treatments was 36, 34, and 27% greater, respectively, than plants grown with the control treatment, there was no difference (P=0.35) determined by ANOVA at the 5% level of significance (Table 4.6). It was suspected that irregular variation within the trial site, which could not be explained by the blocking effect, contributed to this effect. Furthermore, other yield parameters also did not differ at the 5% level of probability for tomato plants grown with the various bio-additive treatments.

Table 4.6 Bio-additive treatment effects on yield parameters of tomato cv. Mercedes at Bundaberg in 2000. There were no differences between treatments for any of the parameters at P=0.05.

Treatment	Weight of marketable fruit (t/ha)	Number of marketable fruit (per ha)	Total weight of all fruit (t/ha)	Average weight of a marketable fruit (g/ fruit)	% Marketable fruit (by weight)
Control	30.4	303,000	33.7	100.3	89.8
Nutri-Tech	35.1	354,000	39.6	99.3	88.8
Humega	37.1	371,333	41.5	99.7	89.0
E2001/Multibacter	38.5	396,000	42.8	96.8	90.0
Kelpak	40.7	403,667	45.4	100.6	89.3
Trichodry/grow	41.4	407,000	46.0	101.3	89.4
l.s.d. (P=0.05)	11.1	104,158	11.9	5.4	4.0

4.3.4 2000 Bundaberg trial – Rockmelon cv. Eldorado – spring season

For the yield parameters measured, there were no differences between treatments at P=0.05 (Table 4.7). Continual rain from 13 to 22 November 2000 limited the efficiency of fungicide applications. A severe outbreak of powdery mildew became endemic throughout the trial. The vigour of vines was limited by the fungal disease, and the resultant size of melon fruit was compromised.

Table 4.7 Bio-additive treatment effects on yield parameters of rockmelon cv. Eldorado at Bundaberg in 2000. There were no differences between treatments for any of the parameters at P=0.05.

Treatment	Weight of marketable fruit (t/ha)	Number of marketable fruit (per ha)	Total weight of all fruit (t/ ha)	Average weight of a marketable fruit (g/ fruit)	% marketable fruit by weight	Average Brix°
Untreated Control	30.7	16,333	41.8	1,878	73.3	5.42
Trichodry/Trichoflow`	33.8	19,000	44.5	1,773	74.0	5.60
E2001/Multibacter	34.0	18,667	41.4	1,815	82.4	6.26
Nutri-Tech	35.5	19,333	43.3	1,856	80.1	5.55
Humega	40.8	22,000	48.6	1,858	84.0	5.52
Kelpak	44.5	25,000	51.7	1,784	86.4	5.88
l.s.d. (P=0.05)	13.2	7,053	12.3	139	14.4	1.18

4.3.5 2001 Bundaberg trial – Tomato cv. Queensland Red – autumn season

There were no differences between treatments at P=0.05 for any of the yield or fruit quality (Brix°) parameters measured (Table 4.8). Several transplanted seedlings were replaced due to scorching around the base of the plants on 26/2/01. There was an endemic presence of pith necrosis or hollow stem on plants late in the trial. This disease is caused by the bacterium *Pseudomonas corrugata*. Dark brown to black necrotic areas appeared on the stems of some plants accompanied by profuse development of adventitious roots. Typically, a high incidence of the disease is associated with succulent plant growth caused by high N fertilisation and low night temperatures as winter approached. However, the disease symptoms were not associated with any treatment in particular and were not deemed to have caused any biased reduction in yield.

Table 4.8 Bio-additive treatment effects on yield parameters of tomato cv. Queensland Red at Bundaberg in 2001. There were no differences between treatments for any of the parameters at P=0.05.

Treatment	Weight of marketable fruit (t/ha)	Number of marketable fruit (per ha)	Total weight of all fruit (t/ ha)	Average weight of a marketable fruit (g/ fruit)	% marketable fruit by weight	Average Brix°
Untreated Control	54.5	448,333	64.5	121	84.5	4.59
Humega	58.4	455,333	68.7	128	84.9	4.71
Nutri-Tech	59.5	519,333	71.0	115	83.4	4.51
Trichodry/grow	61.8	507,000	73.9	122	83.8	4.76
E2001/ Multibacter	62.8	527,333	75.4	119	83.2	4.66
Kelpak	70.8	576,667	82.8	123	85.5	4.47
l.s.d. (P=0.05)	14.4	98,008	16.2	10.6	2.33	0.28

4.3.6 2001 Bundaberg trial – Rockmelon cv. Eastern Star – spring season

For the various parameters measured, including fruit yield and quality and petiole sap nitrate concentrations, there were no differences between treatments at P=0.05 (Table 4.9). The sap nitrate measurements at early (12/9/01) and late (21/9/01) fruit set indicated that N was not limiting to the optimal growth of the melon crop (DPI Agrilink kit).

Table 4.9 Bio-additive treatment effects on yield parameters of rockmelon cv. Eastern Star at Bundaberg in 2001. There were no differences between treatments for any of the parameters at P=0.05.

Treatment	Wt of mkt fruit (t/ha)	Number of marketable fruit (per ha)	Total weight of all fruit (t/ ha)	Av wt of a mkt fruit (g/ fruit)	% mkt fruit by weight	Brix°	Sap nitrate 12/9/01 (mg/L)	Sap nitrate 21/9/01 (mg/L)
Trichodry/grow	39.3	28,333	43.7	1,418	90.4	9.9	5,390	4,065
Nutri-Tech	41.3	30,333	43.2	1,366	95.7	10.2	4,895	3,820
Humega	41.4	30,333	46.6	1,362	88.4	10.3	5,625	3,910
Untreated Control	42.8	29,667	45.9	1,439	93.3	9.9	5,225	3,985
Kelpak	44.4	32,000	47.7	1,386	92.7	10.7	5,195	3,755

E2001/ Multibacter	47.7	33,333	49.9	1,430	95.4	10.3	5,275	4,040
l.s.d. (P=0.05)	8.1	7,089	8.6	117	5.8	1.3	567	299

4.4 Discussion

Despite the fact that Kelpak was ranked in the top 2 treatments for weight of marketable fruit in 5 out of the 6 trials, differences were only significant for the 2001 tomato trial at Bowen, and then only between Kelpak and Supa Humus. This lack of difference between treatments for the yield and quality parameters that were measured indicated that growers using these products would be unlikely to see any visible effects to the crop.

Considerable variation was experienced in the trials. Although the weight of marketable fruit was ranked highest for melon plants grown with the untreated control treatment at Bowen in 2000, this parameter was ranked lowest for the same treatment in 3 of the 4 trials conducted at Bundaberg.

It was planned that the field trials at Bowen and Bundaberg would continue for another 2 years, with treatments being applied to the same plots to allow for any longer term effects to show. Furthermore, exposure to a greater range of seasonal and environmental conditions than was encountered in these trials, would have provided a more comprehensive assessment of the products. The test crops would have been alternated between rockmelon and tomato at Bowen, although at Bundaberg, it was envisaged that both a tomato and a rockmelon crop would be grown each year.

The reason for allocating permanent beds to each bio-additive treatment was to assess the cumulative and temporal effects for tomato and melon crops in different seasons and environmental conditions. It was expected that one particular product would not be best in all situations. For example, a *Trichoderma* product may be beneficial during a particularly wet season which might favour the soil-borne pathogen *Pythium*, but may have little effect during a normal season. The premature termination of these bio-additive trials at Bowen and Bundaberg have therefore limited the ability to make a full assessment of the commercial products under investigation.

Despite anecdotal evidence aplenty, the absence of comprehensive field studies on bio-additive products of the kind undertaken in this project can be explained by the quantity and meticulous nature of the work required to conduct such experiments (refer to Appendix 1). The fact that our team conducted the work at 2 geographically diverse sites makes the work even more extraordinary.

5. Lycopene assessment

An initial assessment of the lycopene concentration of tomato fruit at different stages of maturity was conducted under different storage conditions. It was proposed that this work would be used as a reference for more comprehensive lycopene assessment later in the project.

The change in concentrations of lycopene was measured at six stages of maturity, based on the DPI colour grading chart; viz., mature green, ¼ colour, ½ colour, ¾ colour, coloured, and full colour.

Three ripening treatments were tested:
(1) fruit ripened on the vine,
(2) fruit ripened in darkness at 20 °C in a standard cool room or
(3) fruit ripened in darkness at 20 °C in a cool room with a modified atmosphere of 200 mg/ L ethylene.

The tomato cv. Danielle plants were grown under standard cultural practices at Bundaberg Research Station (24°51'S, 152°24'E), Queensland, Australia. The plants were trellised and were grown in 9 rows of length 95 m running in a N-S direction. The field area was divided into 4 blocks/ replicates, each comprising 9 adjacent rows of length 20 m. Only fruit exposed to direct sunlight, either fully or partially at some stage during the day, were harvested from the lowest truss of each plant.

For each block/ replicate of tomato plants, fruit were randomly harvested at the mature green stage and placed in 1, 5, or 7 crates of 50 fruit for Treatments 1, 2, or 3, respectively. The crate of fruit selected for Treatment 1 was representative of the mature green stage and was immediately measured. For Treatments 2 and 3, the crates of fruit were grouped vertically into separate stacks in the appropriate cool room (crate order in stack re-arranged daily) corresponding with the replicate/ block of plants from where the fruit were harvested. For each block (stack) x treatment combination, one crate of fruit was selected randomly for fruit measurement at each of the 5 remaining designated stages of maturity. To simulate commercial practice for gas ripening, the 2 extra crates of fruit in each block/ replicate of Treatment 3 were transferred to Treatment 2 when the tomato fruit attain ¾ colour; the 2 crates of fruit from each replicate were then be randomly assigned for measurement at the coloured and full colour stages.

For Treatment 1, 50 vine-ripened fruit were harvested from each block/ replicate at each stage of maturity and processed immediately.

For each block x treatment combination, the 14 most-uniformly-ripened fruit per crate which most closely match the designated stage of maturity were selected for measurement. Three equatorial sections (3 mm thick) were then cut from each of the selected fruit. The sections cut from the middle of each of the 14 fruit were frozen immediately with liquid N, stored at –18 °C, and analysed for lycopene. Lycopene was determined by HPLC under subdued light conditions.

The factorial design comprised 3 ripening treatments x 6 stages of fruit maturity with 4 replicates/ blocks. Additional comparisons were made from the extra fruit which were transferred from Treatment 3 to Treatment 2 at ¾ colour.

Lycopene concentration increased exponentially with each stage of colour ripeness, irrespective of ripening treatment (Table 5.1). At the full colour stage, the highest lycopene concentrations appeared to be associated with vine ripened fruit, whereas storage in a cold room, and ethylene room, or a combination of the 2 did not affect the values obtained. The work showed that the best time to sample fruit for lycopene assessment would be at the full colour stage from vine ripened fruit.

Table 5.1 Summary of lycopene concentration (mg/ kg) of tomatoes stored under different conditions and vine ripened.

Treatment	Block	Mature green	¼ colour	½ colour	¾ colour	Coloured	Full Colour
Vine	1	0	1.9	2.8	4.5	6.0	27.9
Ripened	2	0	1.0	3.0	4.7	6.6	25.0
	3	0	1.2	3.2	3.7	6.6	27.0

	4	0	1.0	2.8	3.9	6.7	19.8
Cold Room (20°C)	1	-	1.9	3.3	2.4	5.1	17.2
	2	-	2.1	3.2	2.5	6.3	19.1
	3	-	1.4	2.0	1.2	4.5	10.3
	4	-	1.1	1.0	1.4	3.1	14.4
Ethylene (20°C)	1	-	1.2	2.2	3.6	4.3	15.4
	2	-	1.5	2.5	3.0	4.9	11.8
	3	-	1.3	1.6	3.8	5.0	13.1
	4	-	0.9	2.2	4.4	5.5	13.0
Ethylene → 20° Room	1	-	-	-	-	6.0	14.7
	2	-	-	-	-	6.1	15.6
	3	-	-	-	-	5.0	12.2
	4	-	-	-	-	4.3	19.8

6. Technology transfer

Much of the extension activities for this project were scheduled to take place near the end of the project, once the full effect of the treatments were to have had their best chance to take effect. Indeed, the Milestone 6, due on 31 October 2002, was to report on field days to disseminate the findings of the project to industry. These field days were to be held at the Bowen and Bundaberg trial sites during the 2002 cropping season.

One of the main extension strategies proposed for the project was the do our own research (DOOR) methodology. Information relating to setting up the DOOR methodology in this project has been previously reported in milestone 3 (report submitted on 31 January 2001), which has been forwarded to QFVG and Horticulture Australia Ltd.

In order to identify potential DOOR participants, the names and addresses of 103 tomato and/ or melon growers in the Bundaberg district were sourced from records of local grower associations. A covering letter and the DOOR questionnaire were mailed to each grower on 27 April 2000, along with a stamped envelope addressed to Dr Hunter (DOOR coordinator). There were 16 respondents, 6 of whom were interested in registering for the DOOR workshop. Results from the questionnaire showed that only one-quarter of the respondents derived 100% of their annual income from fruit and/or vegetable production, and the most important topics requiring research were deemed to be integrated pest management, disease control, and nutrition.

For research personnel, both within and external to the project who wished to be involved in the DOOR process as consultants (viz. providing input in designing experiments, analysing and interpreting data, and working with operators to clarify issues, make decisions and formulate recommendations), a workshop (5 participants) was conducted by Dr Hunter at Bundaberg Research Station on 26 June 2000.

Despite contacting by phone the week before the DOOR operators' workshop the 6 growers who indicated that they were interested in participating, only 3 attended. An experiment, which had been designed and set up to show the components of a typical factorial trial, was demonstrated to participants. Methods for obtaining information were discussed by DPI Information Extension Officer Vikki Lane, and details of the DOOR process were presented by Dr Mal Hunter. All participants were enthusiastic about being involved in the DOOR process, however, follow-up phone conversations with the growers a couple of weeks later indicated that a paucity of available time would be a major impediment to their involvement. The project leaser pursued the matter further with a leading tomato grower in the district, who said that the complexities of undertaking research and the time demand on growers who are already time-limited were constraints to growers becoming involved in the DOOR process. Indeed, the grower stated that if operators of large farms were interested in conducting research, they would employ someone to do the job, rather than do it themselves. The fact that only one-quarter of the respondents to the DOOR questionnaire derived 100% of their annual income from fruit and vegetable production indicated that managers of large farms did not give a high priority to participating in the DOOR process.

The DOOR model has been used successfully in the nursery industry for simple experiments conducted in small pots which can be replicated easily. However, fully replicated factorial experiments conducted with large field plots on commercial farms take considerable time and effort to set up and maintain. For field trials involving regular applications of substances injected through the trickle irrigation system, as would be the case in assessing the effect of commercial bio-additive products on soil health, a complex plumbing system would be required and considerable time and effort would be necessary each week to apply treatments. Therefore, the DOOR model would appear to lend itself more readily to some industries than to others, based on the ease with which experiments could be conducted.

Despite the effort that was invested into the DOOR component of the project, it was apparent that the model was not adopted by the grower groups that were selected. Since the DOOR method required growers to undertake research work themselves, for themselves, their lack of available time seemed to be the major impediment to the adoption process.

Therefore, the funds allocated for the DOOR component of VX99043 were given back to Horticulture Australia in 2001, and the DOOR methodology was removed from the project. Dr Hunter indicated that should any grower be interested in the future in conducting their own research, he could provide them with a recipe to conduct an experiment.

7. Recommendations

That the money for the full term of the project be provided to complete the work.

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Appendix 1. Application methodology used in the bio-additive field trials (schedule for the first tomato crop grown at the Bundaberg).

E2001/MULTIBACTER

Bio-additive trial – methodology for application of E2001/ Multibacter

INSTRUCTIONS FOR MAKING UP THE FORMULATIONS:

Use the following methodology to produce the ***brewed E2001/ Multibacter formulation***:

1. Mix E2001:Multibacter: bore water in the ratio of 1:1:100, by volume.
For a rate of 1L E2001 and 1 L Multibacter per ha (10,000 m^2),
=➔ mix 4.5 mL E2001, 4.5 mL Multibacter and 450 mL bore water for each 45 m^2 plot.
2. Let the mixture stand for 3 days, out of direct sunlight.
3. Add more Multibacter to the mixture at the rate of 5 L/ ha
=➔ add 22.5 mL Multibacter to the mixture for each 45 m^2 plot.
4. Make the mixture up to a volume of 10 L with bore water and apply to each plot through the trickle system.

Use the following methodology to produce the ***straight Multibacter formulation***:

1. For a rate of 3 L Multibacter per ha (10,000 m^2),
=➔ mix 13.5 mL Multibacter and 10 L bore water for each 45 m^2 plot.
2. Apply the mixture to each plot through the trickle system.

INSTRUCTIONS FOR APPLYING THE FORMULATIONS:

For gourmet tomatoes, apply to moist soil through the trickle system the ***brewed E2001/ Multibacter formulation*** at planting and again at 3 weeks prior to the first fruit harvest. Apply through the trickle system at weekly intervals the ***straight Multibacter formulation*** from 3 weeks after transplanting to 3 weeks before the final fruit harvest.

Bio-additive E2001 / Multibacter Work Schedule		
DATE	**TASK**	**RATE**
08-Apr-00	Brew E201/Multibacter solution for 3 days.	Mix 4.5mL E2001 + 4.5mL Multibacter to 450mL of bore water per 45 m^2 plot
11-Apr-00	Plant tomato seedlings	Plant tomatoes @ 50cm spacings using water wheel planter.
11-Apr-00	Add to the brewed solution 22.5mL of Multibacter and inject into trickle plot lines	Add 22.5mL of Multibacter to the brewed solution.
03-May-00	Inject straight Multibacter formulation into trickle plot lines.	Mix 13.5mL of Multibacter to 10L of bore water per 45m^2 plot.
10-May-00	Inject straight Multibacter formulation into trickle plot lines.	Mix 13.5mL of Multibacter to 10L of bore water per 45m^2 plot.
17-May-00	Inject straight Multibacter formulation into trickle plot lines.	Mix 13.5mL of Multibacter to 10L of bore water per 45m^2 plot.
24-May-00	Inject straight Multibacter formulation into trickle plot lines.	Mix 13.5mL of Multibacter to 10L of bore water per 45m^2 plot.
31-May-00	Inject straight Multibacter formulation into trickle plot lines.	Mix 13.5mL of Multibacter to 10L of bore water per 45m^2 plot.
07-Jun-00	Inject straight Multibacter formulation into trickle plot lines.	Mix 13.5mL of Multibacter to 10L of bore water per 45m^2 plot.
14-Jun-00	Inject straight Multibacter formulation into trickle plot lines.	Mix 13.5mL of Multibacter to 10L of bore water per 45m^2 plot.

21-Jun-00	Inject straight Multibacter formulation into trickle plot lines.	Mix 13.5mL of Multibacter to 10L of bore water per 45m² plot.
28-Jun-00	Inject straight Multibacter formulation into trickle plot lines.	Mix 13.5mL of Multibacter to 10L of bore water per 45m² plot.
05-Jul-00	Inject straight Multibacter formulation into trickle plot lines.	Mix 13.5mL of Multibacter to 10L of bore water per 45m² plot.
12-Jul-00	*Brew E201/Multibacter solution for 3 days*	*Mix 4.5mL E2001 + 4.5mL Multibacter to 450mL of bore water per 45m² plot*
14-Jul-00	*Add to the brewed solution 22.5mL of Mutltibacter and inject into trickle plot lines*	*Add 22.5mL of Multibater to the brewed solution.* ***(3 weeks before final harvest).***
19-Jul-00	Inject straight Multibacter formulation into trickle plot lines.	Mix 13.5mL of Multibacter to 10L of bore water per 45m² plot.
26-Jul-00	Inject straight Multibacter formulation into trickle plot lines.	Mix 13.5mL of Multibacter to 10L of bore water per 45m² plot.
02-Aug-00	Inject straight Multibacter formulation into trickle plot lines.	Mix 13.5mL of Multibacter to 10L of bore water per 45m² plot.
09-Aug-00	Inject straight Multibacter formulation into trickle plot lines.	Mix 13.5mL of Multibacter to 10L of bore water per 45m² plot.
16-Aug-00	Inject straight Multibacter formulation into trickle plot lines.	Mix 13.5mL of Multibacter to 10L of bore water per 45m² plot.
23-Aug-00	Inject straight Multibacter formulation into trickle plot lines.	Mix 13.5mL of Multibacter to 10L of bore water per 45m² plot.
30-Aug-00	Inject straight Multibacter formulation into trickle plot lines.	Mix 13.5mL of Multibacter to 10L of bore water per 45m² plot.

Ceased injection of Straight Multibacter formulation 3 weeks before final fruit harvest
Three weeks prior to first fruit harvest, E2001 / Multibacter formulation was applied.

TRICHODRY/ TRICHOGROW

Bio-additive trial—methodology for application of Trichodry/ Trichogrow

INSTRUCTIONS FOR MAKING UP THE FORMULATIONS:

Use the following methodology to mix Trichodry in growing media or compost

1. Make up growing media as per formulation.
2. Incorporate Trichodry to the growing media at the rate of 1kg of Trichodry per cubic metre.
=>> mix 1g of Trichodry to 1,000 cm³ of growing media for each 45m² plot.
3. Sow seeds into Trichodry growing media formulation.

Use the following methodology to pre mix Trichogrow:

1. Trichogrow to be applied in the planting water at the rate of 150g per 500 L water.
2. 100mL to 250mL to drench the planting hole as each tomato seedling is planted.

APPLYING TRICHOGROW FOLLOWING TRANSPLANTING(RATES & METHODS):

1. Apply Trichogrow at the rate of 1.5kg/ha every 21 days throughout the growing period. Apply 6.75g diluted in 5L of water to each treatment plot every 21 days.
2. Trichogrow to be injected through the trickle line.
3. NOTE: WHEN CROP IS UNDER STRESS, APPLICATION SHOULD BE REDUCED TO 14 DAYS BETWEEN APPLICATIONS.

Application of Trichogrow

DATE	TASK	RATE
11-Apr-00	Drench each tomato seedling hole with Trichgrow as seedling is planted with the waterwheel.	21g of Trichogrow diluted in 70L water applied @ 250mL per waterwheel planting hole.

3-May-00	Inject Trichogrow through the trickle line.	=>mix 6.75g of Trichogrow diluted in 10L of water for each 45m² plot.
24-May-00	Inject Trichogrow through the trickle line.	=>mix 6.75g of Trichogrow diluted in 10L of water for each 45m² plot.
14-Jun-00	Inject Trichogrow through the trickle line.	=>mix 6.75g of Trichogrow diluted in 10L of water for each 45m² plot.
5-Jul-00	Inject Trichogrow through the trickle line.	=>mix 6.75g of Trichogrow diluted in 10L of water for each 45m² plot.

NUTRI-TECH SOLUTIONS

Nutri-Tech Solutions Methodology Program

Nutri-Tech Solutions program – Graeme Sait. These Methods confirmed by Graeme Sait by phone 10 April 2000.

Nutri –Tech Solutions trial plot (4 plots, each at $45m^2 = 180m^2$).
SEEDLING TREATMENT:---before planting

1. To 2 L of water, add 90mL of **Tom – Tech Triple Ten** and 90mL of molasses.
2. Add to the mixture 18mL of **Nutri – Life Bio-N** and 18mL of **Nutri – Life Bio-P.**
3. Stand in the mixture for 3 hours one tray of 198 seedlings, immediately before transplanting.

N.B. It is important that the **Tom – Tech Triple Ten** and the molasses be diluted in the 2 L water before adding the **Bio-N and Bio-P.** This procedure is important because the Triple Ten concentrate contains a stabiliser which could affect the microbes in the undiluted form.

Soil treatment: (this is applied once only after transplanting).

Note: The **Nutri – Life 4/20** needs to be brewed for 24 hours before application.
1. To 10 L of warm water, add 12.5 g **Nutri – Life 4/20** and 75 g of **Microbe Food**. Aerate the mixture with an aquarium pump for 24 hours.
2. To the brewed solution, add
   - 45 mL of **Nutri – Life Bio-N**
   - 45 mL of **Nutri – Life Bio-P**
   - 180 mL of **Cal – Tech Amino Chelated Liquid Calcium**
   - 450 mL of **Molasses**, and mix thoroughly.
3. Divide the above brewed solution into 4 equal volumes, and dilute each part to 10 L with water.
4. Immediately after transplanting, apply through the irrigation the 4 dilutions, one to each $45m^2$ plot.

Soil Treatment: (every 3 wks)
1. Apply 45 mL **Cal – Tech** and 67.5 mL **molasses**, diluted to 10 L with water, to each $45m^2$ plot every 3 weeks through the irrigation. (Note: These repeat applications are intended as an ongoing food source for the Micro-organisms to stimulate maximum activity. The soluble Calcium input is also intended to help counteract the poor Calcium/Magnesium ratio in the trial plot.)

Foliar program:
Begin 2 weeks after transplanting and repeat every 20 days.
Note: The **Nutri – Life 4/20** needs to be brewed for 24 hours before application.
1. To 2.7 L of warm water, add 7 g **Nutri – Life 4/20** and 20.3 g of **Microbe Food**. Aerate the mixture with an aquarium pump for 24 hours.
2. Add the following ingredients to 5.4 L of water:
   - 9 mL of **Nutri – Life Bio-Plex**
   - 2.7 L of brewed **Nutri – Life 4/20**
   - 45 mL of **Molasses**
   - 54 mL of **Tom – Tech Triple Ten**
3. Divide the above mixture into 4 equal volumes.
4. Apply the 4 equal volumes (2 L) onto the foliage of the tomato plants growing in each of the $45m^2$ plots.

Important Handling Instructions:
The **Nutri – Life** range will be supplied in 250 mL containers. This product is stable for up to 2 years until the container is opened. After the seal has been broken there is the potential of contamination and the opened container should be refrigerated after use.

NUTRI-TECH SOLUTIONS APPLICATION SCHEDULE

DATE	***TASK***	***RATE***
10-Apr-2000	Brew up soil treatment using Nutri-Life 4/20 for 24 hrs as per soil treatment program.	As per NUTRI-TECH soil treatment program.
11-Apr-2000	3 hrs prior to planting, soak seedling trays in NUTRI-TECH seedling treatment solution	As per NUTRI-TECH seedling treatment program.
11-Apr-2000	To brew soil treatment solution add the other soil treatment ingredients as per program and divide into four equal volumes and inject each into the four plots.	As per NUTRI-TECH soil treatment program.
27-Apr-2000	24 hrs prior to applying foliar spray brew up NUTRI-TECH solution as per Foliar program	AS per NUTRI-TECH foliar treatment program.
28-Apr-2000	Divide brewed foliar treatment solution into four equal volumes and apply to foliage of tomatoes in treatment plots.	AS per NUTRI-TECH foliar treatment program.
03-May-2000	Apply soil treatment solution (Cal-Tech & molasses) as per soil treatment program by injection into trickle line.	As per NUTRI-TECH soil treatment program.
17-May-2000	24 hrs prior to applying foliar spray brew up NUTRI-TECH solution as per Foliar program	AS per NUTRI-TECH foliar treatment program.
18-May-2000	Divide brewed foliar treatment solution into four equal volumes and apply to foliage of tomatoes in treatment plots.	AS per NUTRI-TECH foliar treatment program.
24-May-2000	Apply soil treatment solution (Cal-Tech & molasses) as per soil treatment program by injection into trickle line.	As per NUTRI-TECH soil treatment program.
06-Jun-2000	24 hrs prior to applying foliar spray brew up NUTRI-TECH solution as per Foliar program	AS per NUTRI-TECH foliar treatment program.
07-Jun-2000	Divide brewed foliar treatment solution into four equal volumes and apply to foliage of tomatoes in treatment plots.	AS per NUTRI-TECH foliar treatment program.
14-Jun-2000	Apply soil treatment solution (Cal-Tech & molasses) as per soil treatment program by injection into trickle line.	As per NUTRI-TECH soil treatment program.
26-Jun-2000	24 hrs prior to applying foliar spray brew up NUTRI-TECH solution as per Foliar program	AS per NUTRI-TECH foliar treatment program.
27-Jun-2000	Divide brewed foliar treatment solution into four equal volumes and apply to foliage of tomatoes in treatment plots.	AS per NUTRI-TECH foliar treatment program.
05-Jul-2000	Apply soil treatment solution (Cal-Tech & molasses) as per soil treatment program by injection into trickle line.	As per NUTRI-TECH soil treatment program.
18-Jul-2000	24 hrs prior to applying foliar spray brew up NUTRI-TECH solution as per Foliar program	AS per NUTRI-TECH foliar treatment program.
19-Jul-2000	Divide brewed foliar treatment solution into four equal volumes and apply to foliage of tomatoes in treatment plots.	AS per NUTRI-TECH foliar treatment program.
26-Jul-2000	Apply soil treatment solution (Cal-Tech & molasses) as per soil treatment program by injection into trickle line.	As per NUTRI-TECH soil treatment program.
08-Aug-2000	24 hrs prior to applying foliar spray brew up NUTRI-	AS per NUTRI-TECH foliar treatment

	TECH solution as per Foliar program	program.
09-Aug-2000	Divide brewed foliar treatment solution into four equal volumes and apply to foliage of tomatoes in treatment plots.	AS per NUTRI-TECH foliar treatment program.
16-Aug-2000	Apply soil treatment solution (Cal-Tech & molasses) as per soil treatment program by injection into trickle line.	As per NUTRI-TECH soil treatment program.

HUMEGA (HUMIC ACID)

Bio-additive trial - methodology for application of BioFlora Humega (Humic Acid).

INSTRUCTIONS FOR MAKING UP THE FORMULATION

1. Pre-plant rates for Humega:- 20 litres per Acre => mix 222.5mL of Humega to 10L of water for each 45m² plot.
2. After planting rates for Humega:-5 litres per Acre. => mix 55.5 mL of Humega to 10L of water for each 45m² plot.

INSTRUCTIONS FOR APPLING HUMEGA:

Apply pre-plant rate by injecting through the trickle irrigation system prior to planting.
Apply after planting by injecting through the irrigation system at weekly intervals until the end of harvest.

Bio-additive Humega Work Schedule

DATE	TASK	RATE
11-Apr-00	Per-plant injection of Humega into trickle irrigation system.	=> mix 222.5mL of Humega to 10L of water for each 45m² plot.
19-Apr-00	Field injection of Humega into trickle irrigation system.	=> mix 55.5 of Humega to 10L of water for each 45m² plot.
26-Apr-00	Field injection of Humega into trickle irrigation system.	=> mix 55.5 of Humega to 10L of water for each 45m² plot.
03-May-00	Field injection of Humega into trickle irrigation system.	=> mix 55.5 of Humega to 10L of water for each 45m² plot.
10-May-00	Field injection of Humega into trickle irrigation system.	=> mix 55.5 of Humega to 10L of water for each 45m² plot.
17-May-00	Field injection of Humega into trickle irrigation system.	=> mix 55.5 of Humega to 10L of water for each 45m² plot.
24-May-00	Field injection of Humega into trickle irrigation system.	=> mix 55.5 of Humega to 10L of water for each 45m² plot.
31-May-00	Field injection of Humega into trickle irrigation system.	=> mix 55.5 of Humega to 10L of water for each 45m² plot.
07-Jun-00	Field injection of Humega into trickle irrigation system.	=> mix 55.5 of Humega to 10L of water for each 45m² plot.
14-Jun-00	Field injection of Humega into trickle irrigation system.	=> mix 55.5 of Humega to 10L of water for each 45m² plot.
21-Jun-00	Field injection of Humega into trickle irrigation system.	=> mix 55.5 of Humega to 10L of water for each 45m² plot.
28-Jun-00	Field injection of Humega into trickle irrigation system.	=> mix 55.5 of Humega to 10L of water for each 45m² plot.
5-Jul-00	Field injection of Humega into trickle irrigation system.	=> mix 55.5 of Humega to 10L of water for each 45m² plot.
12-Jul-00	Field injection of Humega into trickle irrigation system.	=> mix 55.5 of Humega to 10L of water for each 45m² plot.
19-Jul-00	Field injection of Humega into trickle irrigation system.	=> mix 55.5 of Humega to 10L of water for each 45m² plot.

26-Jul-00	Field injection of Humega into trickle irrigation system.	=> mix 55.5 of Humega to 10L of water for each 45m² plot.
02-Aug-00	Field injection of Humega into trickle irrigation system.	=> mix 55.5 of Humega to 10L of water for each 45m² plot.
09-Aug-00	Field injection of Humega into trickle irrigation system.	=> mix 55.5 of Humega to 10L of water for each 45m² plot.
16-Aug-00	Field injection of Humega into trickle irrigation system.	=> mix 55.5 of Humega to 10L of water for each 45m² plot.
23-Aug-00	Field injection of Humega into trickle irrigation system.	=> mix 55.5 of Humega to 10L of water for each 45m² plot.
28-Jun-00	Field injection of Humega into trickle irrigation system.	=> mix 55.5 of Humega to 10L of water for each 45m² plot.

KELPAK

Methodologies for the mixing and applying KELPAK. Tomatoes Autumn 2000

RATES FOR TRANSPLANTING:
Use the following methodology to make up the Kelpak:

1. Mix 1mL of Kelpak to 100mL of water for each 45m² plot to be applied at transplanting.

RATES FOR FOLIAR APPLICATION:

1. Mix Kelpak @ the rate of 2.0L/ha and apply as a foliar spray. For each 45m² plot, mix 9mL of Kelpak to 2.5L of water and apply as a foliar spray.(FOLIAR SPRAY TO BE APPLIED 14 DAYS AFTER TRANSPLANTING AND REPEAT THE APPLICATION TWICE AT 14-DAY INTERVALS.)

INSTRUCTIONS FOR APPLING KELPAK:

1. At transplanting, apply Kelpak at 1mL to 100mL of water. Using a 60mL syringe, applying two 50mL drenches to each seedling making 100mL per plant.
2. To apply Kepak as a foliar spray mix the 9mL of Kelpak to 2.5mL of water and apply to each 45m² plot using a knapsack and lance.

DATE	TASK	RATE
11-Apr-00	Apply Kelpak to tomato seedlings after transplanting with a 60mL syringe.	1mL of Kelpak diluted in 100mL of water.
28-Apr-00	Apply foliar spray to treatment plots with knapsack.	9mL of Kelpack diluted in 2.5L of water.
12-May-00	Apply foliar spray to treatment plots with knapsack.	9mL of Kelpack diluted in 2.5L of water.
26-May-00	Apply foliar spray to treatment plots with knapsack.	9mL of Kelpack diluted in 2.5L of water
09-Jun-00	Apply foliar spray to treatment plots with knapsack.	9mL of Kelpack diluted in 2.5L of water.
23-Jun-00	Apply foliar spray to treatment plots with knapsack.	9mL of Kelpack diluted in 2.5L of water.
07-Jul-00	Apply foliar spray to treatment plots with knapsack.	9mL of Kelpack diluted in 2.5L of water.

CONTROL

Bio-additives methods and application for Control treatment
No additives to be applied to the Control plot.
Fertiliser to be applied as per fertigation program.