

Promoting sustainable and productive agriculture with organic and non-toxic solutions

**Suitable for use in all growing situations
including hydroponics**

Contents

- 1) Introduction
- 2) Composition of BioAlexin
- 3) Features and benefits
- 4) An insight into the technology
- 5) Areas of application and examples of commercial use
- 6) Examples of successful containment of various pathogens and parasites using ProAlexin as a plant nutrient synergist/fertiliser.
- 7) Examples of successful field trials
 - Apples
 - Strawberries
 - Cucumbers
 - Tomatoes
 - Plums
 - Grapes
- 8) A list of relevant literature references

1. Introduction

Biological Solutions has developed technologies (patent applied for) to assist Agronomists to utilise naturally derived materials as replacements for conventional synthetically produced products.

The product range is trade marked as **BioAlexin** and has now been successfully commercialised in different parts of the world, including the European Union, South Africa, India, New Zealand and Australia.

The following brochure is designed to give more detail on the products (**ProAlexin™**) and further background to the technology and its use in various agronomic applications.

If you should require any further information, please contact us via our website where contact details are available.

1. Composition of BioAlexin

A Mixture of:

- Citrus biomass
- Botanical extracts rich in vitamin C
- Palm oil extracts
- Inert carriers

2. Features and Benefits

Features

- Derived from natural resources
- A natural alternative to pesticides and fungicides.
- **BioAlexin** products aim to assist the Agronomist to achieve gains without the use of synthetic and often toxic chemicals, pesticides and fungicides.
- **BioAlexin** is designed to activate, and enhance synergistically, the plants natural production of phytoalexins, allowing the plant to respond to the risk of damage from plant-attacking pathogens.

Benefits

- Activates the production of phytoalexins by supplying the basic nutrients.
- Improves plant health and vigour
- Improves the intake of foliar applied nutrients
- Increases crop quality and yields
- Reduces the dependency on traditional agrochemicals (synthetic fertilisers, fungicides and pesticides)
- Leads to positive effect on brix levels
- Manufactured using natural ingredients
- Far less impact on the environment: organic compliant and completely biodegradable
- Can be applied just before or during harvest
- Does not alter the taste of produce, has no irritant odour and has no residual withholding periods when used at manufacturers recommendations
- Non-toxic, non-corrosive to expensive plant and equipment, reducing maintenance costs

Enhanced Nutrient Properties

TISSUE ANALYSIS			
Analysis	Test Plot (1) Treated with Fertilisers (only)	Test Plot (2) Treated with Fertilisers and ProAlexin™	% Variance between Plots (1) and (2)
PROTEIN	10.4%	15.0%	+44%
NITROGEN	1.7%	2.32%	+35%
CALCIUM	0.25%	0.28%	+12%
MAGNESIUM	0.15%	0.21%	+40%
PHOSPHOROUS	0.6%	0.65%	+8%
POTASSIUM	2.7%	2.8%	+4%
IRON	80ppm	85ppm	+6%
MAGANESE	18ppm	20ppm	+11%
COPPER	9ppm	10ppm	+11%
ZINC	30ppm	35ppm	+17%
BORON	12ppm	16ppm	+33%
SULPHUR	0.15%	0.17%	+13%

3. An Insight to the Technology

Bioflavonoids and their synergistic effects in combination with Vitamin C

Flavonoids are bio-chemical compounds (polyphenols) that are naturally produced by plants. Once produced by the plant naturally they have antioxidant and anti-microbial properties, and also help decrease capillary fragility and restore normal permeability in plant structures.

Citrus flavonoids are found in citrus plant varieties, e.g. grapefruit (Citrus Paradise), bitter orange (Bergamot), tangerine (Citrus Reticulata), sweet orange (Citrus Sinensis), etc.

- Flavonoids in combination with Vitamin C (Ascorbic Acid) show synergistic effects, allowing the plant naturally to suppress pathogenic attack and preserving the natural flavour of vegetables and fruits.
- The high reducing power of Ascorbic Acid gives it the character of a stabilizer towards readily oxidizable substances that are present in the fruit and vegetables. There are also important metabolism relationships between Ascorbic Acid, other vitamins and enzymes, which manifest themselves in different actions. The micro-biological activity of Ascorbic Acid, which is based, in particular on inactivation of toxins, is possibly connected with these mechanisms.
- The use of extracts from palm kernel oil produces a buffering effect which optimizes the specific effects between flavonoids and vitamin C (Ascorbic Acid).
- Underpinning Scientific research was initiated in the 1930's by Prof. Szent-Gyorgyi (awarded Nobel Prize for medicine) and Prof. Gabor (discovered the biological importance of flavonoids). Gabor demonstrated the synergistic qualities of bioflavonoids when used in conjunction with Vitamin C (Ascorbic Acid). The combination produces:- powerful antioxidants produced within the plant, absorbers of free radicals and significant anti-microbial, anti-viral properties that are produced naturally by the plant.
- The application of Vitamin C (Ascorbic Acid) and flavonoids in fruit and vegetables can result in higher growth rates in plants and improved micronutrient uptake. The Vitamin C (Ascorbic

Acid) is considered very important and essential for growth promotion in plants.

- It has been discovered that vitamin C (Ascorbic Acid) acts in a positive and beneficial way to support the nodulation of legumes, promoting the synthesis of nucleic acids and proteins thus increasing nodule growth. Vegetables treated with vitamin C (Ascorbic Acid) and Flavonoids increased the concentration of nitrogen into nodules, branches, leaves and seeds. (Chinoy 1984).
- Scientific studies developed by Prof. Dale Norris of Wisconsin University USA has also verified that foliar spraying with low concentrations of Vitamin C and Flavonoids reduced about 70% of the damage caused by insects in soya bean cultivation. Similar positive and beneficial results in beans and broccoli cultivations were also achieved.

It has also been discovered that Vitamin C and Flavonoids help to build up leaf protein thus protecting the plants.

The Phytoalexin Effect

Phytoalexins are natural antimicrobial organic compounds, naturally produced in plants, which tend to accumulate after attacks on the plants by pathogens. Examples of Phytoalexins are:

Sesquiterpenoids, Phenylpropanoids, Stibenes, Furocoumarins, Isoflavanoids.

Some naturally occurring chemical products can induce plants to produce phytoalexins, and these substances are known as elicitors or phytoalexin precursors. Examples of such natural chemicals are combinations of flavonoids in combination with Vitamin C (Ascorbic Acid) and other specifically selected buffering agents.

In their fundamental mode of action, phytoalexins can be compared with animal anti-bodies, but with the following differences: -

- Antibodies are specific to a single disease, whereas phytoalexins are non-specific.
- Animals produce many types of anti-bodies, each working against a particular kind of microorganism, while plants produce phytoalexins, which are effective against many types of microorganisms.
- In animals a particular antibody is produced by a specific stimulant (antigen), whereas phytoalexins can be produced by several types of stimulant (biotic or abiotic).
- Antibodies remain in an animal for life, while phytoalexins gradually reduce in concentration once the disease attack has been dealt with. It is therefore very important to stimulate the production of Phytoalexins in plants by the

use of elicitors thereby producing a “top up” capability when opportunistic pathogen intrusion occurs.

Thus the action of phytoalexins can be seen as an ongoing battle against microorganisms, initiated by a pathogen attack on the plant. Phytoalexins are not a universal solution to the problem of pathogen attacks on plants, but they are the most powerful weapon against these attacks. When the plant's production of phytoalexins is higher than the degradation capability of the attacking pathogen, then infection either does not occur or its spread is minimised.

Modern agriculture strives continually for higher productivity by means of the intensive use of nitrogenous fertilizers, and by the use of potentially toxic substances such as herbicides, insecticides, fungicides and bactericides. All of these techniques are inimical to the natural production of phytoalexins and it has been shown that plants treated regularly with these substances lose their natural ability to produce phytoalexins.

Flavonoids, and Vitamin C (Ascorbic Acid), both originating in citrus plant varieties such as grapefruit and oranges, are extremely powerful elicitors of phytoalexins, and they form fundamental elements in the make-up of our BioAlexin products. As well as their effect as elicitors for phytoalexins, they act in combination as stabilisers towards oxidisable substances present in fruit and vegetables.

4. Areas Of Application and Application

- **Fruits**

- a. **Stoned fruits**: apricots, nectarines, plums, etc
- b. **Deciduous fruits**: apples, pears, etc
- c. **Citrus fruits**: grapefruit, lemons, oranges, etc
- d. **Berries**: blueberries, strawberries, etc
- e. **Exotic/tropical fruits**: banana & plantain, coconuts, guava, mangos, passion fruits, pineapples, pomegranates, etc

- **Vegetables**

- f. **Leafy/flowery vegetables**: broccolis, cauliflowers, lettuces, etc
- g. **Root vegetables**: carrots, onions, potatoes, etc
- h. **Other vegetables**: cucumber, onions, peppers, tomatoes, etc
- i. **Aromatic herbs**: lavender, thyme, etc

- **Other produce**

- j. **Cereals**: corn/maize, hay, wheat, etc
- k. **Table and wine grapes**
- l. **Ornamental flowers**: orchids, chrysanthemums, gerbera, roses, petunias, etc
- m. **Turf and lawns**

Application rates and Directions for Use

Directions for Use

BioAlexin is a foliar spray and can be applied with standard spraying equipment.

Mix into water before mixing with (if required) foliar fertilisers or calcium sprays.

If rain occurs within 6 hours of application you will need to reapply. Do not in the heat of the day.

Time Of Application	Application rates per ha BioAlexin
<ul style="list-style-type: none">• Post-harvest Directly after harvest Before leaf changes colour During pruning• Budswell (dormancy spray)• Green point• 10% bloom• 80% full bloom• 100% full bloom• Petal fall• Fruit set• Every fortnight up till harvest depending on the disease pressure	<p>Indicative use – 70 - 200ml per 100l of water</p> <p>Notes on Application;</p> <ul style="list-style-type: none">• Variations on application rates and intervals may occur, depending on the intensity of pathogen attack.• Water volume per hectare depends on tree row volume (high volume application) and the amount of foliage on the trees. The aim is to get good coverage to wet all the surfaces.• 70ml per 100 litres water applicable on trees up till 3 years of age.• BioAlexin has a low pH so cannot be pre-mixed with high pH fertilisers (NKP).• Works well with micronutrient fertilisers including calcium sprays.• BioAlexin can be applied in an integrated programme (I.P.M) along with synthetic or organic fertilisers to optimise plant development through the various growing stages

Precautions

- Do not apply with high levels of Nitrogen, and always consider the Calcium, Brix levels and pH status of the plant
- Do not apply in the heat of the day
- Do not apply if rain is expected within 6- 8 hours. If rain occurs re-apply after rain ceases
- Phyto-toxicity might occur when applied together with high dosage of nitrogen containing compounds

5. Examples Of Successful Containment Of Various Pathogens and Parasites using the BioAlexin plant nutrient synergist/fertiliser by allowing the plant to respond naturally to attacks

Pre-Harvest – Field Trials

CITRUS

DISEASE	PATHOGENS
Alternaria Rot	Alternaria citri
Anthracnose	Colletotrichum gloeosporioides
Bacterial Blast (Citrus Blast)	Pseudomonas syringae
Botrytis Rot	Botrytis cinerea
Brown Rot	Phytophthora spp.
Septoria Spot	Septoria citri

STONE FRUITS

DISEASE	PATHOGENS
Bacterial Canker	Pseudomonas syringae
Brown Rot Blossom & Twig Blight	Monilinia laxa Monilinia fructicola
Phytophthora root and crown rot	Phytophthora spp.
Powdery Mildew	Sphaerotheca pannosa Podosphaera tridactyla Podosphaera cladeana
Jacket Rot	Botrytis cinerea Sclerotinia sclerotiorum
Ripe Fruit Rot	Monilinia laxa Monilinia fructicola Botrytis cinerea Rhizopus spp
Peach leaf curl	Taphrina deformans
Scab	Cladosporium carpophilum

POTATOES

DISEASE	PATHOGENS
Bacterial Soft Rot & Black leg	Pectobacterium carotovorum var. carotovora Pectobacterium chrysanthemi
Early Blight	Alternaria solani
Fusarium Dry Rot & Seed Piece Decay	Fusarium spp.
Late Blight	Phytophthora infestans
Leak (Water Rot)	Pythium spp.
Pink Rot	Phytophthora erythroseptica
Powdery scab	Spongospora subterranea
Sclerotium Stem Rot	Sclerotium rolfsii
Stem and Stolon canker	Rhizoctonia solani
White Mold	Sclerotinia sclerotiorum

CUCURBITS (cucumber, melons, watermelons, squash, pumpkin)

DISEASE	PATHOGENS	
Angular leaf spot	Pseudomonas syringae	
Downy mildew	Pseudoperonospora cubensis	
Monosporascus Root rot	Monosporascus cannonballus	
Fusarium Wilt (watermelon)	Fusarium oxysporum Fusarium sp. niveum	
Powdery mildew	Sphaerotheca fuliginea Erysiphe cichoracearum	
Seedling Root Rots (Damping-off)	Pythium spp Rhizoctonia solani Fusarium solani Thielaviopsis basicola	Phytophthora spp. Acremonium spp. Fusarium equiseti

TOMATOES

DISEASE	PATHOGENS
Bacterial canker	Clavibacter michiganensis pv. Michiganensis
Bacterial Speck	Pseudomonas syringae pv. tomato
Bacterial Spot	Xanthomonas campestris pv. rescatoria
Black Mold	Alternaria alternata
Damping-off	Phytophthora Pythium spp. Rhizoctonia spp.
Early Blight	Alternaria solani
Gray mold	Botrytis cinerea
Phytophthora root Rot	Sclerotium rolfsii
Stem and Stolon canker	Phytophthora parasitica Phytophthora capsici
Powdery Mildew	Leveillula taurica Oidium neolycopersici

LETTUCE

DISEASE	PATHOGENS
Bacterial Leaf spot	Xanthomonas campestris p.v. vitians
Downy Mildew	Bremia lactucae
Lettuce drop	Sclerotinia minor Sclerotinia sclerotiorum
Phoma Basal Rot	Phoma exigua
Powdery mildew	Erysiphe cichoracearum

SMALL GRAINS (rye,wheat,barley,oat)

DISEASE	PATHOGENS
Covered smut of wheat	Tilletia caries Tilletia foetida
Covered smut of barley & oat	Ustilago nordei
Karnal Bunt of wheat	Tilletia indica
Loose smut of wheat, triticale and rye	Ustilago tritici
Loose smut of barley	Ustilago nuda
Black loose smut of barley & oat	Ustilago nigra
Powdery mildew	Erysiphe graminis
Blotch of wheat	Septoria tritici
Stripe Rusts of wheat & barley	Puccinia striiformis

STRAWBERRY

DISEASE	PATHOGENS
Angular leaf spot	Xanthomonas fragariae
Anthracnose	Colletotrichum acutatum
Botrytis fruit rot	Botrytis cinerea
Common leaf spot	Ramularia tulasnei
Leather rot	Phytophthora cactorum
Phytophthora crown rot	Phytophthora cactorum Phytophthora citricola Phytophthora parasitica Phytophthora megasperma
Powdery mildew	Sphaerotheca macularis
Verticillium wilt	Verticillium dahliae

APPLES & PEARS

DISEASE	PATHOGENS
Apple & Pear Scab	Venturia inaequalis
Bacterial Blossom Blast	Pseudomonas syringae
European canker	Nectria galligena
Fire blight	Erwinia amylovora
Phytophthora Root and crown rot	Phytophthora spp.
Powdery mildew	Podosphaera leucotricha

GRAPES

DISEASE	PATHOGENS
Armillaria Root Rot (Oak root fungus)	Armillaria mellea
Botrytis Bunch Rot	Botrytis cinerea
Downy Mildew	Plasmopara viticola
Eutypa Dieback	Eutypa lata Eutypa leptoplaca
Measles (Esca)	Togninia minima
Phomopsis cane & leafspot	Phomopsis viticola
Powdery mildew	Erisphe necator
Summer Bunch Rot (Sour rot)	Aspergillus niger Aternaria tenuis Botrytis cinerea Cladosporium herbarum Rhizopus arrhizus Penicillium spp.

COLE CROPS (Broccoli, Cabbage, Cauliflower)

DISEASE	PATHOGENS
Alternaria leafspot	Alternaria brassicae Alternaria brassicola
Bacterial Blight	Pseudomonas syringae pv. alisalensis
Black Rot	Xanthomonas campestris pv. campestris
Downy Mildew	Peronospora parasitica
Phytophthora Root rot	Phytophthora megasperma
Ringspot	Mycosphaerella brassicola
White Rust	Albugo candida

OLIVES

DISEASE	PATHOGENS
Armillaria Root rot	Armillaria mellea
Leaf spot	Mycocentrospora cladosporioides
Peacock spot	Spilocaeca oleaginea

FLORICULTURE

DISEASE	PATHOGENS
Cottony Rot	Sclerotinia sclerotiorum
Damping off	Rhizoctonia solani Pythium spp.
Downy Mildew	Peronospora spp. Plasmopara spp. Bremia spp.
Phytophthora root and crown Rots	Phytophthora spp.
Powdery mildew	Erysiphe spp. Leveillula spp. Oidium spp Sphaerotheca spp.
Pythium Root rot	Pythium spp.
Rust	Puccinia spp.
Thielaviopsis Root Rot	Thielaviopsis basicola

NUTS (Almonds, Pistachio, Walnuts)

DISEASE	PATHOGENS
Alternaria leaf spot & late Blight	Alternaria alternata
Anthrax nose	Colletotrichum acutatum
Armillaria Root Rot	Armillaria mellea
Bacterial canker	Pseudomonas syringae
Brown Rot Blossom Blight	Monilinia laxa Monilinia Fructicola
Green Fruit Rot	Botrytis cinerea Sclerotinia sclerotiorum Monilinia laxa
Leaf Blight	Seimatosporium lichenicola
Phytophthora Root and crown rot	Phytophthora spp.
Powdery mildew	Podosphaera panosa Pososphaera tridactyla Podosphaera leukotricha Oidium spp.
Rust	Tranzchelia discolor
Scab	Cladosporium carpophilum
Shot Hole	Wilsonomyces carpophilus
Pistachio Blossom & Shoot Blight	Botrytis cynerea
Panicle & Shoot Bilght	Botryosphaeria dothidea Fusicoccum spp.
Walnut Blight	Xanthomonas pv. juglandis

7. Examples Successful Field Trials

Apples (South Africa)

The Problem

Apple scab (*Venturia inaequalis*) is of major economic importance to apple growers causing extensive losses, especially in the humid and cool conditions of spring. The first signs of disease are often seen on the lower surfaces of leaves as they emerge and later both surfaces can become infected as the leaves unfold. The disease manifests itself first as brown coloured lesions which can coalesce with time. As the infected leaf ages, tissues close to the lesion thicken causing the leaf shape to become curled and distorted. If leaf or flower stalks become infected premature leaf or fruit fall may occur. Infected fruit can become cracked or deformed as corky tissue develops around fungal lesions. Infections of fruit that occur late in the growing season may not be visible until the fruit are already in storage and develop into rough black lesions referred to as "pin-point" scab.

The Strategy for Control of this Problem

Dr. W. Schwabe, an expert on the Apple Scab fungus, conducted field trials using MM109 rootstock apple trees to test the effectiveness of the ProAlexin™ to control fungal attack. Both preventative and curative action were assessed. Trials were conducted in the late summer.

For assessment of preventative action: apple trees were sprayed to run off with ProAlexin™ at various concentrations alone and in combination, one day before inoculation with a suspension of the Apple Scab fungus *Venturia inaequalis*. Unsprayed control trees were also inoculated. Permanent wetness was maintained throughout the trial by an overhead irrigation system and the temperature was maintained at around 20°C. Ten potted trees were used per treatment which equaled 140 trees in total.

For assessment of curative action: apples trees were inoculated with a suspension of *Venturia inaequalis* and permanent wetting was maintained as above. Twenty-four and 96 hours after inoculation, 12 trees for each treatment were sprayed with the relevant treatment of ProAlexin™. Twelve unsprayed control trees were also used for each treatment making a total of 336 trees.

Results

Preliminary results show that ProAlexin™ provided perfect control of the fungus compared to control plants, as well as showing a "kickback" period of at least two days (residual protection provided after infection). ProAlexin™ therefore provide a very effective solution for the organic producer and the grower wishing to move towards a more natural method of fungal control on Apple trees.

Cucumbers (South Africa)

Testimonial

C.C.PAGE.

Manger of Hertzberger Farm, SA

"After having used ProAlexin™ for almost two years we have achieved Eurepgap Certification in 2005 and again in 2006. ProAlexin™ comes in as the only fertiliser for micro-organism total control agents in cucumber for Eurepgap Certification as conventional chemical companies have not bothered to register any chemicals of total significance on minor crops such as cucumbers and some others. For the few that are registered on cucumbers one would have to do continuous spraying of the different chemicals to be able to keep up with the micro-organism pressure. And then you still would not achieve Eurepgap Certification as there are some diseases caused that can only be controlled by unregistered product.

When you use ProAlexin™ you bypass all these obstacles. You get total control in just one spray application. And you are immediately Eurepgap compliant as ProAlexin™ is totally organic and totally non residual and totally safe for human consumption. During the six month life cycle of the cucumber crop it is only needed to do eight applications of ProAlexin™. If you use conventional chemical you would be looking at 20 + applications. It should not be difficult to work out which is the most efficient protocol to follow. On the above note all the micro-organism threats have been eliminated.

However, we still have the insects to deal with. And when it comes to cucumbers, once again the chemical companies have not bothered to register much on cucumbers. So once again we have to go Organic. Fortunately there is a reasonably good range of organic products that can be used on cucumbers to fight off the insect attacks. Between these and the effects of ProAlexin™ we are able to naturally ward off all the insects that attack cucumbers, with the exception of one. The White Fly. Because White Fly breed exponentially and we are not allowed to use unregistered insect growth control regulants, we can never eliminate the White Fly totally. However, thanks to ProAlexin™ and the other Organic based insecticides we use, we are able to limit the White Fly population down to acceptable limits. The White Flies that do survive unfortunately do infect the plant with the cucumber leaf yellowing virus. This is where ProAlexin™ again comes in very handy, putting us in a position to still get acceptable production.

Altogether, if it were not for ProAlexin™ we as cucumber growers would not have been able to survive being Eurepgap compliant. Therefore, thanks to ProAlexin™ we can and are Eurepgap compliant."

Strawberries (South Africa)

The Problem

Strawberries (*Fragaria x ananassa* Duch.) are highly susceptible to post-harvest decay and particularly the effects of *Botrytis cinerea*, also known as grey mould. This unfortunately means that they have a relatively short shelf-life, typically measured in terms of days. Good temperature management is currently the best way to ensure that an acceptable product is delivered to the market. The strawberries have to be rapidly cooled as soon as possible after harvest, and maintained at a temperature of approximately 0°C for as long as possible during post-harvest distribution. Alas, grey mould is able to withstand even these low temperatures, and if not managed correctly, condensation on the berries due to low temperatures will often be followed by rapid development of the fungus.

This trial was intended to assess the potential of ProAlexin™ to replace the conventional pre-harvest chemical fungicide spray program on strawberries. It was hypothesized that control of post-harvest decay development would be negatively affected by replacement with these organic products.

The Strategy

The trials were conducted on a commercial strawberry farm between Wemmershoek and Paarl, Western Cape, using the cultivar "Eris". Prior to planting, all plants were dipped in Ridomil Gold, a commercial fungicide, for *Phytophthora* control, and dipped in Chronos (imidazole) for *Anthraco*se control. This was followed by a single spray application of the same chemicals to all plants 13 days later on 25 May. The Trial commenced on 7 June. Normal irrigation and fertilization practices were followed on all plants.

The Control plants, treatment 1, received the conventional spray program applied by the grower. This consisted of 17 individual chemicals (11 insecticides and 6 fungicides) applied 21 times during the growing season between 23 June and 6 December.

In the second treatment, ProAlexin™ plant nutrient synergist/fertilizer was applied at the recommended rate of 150 ml/hectare (7 June, approximately 21 days after transplant), 225 ml/hectare (15 June) and 300 ml/hectare (on an approximately weekly basis until 18 November, equaling 18 applications in total). Commencing on 3 August, a further treatment of ProAlexin™ was added to this spray mix at a concentration of 200mls/hectare. While none of the conventional fungicides were used, insecticide sprays were applied as normal when there were no fungicides in the tank mix.

Berries were harvested on three occasions from mid November to early December at the commercially-ripe stage. Fruit were immediately transported to Stellenbosch where the punnets were weighed and then kept in an air-conditioned laboratory for simulated shelf life evaluation. Fruit were monitored daily over five days to record the incidence of decay development in each punnet. Decay development was expressed as a percentage of the fruit fresh weight at the time of harvest. The trial was designed and analysed as a complete, randomised block design. The three harvests were analysed separately as distinct trials.

The Results

	Treatment	% (by weight) of decayed berries				
		Day 1	Day 2	Day 3	Day 4	Day 5
Harvest 1	Conventional Spray Program	0.0	0.5	3.4	9.1	27.6
	ProAlexin™	0.0	1.8	4.7	8.8	24.2
	P>0.05	0.955	0.687	0.575	0.846	0.748
Harvest 2	Conventional Spray Program	0.3	2.2	8.7	16.7	35.1
	ProAlexin™	0.0	0.9	6.5	19.7	28.4
	P>0.05	0.843	0.662	0.497	0.216	0.448
Harvest 3	Conventional Spray Program	0.0	4.4	17.5	39.4	67.5
	ProAlexin™	0.4	5.8	14.5	36.8	74.8
	P>0.05	0.898	0.334	0.884	0.457	0.225

As can be seen from the table, for all three harvests, the results were similar and statistically there were no differences between treatments in the rate of decay development (a p-value of <0.05 is a statistically significant result).

Although not relevant to this particular trial, it was observed that there was a strong trend for an increase in decay from Harvest 1 to Harvest 3. This is likely due to increased decay susceptibility with age and warmer weather conditions from July to December. Spore loads in the block would also be expected to increase substantially over time, leading to a higher risk of infection as time passes.

All fruit punnets started showing signs of decay within two to three days of harvest. Once decay development commenced, the spread of disease was rapid, likely facilitated by the warm 20°C temperature. Decayed berries had to be removed from the punnets daily to prevent a larger and more rapid spread of decay which would have harmed the experiment. This demonstrates the extreme susceptibility to decay in

strawberries and illustrates clearly why a good temperature management program is required for the successful post-harvest distribution and sale of this crop.

It was concluded from this trial that the replacement of the conventional pre-harvest chemical fungicide spray program with the organic ProAlexin™ products had no deleterious effect on berry decay development during simulated shelf life evaluation after harvest. This therefore opens the possibility for the grower to replace the chemical program with the organic fertiliser program.

Cherry Tomatoes (South Africa)

Cherry tomatoes (*Lycopersicon esculentum*) are produced year round in South Africa. Many growers utilise greenhouse or glasshouse facilities, particularly in winter when field conditions are not conducive to crop production. Cherry tomatoes have a good post-harvest shelf life and generally tend not to develop many post-harvest decay problems. However, during the fruit production cycle itself fungal organisms often attack the plants, leading to the necessity for regular fungicide applications. Due to the emerging pressure from consumers for “chemical free” fruit and vegetable products, growers are interested in the prospect of replacing conventional chemical pesticides with more organic crop treatments.

This project investigated the potential of ProAlexin™ to replace the conventional pre-harvest fungicide spray program on cherry tomatoes. It was hypothesised that control of post harvest decay development would be negatively affected by such a replacement.

The Strategy

The trials were conducted in a polyethylene-covered tunnel on a commercial cherry tomato farm between Wemmershoek and Paarl, Western Cape, using the cultivar “Josephina”.

Seeds were sown in seedling trays in early May and young seedlings were then transplanted into black bags (approximately 10 litre capacity). On all plants the standard side shoot pruning was conducted.

Control plants, treatment 1, received the conventional fungicide spray program applied by the grower, consisting of Dithane (200g/100L) and Dimildex (300g/100L) applied at approximately 3-weekly intervals during the season from early June to December.

In the second treatment, the fungicide sprays were substituted with ProAlexin™ and was applied at the recommended rate of 135 ml/hectare (22 June approximately 7-14 days after transplant) and 200 ml/hectare (approximately once weekly from 6 July until 18 November, totally 17 applications). Commencing on 3 August, ProAlexin™ was added to the spray mix at a concentration of 0.2% (volume/volume).

Tomatoes were harvested on two occasions for shelf life evaluation, in mid November and early December. In all cases fruit were harvested at the commercially-ripe stage. Fruit were immediately transported to Stellenbosch, bags of sampled fruit were weighed and then kept in an air-conditioned laboratory for simulated shelf life evaluation, at

approximately 20°C. Fruit were monitored daily over 10-16 days (Harvest 1 and Harvest 2, respectively) to record the incidence of decay development in each bag. Decay development was expressed as a percentage of fresh weight at the time of harvest. The trial was designed and analysed as a complete, randomised block design, with 8 replicates per treatment and two treatments (control and organic citrus extract). The two harvests were analysed separately as distinct trials.

The Results

			% (by weight) of decayed tomatoes	
	Treatment	Days of first appearance of decay	Day 10	Day 10
Harvest 1	Conventional fungicide program	8	0.8	-
	ProAlexin™	-	0.0	-
	P>0.05	-	0.994	-
Harvest 2	Conventional fungicide program	9	-	53.7
	ProAlexin™	-	-	0.0
	P>0.05	-	-	0.001

As can be seen from the table, in the case of the first harvest, the first observation of decay appeared in the Control treatment on the 8th day of evaluation. No decay was evident in the ProAlexin™ treated fruits over the 10 day evaluation period. Despite these observations decay levels of the Control fruits were low overall in this harvest so statistically there were no differences between the treatments.

In the second harvest, however, dramatic differences were evident. Fruit in the Control bags started to show signs of soft decay on Day 9. Rather than terminating the investigation at that stage, it was decided to wait for the ProAlexin™ treatment to also develop decay. In the control, decay spread rapidly in the bags and as decayed fruit were not removed the contents became very soggy. The observations were continued for 16 days, at which time there was still zero decay in the ProAlexin™ treatment. By this time all eight replicates of control fruit were totally degraded. It was decided to terminate the investigation at this point, and the sound fruit in the control treatment were weighed as it was almost impossible to weigh the decayed fruit.

Conclusion

Based on these results, one can conclude that the replacement of the conventional chemical fungicide spray program with ProAlexin™ during pre-harvest production of tomatoes had a very positive effect on decay development during simulated shelf life evaluation after the second harvest. It is unfortunate that the two chemical fungicide sprays were inadvertently applied during the season, but nevertheless the results were still extremely promising. This opens up the possibility for the grower to replace the conventional pre-harvest chemical program with the organic program.

Plums (South Africa)

Mr Derick van Zye – Applethwait Farm
7th August 2008

We have been involved with the product ProAlexin™ for more than 2 years. Initially I looked at the product as a natural control utilising the plant own defence mechanism for Xanthamona on plums as all other known products yielded insufficient results. Following from the initial sprays on plums we also treated various young non producing orchards to see if we can improve the vegetative growth of the plants.

Some outstanding results:
Plums – Orchard 107

These plums were, due to stress conditions, heavily invested with Zanthamonas and Pseudomonas bacteria. Due to the three applications post harvest followed up with one dormancy spray we have succeeded to suppress the amount of visible spring cancers.

- Stronger base to start
- Plant growth thru seasons is better
- Fast recovery after “stress”
- No with holing time before harvests
- Increased amount of class one fruit from these trees

Sunkiss 2007 10t/ha with 20% cull
 a. 15t/ha with 14% cull

Fortune 2007 4t/ha with 16% cull
 2008 14t/ha with 13% cull

- Less rotting and post harvest decay
- No product returned be less than 5%
- Less scares on re-growth for next season
- Health plant
- Use of preening decontaminant

In future we must use this product in a regular programme with emphasis on wet period.

We will continue with trials.

Orchards 23 Souviner plums

- Increased yields
- Better fruit size
- Less post harvest disorders

SOU	2006	11t/ha	11% orchard cull with 6% decay
	2007	14tt/ha	0% orchard cull with no post harvest disorders
	2008	29t/ha	0.1% orchard cull with no post harvest disorders

Better fruit size in 2nd year. All fruit size A and better with a peak in AA and AAA.

Not a silver bullet and must be used in a normal programme.
Enhances the uptake of nutrients resulting in better vegetative growth.

Grapes (Greece)

Application of ProAlexin™ to suppress Esca and Black Measles.
Nemea, Greece, 2006-7
(Trial conducted by Polypan Group S.A.)

Before ProAlexin™ (2005)... The disease situation was so serious, the farmer wanted to plant a new vineyard

After ProAlexin™ treatment (2006-7)...

Season	Chronic symptoms	Acute symptoms (apoplexy)
2005	≈ 50	10
2006	≈ 20	1
2007	≈ 10	0

...even from the first year of application there was an important decrease in Esca incidence, and there was a significant decrease in the sudden death of plants.

8) A List Of Literature References

1. Altman, J. and Campbell, C.L.
Effect of Herbicides on plant diseases. *Ann.Rev.Phytopathol.* 1977 15:361-85.
2. Chinoy,J.J.
The role of ascorbic acid in growth, differentiation, and metabolism of plants.
Martinus Nijhoff/Dr.W.Junk Publishers, Boston, 1984. 322p.
3. Kuc,J.
Phytoalexins, Stress Metabolism, and Disease Resistance in plants. *Annu. Rev. Phytopathol.* 33:275-297. 1995.
4. Kuc,J.;Rush,J.S.
Phytoalexins. *Archives of Biochemistry and Biophysics.* Vol 236, N°2,pp.455-472,1985.
5. Lydon, J and Duke, S.O.
Pesticide Effects on Secondary metabolism of higher Plants. *Pesticide science.* 1989, 25, 361-373.
6. Osbourn, E.A.
Performed Antimicrobial compounds and Plant Defences Against Attack. *The plant cell*, Vol 8, 1821-1831 – 1996.
7. Phillips, D.A.
Flavonoids: plant signals to microbes, in: Stafford, H.A. and ibrahin, R.K., eds. *Metabolism in Planto.* New York, Plenum Press, 1992. P.201-231.
8. Smith, C.J.
Tansley Review N°86. Accumulation of phytodexins: defence mechanism and stimulus response system. *New Phytol.* (1996) 132, 1 45.
9. Van Etten H.D.
Antifungal activity of pterocarpans and others selected isoflavonoids.
Phytochemistry 15: 655-656, 1976.
10. *Clinical Microbiological Reviews*, October 1999, p.564-582, 12, No 4. "Plant Products as Antimicrobial Agents".
11. Dixon et al. 1983 Phytoalexins: enzymology and molecular biology. *Adv. Enzymol.* 55: 1-69.